SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-089514- -00000-0000
Fecha: 2013-04-05 14:18:14 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 156

Delegatura de Propiedad Industrial
División de Nuevas Creaciones

SOLICITUD DE

PATENTE DE INVENCION
PCT ENTRADA EN FASE NACIONAL

21. EXPEDIENTE No. _____

54. TITULO: **COMPOSICION FARMACEUTICA**

52. CLASIFICACION INTERNACIONAL: **C07K 16/18, G01N 33/68, A61K 39/00**

71. SOLICITANTE: **1. AC IMMUNE S.A.**
2. KATHOLIEKE UNIVERSITEIT LEUVEN

DOMICILIO: **1. EPFL-PSE Building B, CH-1015 Lausanne, SUIZA**
2. KU Leuven Research & Development, Waaistraat 6, Box 5105, B-3000 Leuven, BELGICA

74. APODERADO: **Dra. MARGARITA CASTELLANOS A.**

22. BOGOTA, D.C., 5 ABR. 2013

Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-089514- -00000-0000
Fecha: 2013-04-05 14:18:14 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 156

DIRECCION DE NUEVAS CREACIONES
SOLICITUD FASE NACIONAL - PCT

1	(12) TIPO DE SOLICITUD	X Patente de Invención X Capítulo I	____ Patente de Modelo de Utilidad ____ Capítulo II
2	DATOS SOLICITUD INTERNACIONAL PCT (86)		
	Solicitud Internacional No.	PCT/EP2011/067604	Fecha 07/10/2011
	Publicación Internacional No.	WO 2012/045882 A3	Fecha 12/04/2012
3	(54) TITULO DE LA INVENCION (200 caracteres o espacios máximos)		
	COMPOSICION FARMACEUTICA		
4	CLASIFICACION INTERNACIONAL (51) CIP		
	C07K 16/18, G01N 33/68, A61K 39/00		
5	(71) SOLICITANTE(S) ____ Esta persona también es inventor	Para datos adicionales utilizar hoja de información adicional	

APELLIDOS O RAZON SOCIAL	NOMBRE	IDENTIFICACION	TIPO
AC IMMUNE S.A.			

6	DATOS DEL SOLICITANTE		
	DIRECCION	EPFL-PSE Building B	No. TELEFONO
	CIUDAD	CH-1015 Luasanne	E-MAIL
	DEPARTAMENTO/ESTADO		NACIONALIDAD O LUGAR DE CONSTITUCION SUIZA
	PAIS DE RESIDENCIA	SUIZA	
7	(72) INVENTOR(ES)	Para datos adicionales utilizar hoja de información adicional	

	APELLIDOS	NOMBRES	NACIONALIDAD
1.	PFEIFER	Andrea	Alemana
2.	MUHS	Andreas	Alemana
3.	VAN LEUVEN	Fred	Belga
4.	PIHLGREN	Maria	Sueca
5.	ADOLFSSON	Oskar	Islandés

DIRECCION DE CORREO ELECTRONICO:

8	DATOS INVENTOR(ES)			
	PAIS RESIDENCIA	DEPARTAMENTO/ESTADO	CIUDAD	DIRECCION
1.	SUIZA		CH-1806 St-Légier	Route de Fenil 16A
2.	SUIZA		CH-1009 Pully	Avenue des Cerisiers 39b
3.	BELGICA		B-3210 Linden	Hertogenlaan 18
4.	SUIZA		CH-1052 Montsur-Lausanne	Chemin du Tessin 6B
5.	SUIZA		CH-1038 Bercher	Chemin de Larry 16

OTRO(S) SOLICITANTE(S) Y/U OTRO(S) INVENTOR(ES)

____ Los demás solicitantes y/o demás inventores se indican en una hoja a continuación

9	(74) ____ REPRESENTANTE	X APODERADO	
	APELLIDOS	NOMBRES	IDENTIFICACION
	CASTELLANOS ABONDANO	MARGARITA	C.C. 39.779.654 T.P. 70.819
	DIRECCION Calle 94A No. 7A-09		No. TELEFONO 7560770
	CIUDAD BOGOTA		E-MAIL info@castellanosyco.co
	PAIS COLOMBIA		No. DE PROTOCOLO EN LA OFICINA

10	(30) DECLARACIONES DE PRIORIDAD	X SI	NO

	(33) PAIS DE ORIGEN	CODIGO PAIS	(31) NUMERO	(32) FECHA (AAA/MM/DD)
1.	OFICINA EUROPEA DE PATENTES	EP	10186810.7	2010/10/07
2.	OFICINA EUROPEA DE PATENTES	EP	11174248.2	2011/07/15
3.				

11 DECLARACION SOBRE USO DE RECURSOS GENETICOS O BIOLOGICOS

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de recursos genéticos o biológicos de los que cualquiera de los paises miembros de la Comunidad Andina es país de origen.

_____ SI __X__ NO

NOTA: En caso afirmativo deberá anexar copia del contrato de acceso de recursos genéticos o productos derivados, o certificado o número de registro expedido por la Autoridad competente.

12 DECLARACION SOBRE USO DE CONOCIMIENTOS TRADICIONALES

Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de conocimientos tradicionales de comunidades indígenas, afroamericanas o locales de países miembros de la Comunidad Andina.

_____ SI __X__ NO

NOTA: En caso afirmativo deberá anexar la licencia o autorización de uso de conocimiento tradicional, o certificado, o número de registro expedido por la Autoridad competente.

13 REDUCCION DE TASAS

Declaro que carezco de medios económicos para presentar la solicitud de patente

_____ SI __X__ NO

NOTA: En caso de ser persona natural y carecer de medios económicos, y por lo tanto, aplique la reducción de tasas a que se refiere la resolución vigente en tarifas, debe firmar la presente solicitud bajo la gravedad de juramento.

Micro, pequeñas y medianas empresas	Universidades públicas o privadas	Entidades sin animo de lucro
_____ Copia simple de la declaración de renta del año inmediatamente anterior, o en su defecto prueba documental idónea. _____ Documento de constancia de cumplimiento con lo establecido en la ley 905 de 2004.	_____ Copia acto de reconocimiento institucional emitido por el Ministerio de Educación. Acreditación Colciencias.	_____ Copia de registro vigente en Cámara de Comercio. _____ Hoja de información complementaria. _____ Otros, especificar.

14 AUTORIZACION DE NOTIFICACION EN LINEA _____ SI __X__ NO

Manifiesto que he leído y entendido perfectamente los términos y condiciones de uso de medios electrónicos para las notificaciones en linea a través de Internet de los actos administrativos proferidos por la Superintendencia de Industria y Comercio que deber ser notificados personalmente y, en consecuencia, autorizo el servicio de notificación a través de internet.

14	COMPROBANTE DE PAGO O PAGO ELECTRONICO	N° 13-0031210 y 13-0031211	Fecha: Abril 3 de 2013

15 FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL

Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)

MARGARITA CASTELLANOS ABONDANO - APODERADO - 5 ABR. 2013

16 ORDEN Y RELACION DE DOCUMENTOS QUE ACOMPAÑAN LA SOLICITUD

Documentación Técnica
Solicitud Internacional en Castellano

1. __X__ Descripción — N° de folios: 336
2. __X__ Reivindicaciones — N° de reivindicaciones: 120
3. __X__ Dibujos y/o figuras — N° de folios: 22
4. __X__ Resumen
5. _____ Certificado de depósito de material biológico si fuera el caso.
6. _____ Listado de secuencias de nucleótidos y/o aminoácidos en forma digital si fuera el caso.
7. __X__ Arte final 12 x 12
8. __X__ Anexo formato digital.

SE ANEXA CD QUE CONTIENE:
* TEXTO EN ESPAÑOL DE LA DESCRIPCION Y REIVINDICACIONES
** TEXTO DE LA PUBLICACION INTERNACIONAL DE LA SOLICITUD No. PCT/EP2011/067604
*** LISTADO DE SECUENCIAS EN INGLES

Documentación Jurídica

9. _____ Poderes, si fuera el caso.
10. _____ Documento que legalmente pruebe la cesión del inventor al solicitante o a su causante.
11. _____ Copia del contrato de acceso de recursos genéticos o productos derivados, certificado o número de registro, si fuera el caso.
12. _____ Copia de la licencia o autorización de Conocimientos Tradicionales, certificado o número de registro, si fuera el caso.
13. _____ Documento que acredita la existencia y representación legal, cuando el solicitante es persona jurídica.
14. __X__ Comprobante de pago de la tasa de presentación de la solicitud.
15. _____ Comprobante de pago por reivindicación de prioridad
16. _____ Comprobante de pago de la tasa por concepto de excedente de palabras en la publicación.
17. __X__ Comprobante de pago por reivindicación adicional a 10.

Industria y Comercio
SUPERINTENDENCIA

HOJA DE INFORMACION COMPLEMENTARIA PCT

1	**(12) TIPO DE SOLICITUD**	NUMERO DE RADICACION	
X	Patente de Invención	FECHA DE PRESENTACION	
	Patente de Modelo de Utilidad		

4	**(71) SOLICITANTE(S)**			
	APELLIDOS O RAZON SOCIAL	NOMBRE	IDENTIFICACION	TIPO
2.	**KATHOLIEKE UNIVERSITEIT LEUVEN**			
3.				
4.				

5	**DATOS DEL SOLICITANTE**			
	PAIS DE RESIDENCIA	DEPARTAMENTO/ESTADO	CIUDAD	DIRECCION
2.	**BELGICA**		**B-3000 Leuven**	**KU Leuven Research & Development, Waaistraat 6, Box 5105**
3.				
4.				
	TELEFONO	FAX	E-MAIL	NACIONALIDAD
2.				**BELGA**
3.				
4.				

6	**(72) INVENTOR(ES)**		
	APELLIDOS	NOMBRE	NACIONALIDAD
6.			
7.			
8.			
9.			
10.			
11.			
12.			

7	**DATOS INVENTOR(ES)**			
	DIRECCION	CIUDAD	DEPARTAMENTO/ESTADO	PAIS RESIDENCIA
6.				
7.				
8.				
9.				
10.				
11.				
12.				

9	**(30) DECLARACIONES DE PRIORIDAD**			
	(33) PAIS DE ORIGEN	**CODIGO PAIS**	**(31) NUMERO**	**(32) FECHA (AAA/MM/DD)**
4.				
5.				
6.				
7.				
8.				
9.				
10.				

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 0031210

Bogotá D.C., Abril 03 de 2013 - 15:30:25

RECIBIDO DE : KIENESKIEN EDITORIAL SAS NI 900.380.957

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	444580275	19/03/2013	23.350.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCION	500.000.00	500.000.00
				$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*******-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-089514- -00000-0000
Fecha: 2013-04-05 14:18:14 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 156

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 0031211

Bogotá D.C., Abril 03 de 2013 - 15:30:25

RECIBIDO DE : KIENESKIEN EDITORIAL SAS NI 900.380.957

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	444580275	19/03/2013	23.350.000.00

*** Conceptos Pagados ***

CANT. RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
110 50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	30.000.00	3.300.000.00
			$3.300.000.00

SON: **TRES MILLONES TRESCIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-********-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-089514- -00000-0000
Fecha: 2013-04-05 14:18:14 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 156

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 6C10F9C12A11	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3079

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address: Inhoffenstr. 7 B, D-38124 Braunschweig	Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA PSE-EPFL Building B	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3079
Address:	1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 7C2(2)B9F11D5	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3083

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 08/2006

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

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PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA PSE-EPFL Building B	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3083 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

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PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 2B6G7A12	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3087

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building B Address: 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3087 Date of the deposit or the transfer[1]: 2010-08-25
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2010-08-30 [2]. On that date, the said microorganism was (x)[3] viable ( )[3] no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wirths Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 3A8E12H8	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3085

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wirth Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building B Address: 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3085 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: A6-2G5-30	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3137

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-08-30 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wirths Date: 2011-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3137 Date of the deposit or the transfer[1]: 2011-08-30

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wells Date: 2011-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:
A6-2G5-41

Accession number given by the
INTERNATIONAL DEPOSITARY AUTHORITY:
DSM ACC3138

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-08-30 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address: Inhoffenstr. 7 B
D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

V. Wecko

Date: 2011-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3138 Date of the deposit or the transfer[1]: 2011-08-30

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Werb Date: 2011-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

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PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: A4-2A1-18	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3139

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-08-30 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wecko Date: 2011-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3139 Date of the deposit or the transfer[1]: 2011-08-30

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wecks Date: 2011-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
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INTERNATIONAL FORM

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PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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INTERNATIONAL DEPOSITARY AUTHORITY
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: A4-4A6-48	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3136

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-08-30 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2011-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3136 Date of the deposit or the transfer[1]: 2011-08-30

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weks Date: 2011-09-09

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: A4-2A1-40	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3140

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-08-30 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wels Date: 2011-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3140 Date of the deposit or the transfer[1]: 2011-08-30

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wehs Date: 2011-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 6C10E5E9C12	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3081

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA PSE-EPFL Building B	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3081 Date of the deposit or the transfer[1]: 2010-08-25
III. VIABILITY STATEMENT		
The viability of the microorganism identified under II above was tested on 2010-08-30 On that date, the said microorganism was		
(X)[2] viable ( )[2] no longer viable		
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]		
V. INTERNATIONAL DEPOSITARY AUTHORITY		
Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wecks
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: A6-1D2-12	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3141

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2011-09-06 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Wecks Date: 2011-09-16

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building
1015 Lausanne
Switzerland

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA PSE-EPFL Building Address: 1015 Lausanne Switzerland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3141 Date of the deposit or the transfer[1]: 2011-09-06

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2011-09-06 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2011-09-16

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Karl-von-Frisch-Str.,
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 7C2(1)F10C10D3	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3082

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 08/2006

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PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Karl-von-Frisch-Str.,
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA PSE-EPFL Building B	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	1015 Lausanne SWITZERLAND Karl-von-Frisch-Str., Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3082 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

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SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:
6H1G6E6

Accession number given by the
INTERNATIONAL DEPOSITARY AUTHORITY:
DSM ACC3088

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address: Inhoffenstr. 7 B
D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

V. Weis

Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

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Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: AC Immune SA Address: PSE-EPFL Building B 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3088 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weeks Date: 2010-09-09

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 6H1A11C11	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3080

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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INTERNATIONAL FORM

AC Immune SA
PSE-EPFL Building B
1015 Lausanne
SWITZERLAND
Katholieke Universiteit Leuven
Minderbroedersstraat 8a - Box 5105
B-3000 Leuven

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	PSE-EPFL Building B 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3080 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	V. Wirths Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer)
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

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identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 3A8A12G7	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3086

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: 2010-09-09

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PSE-EPFL Building B
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SWITZERLAND
Katholieke Universiteit Leuven
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I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	PSE-EPFL Building B 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3086 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	V. Wecks Date: 2010-09-09

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identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: 2B6A10C11	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM ACC3084

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2010-08-25 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address: Inhoffenstr. 7 B, D-38124 Braunschweig	Date: 2010-09-09

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
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I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM
Name:	AC Immune SA	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Address:	PSE-EPFL Building B 1015 Lausanne SWITZERLAND Katholieke Universiteit Leuven Minderbroedersstraat 8a - Box 5105 B-3000 Leuven	DSM ACC3084 Date of the deposit or the transfer[1]: 2010-08-25

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2010-08-30 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):
Address:	Inhoffenstr. 7 B D-38124 Braunschweig	Date: 2010-09-09

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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(10) International Publication Number
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G01N 33/68 (2006.01)

(21) International Application Number:
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(22) International Filing Date:
7 October 2011 (07.10.2011)

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(26) Publication Language: English

(30) Priority Data:
10186810.7 7 October 2010 (07.10.2010) EP
11174248.2 15 July 2011 (15.07.2011) EP

(71) Applicants *(for all designated States except US)*: **AC IMMUNE S.A.** [CH/CH]; EPFL-PSE Building B, CH-1015 Lausanne (CH). **KATHOLIEKE UNIVERSITEIT LEUVEN** [BE/BE]; KU Leuven Research & Development, Waaistraat 6, Box 5105, B-3000 Leuven (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PFEIFER, Andrea** [DE/CH]; Route de Fenil 16A, CH-1806 St-Légier (CH). **MUHS, Andreas** [DE/CH]; Avenue des Cerisiers 39b, CH-1009 Pully (CH). **VAN LEUVEN, Fred** [BE/BE]; Hertogenlaan 18, B-3210 Linden (BE). **PIHLGREN, Maria** [SE/CH]; Chemin du Tessin 6B, CH-1052 Mont-sur-Lausanne (CH). **ADOLFSSON, Oskar** [IS/CH]; Chemin de Larry 16, CH-1038 Bercher (CH).

(74) Agent: **WERNER BASTIAN**; Vossius & Partner, Siebertstrasse 4, 81675 München (DE).

[Continued on next page]

(54) Title: PHOSPHOSPECIFIC ANTIBODIES RECOGNISING TAU

(57) Abstract: The present invention relates to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

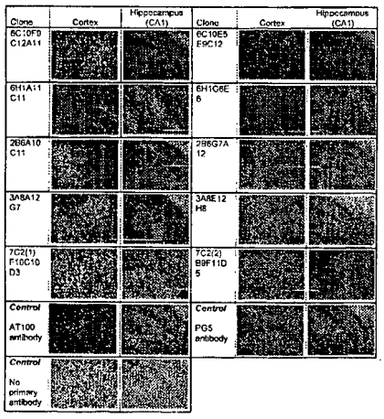


FIGURE 1-1

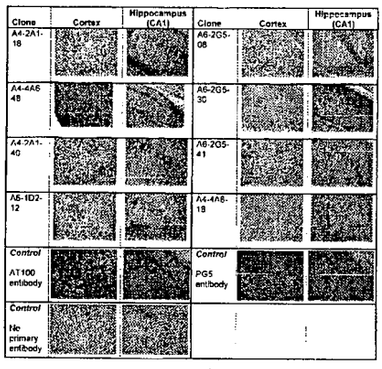


FIGURE 1-2

WO 2012/045882 A3

WO 2012/045882 A3

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report**: 31 May 2012

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(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 April 2012 (12.04.2012)

PCT

(10) International Publication Number
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(51) International Patent Classification:
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G01N 33/68 (2006.01)

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PCT/EP2011/067604

(22) International Filing Date:
7 October 2011 (07.10.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10186810.7 7 October 2010 (07.10.2010) EP
11174248.2 15 July 2011 (15.07.2011) EP

(71) Applicants *(for all designated States except US)*: **AC IMMUNE S.A.** [CH/CH]; EPFL-PSE Building B, CH-1015 Lausanne (CH). **K. U. LEUVEN RESEARCH & DEVELOPMENT** [BE/BE]; Waaistraat 6, Box 5105, B-3000 Leuven (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PFEIFER, Andrea** [DE/CH]; Route de Fenil 16A, CH-1806 St-Légier (CH). **MUHS, Andreas** [DE/CH]; Avenue des Cerisiers 39b, CH-1009 Pully (CH). **VAN LEUVEN, Fred** [BE/BE]; Hertogenlaan 18, B-3210 Linden (BE). **PIHLGREN, Maria** [SE/CH]; Chemin du Tessin 6B, CH-1052 Mont-sur-Lausanne (CH). **ADOLFSSON, Oskar** [IS/CH]; Chemin de Larry 16, CH-1038 Bercher (CH).

[Continued on next page]

(54) Title: PHARMACEUTICAL COMPOSITION

(57) Abstract: The present invention relates to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

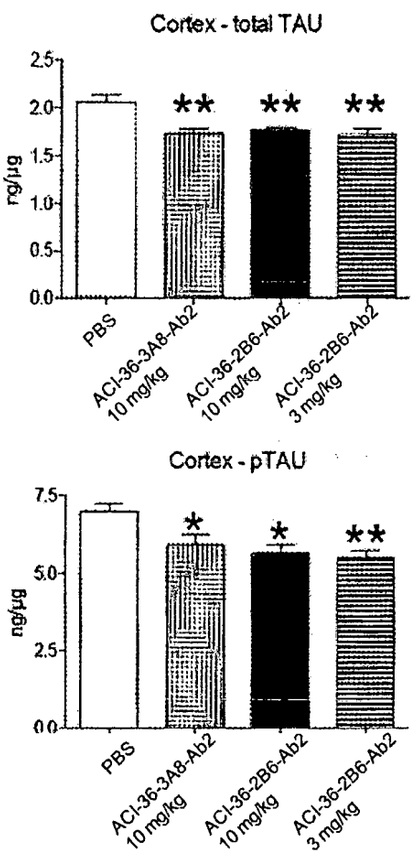


FIGURE 3

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(74) **Agent**: **WERNER BASTIAN**; Vossius & Partner, Siebertstrasse 4, 81675 München (DE).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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PHARMACEUTICAL COMPOSITION

The present invention is related to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

Neurofibrillary tangles and neuropil threads (NTs) are the major neuropathological hallmarks of Alzheimer's Disease (AD). They are composed of the microtubule-associated protein tau that has undergone posttranslational modifications, including phosphorylation, deamidation and isomerization on asparaginyl or aspartyl residues. They originate by the aggregation of hyper-phosphorylated protein tau and its conformers. AD shares this pathology with many neurodegenerative tauopathies, in particularly with specified types of frontotemporal dementia (FTD).

Protein Tau is a freely soluble, "naturally unfolded" protein that binds avidly to microtubuli (MT) to promote their assembly and stability. MTs are of major importance for the cytoskeletal integrity of neurons - and thereby for the proper formation and functioning of neuronal circuits, hence for learning and memory. The binding of tau to MT is controlled by dynamic phosphorylation and de-phosphorylation, as demonstrated mainly *in vitro* and in non-neuronal cells. Due to the large number of possible phosphorylation sites (>80), the exact contribution of each and the identity of the responsible kinases remain largely undefined *in vivo*.

In AD brain, tau pathology develops later than, and therefore probably in response to amyloid pathology, which constitutes the essence of the amyloid cascade hypothesis. This is based on and indicated by studies in AD and Down syndrome patients, and is corroborated by studies in transgenic mice with combined amyloid and tau pathology (Lewis et al., 2001; Oddo et al., 2004; Ribe et al., 2005; Muyllaert et al, 2006; 2008; Terwel et al, 2008).

The exact timing of both pathologies in human AD patients as well as mechanisms that link amyloid to tau pathology remain largely unknown, but are proposed to involve activation of

Claims:

1. An antibody, or a functional part thereof, which antibody recognizes and specifically binds to a phospho-epitope on the mammalian Tau protein or on a fragment thereof, wherein said antibody or antibody fragment has a high binding affinity to soluble and insoluble Tau protein, and modulates soluble and insoluble Tau levels.

2. The antibody of claim 1 or a functional fragment thereof, wherein said antibody or antibody fragment modulates soluble and insoluble Tau levels in the brain of a mammal.

3. The antibody of claim 2 or a functional fragment thereof, wherein said antibody or antibody fragment modulates soluble and insoluble Tau levels in brain cortex and/or hippocampus.

4. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of total soluble tau protein.

5. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of soluble phosphorylated tau protein.

6. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of paired helical filaments containing hyperphosphorylated tau protein.

7. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels total soluble tau protein, soluble phosphorylated tau protein and pTau paired helical filaments.

8. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said mammal is a human.

9. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 10 nM.

10. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 5 nM, at least 2 nM; at least 1 nM, at least 500 pM,at least 300 pM, at least 200 pM, at least 100 pM.

11. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater; of 10^5 $M^{-1}s^{-1}$ or greater, of 10^6 $M^{-1}s^{-1}$ or greater.

12. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 4 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

13. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 3 nM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

14. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 2 nM and an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater.

15. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 1 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

16. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 200 pM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

17. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a

dissociation constant of at least 100 pM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

18. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position (pS214); Tau aa 393-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

19. The antibody of claim 12 or 13 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 15-20 with a phosphorylated Tyr at position 18 (Y18).

20. The antibody of claim 14 or 15 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 405-412 with a phosphorylated Ser at position 409 (pS409).

21. The antibody of claim 16 or 17 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 405-411 with a phosphorylated Ser at position 409 (pS409).

22. The antibody of claim 12 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396).

23. The antibody of claim 12 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404).

24. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains, particularly in sequence, a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, or an amino acid sequence at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%,

least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, or an amino acid sequence at least 60%, particularly at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, 89, 98, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, 115, or an amino acid sequence at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, 91, 100, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

25. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 70% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 90% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 60% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid

sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 80% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 60% identical thereto.

26. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 85% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 80% identical thereto; and/or a antibody domain which contains a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 85% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 85% identical thereto.

27. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto

28. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain (antibody domain) which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid

sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

29. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

30. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 98% identical thereto;. and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 98% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

31. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, a CDR2 with the

amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20.

32. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, or an amino acid sequence at least 76% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, or an amino acid sequence at least 66% identical thereto, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, a DR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66% identical thereto.

33. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 24, or an amino acid sequence at least 88% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 25, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 26, or an amino acid sequence at least 66% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88% identical thereto,and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66% identical thereto

34. The antibody of claim 33 or a functional fragment thereof, comprising a first binding domain wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 27, or an amino acid sequence at least 88% identical thereto.

35. The antibody of claim 33 or a functional fragment thereof, comprising a first binding domain wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 28, or an amino acid sequence at least 88% identical thereto.

36. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 29, a CDR2 with the amino acid sequence shown in SEQ ID NO: 30, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 31, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 15, a CDR2 with the amino acid sequence shown in SEQ ID NO: 16, or an amino acid sequence at least 94% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 17, or an amino acid sequence at least 36% identical thereto.

37. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 32, a CDR2 with the amino acid sequence shown in SEQ ID NO: 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 34, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 19, or an amino acid sequence at least 95% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 20, or an amino acid sequence at least 63% identical thereto.

38. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 73, a CDR2 with the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 75, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 70, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 71, or an amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 72, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least

95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

39. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 81, a CDR2 with the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 80, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

40. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 93, a CDR2 with the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 95, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

41. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 101, a CDR2 with the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 103, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 98, a CDR2 with the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 100, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

42. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 108, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

43. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in

SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 85% identical thereto.

44. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

45. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain (antibody domain) which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

46. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69, 77, 116/92, 97, 105, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68, 76, 88, 96, 104, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

47. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence at least 90% and 94%, respectively, identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, or an amino acid sequence at least 91% identical thereto.

48. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto.

49. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain, which contains the amino acid sequence shown in SEQ ID NO: 9, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3, or an amino acid sequence at least 90% identical thereto.

50. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4, or an amino acid sequence at least 89% identical thereto.

51. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11, or an amino acid sequence at least 98% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5, or an amino acid sequence at least 87% identical thereto.

52. The antibody of any one of the preceding claims or a functional fragment thereof comprising a

a. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or

b. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 7 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or

c. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2; or

d. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 9 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3; or

e. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4; or

f. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5; or a

g. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68; or a

h. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 77 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 76; or a

i. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 116 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a;

j. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 92 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a

k. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 97 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 6; or a

l. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 105 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 104.

53. The antibody of the preceding claim or a functional fragment thereof, which is polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody or a functional fragment thereof.

54. The antibody of any one of the preceding claims or a functional fragment thereof which is of the IgG2b, IgG2a or the IgG3 isotype.

55. A binding peptide or protein of the antibody according to any one of claims 1-54.

56. The binding peptide or protein or the antibody or functional fragment thereof according to any one of the preceding claims, wherein said binding peptide, protein antibody or antibody fragment recognizes and specifically binds to a phospho-epitope on the mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a phospho-epitope on aggregated Tau protein, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

57. A polynucleotide encoding the antibody any one of the preceding claims or a functional fragment thereof.

58. The polynucleotide of claim 57 comprising a nucleic acid molecule selected from the group consisting of

a. a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as depicted in SEQ ID NOs: 35-45,;

b. a nucleic acid molecule comprising a nucleotide sequence that has at least 85% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

c. a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

d. a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

e. a nucleic acid molecule comprising a nucleotide sequence that has at least 98% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

f. a nucleic acid molecule comprising a nucleotide sequence the complementary strand of which hybridizes to the nuclei acid molecule of any of a) – f);

g. a nucleic acid molecule comprising a nucleotide sequence that deviates from the nucleotide sequence defined in any of a) – g) by the degeneracy of the genetic code:

wherein said nucleic acid molecule as defined in any of a) – g) recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said antibody has a high binding affinity to aggregated Tau protein, and modulates soluble and insoluble Tau levels.

59. A pharmaceutical composition comprising a binding peptide or protein, an antibody or a functional fragment thereof or a polynucleotide according to any one of the preceding claims, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

60. The pharmaceutical composition of claim 59, which comprises at least two binding peptides or proteins, antibodies or functional fragments thereof or polynucleotides according to any one of the preceding claims, which recognize and bind to two different phospho-epitope on the mammalian Tau protein.

61. The pharmaceutical composition of claim 59 or 60, which comprises an additional antibody or a functional fragment thereof which regcongizes and binds to a different amyloidogenic protein or peptide.

62. An antibody or a functional fragment thereof, abinding peptide or protein, a polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof, for use in therapy, particularly for use in the treatment of neurodegenerative disorders such as tauopathies in a mammal, particularly a human in need of such a treatment.

63. An antibody or a functional fragment thereof, a binding peptide or protein,, or pharmaceutical composition according to the preceding claim for use in treatment or alleviation of cognitive deficits in a mammal, particularly a human suffering from such a deficit.

64. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim, wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an arrest in the progression of the cognitive deficits.

65. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim,

wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an increase in the retention, particularly a complete restoration of cognitive memory capacity in the treated subject.

66. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or the pharmaceutical composition according to the preceding claim for use in the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

67. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim for use in the treatment of Alzheimer's Disease.

68. A method for the treatment of a neurodegenerative disease or disorder such as tauopathy comprising administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, an antibody or a functional fragment thereof, a binding peptide or protein, a polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

69. The method according to the preceding claim for the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary

lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

70. A method for inducing a passive immune response in an animal, particularly a mammal or a human, suffering from a neurodegenerative disorder such as tauopathy by administering to said animal or human an antibody or a functional fragment thereof, a binding peptide or protein or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

71. A method of diagnosing a tau-protein-associated disease, disorder or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau protein in the sample or specific body part or area.

72. A method for diagnosing a predisposition to tau-protein-associated disease, disorder or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an tau protein-associated disease or condition.

73. A method for monitoring minimal residual disease in a patient following treatment with an antibody or a pharmaceutical composition according to any one of the preceding claims, wherein said method comprises:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

74. A method for predicting responsiveness of a patient being treated with an antibody or a pharmaceutical composition according to any one of the preceding claims comprising

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;

b. allowing the antibody to bind to the tau antigen to form an immunological complex;

c. detecting the formation of the immunological complex; and

d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,

e. comparing the amount of said immunological complex before and after onset of the treatment,

wherein an decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

75. Test kits for detection and diagnosis of tau protein-associated diseases, disorders or conditions comprising an antibody according to any of the preceding claims.

76. Test kit according to the preceding claim comprising a container holding one or more antibodies or functional fragments thereof according to the present invention and instructions for using the antibodies for the purpose of binding to tau antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of tau antigen.

77. An epitope selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); and Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position (pS214), Tau aa 393-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-

400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

78. A cell line producing an antibody according to any of the preceding claims.

79. A cell line according to claim 78, which is hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079.

80. A cell line according to claim 78, which is hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081

81. A cell line according to claim 78, which is hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080.

82. A cell line according to claim 78, which is hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088.

83. A cell line according to claim 78, which is hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084.

84. A cell line according to claim 78, which is hybridoma cell line 2B6G7A12 deposited on March 10, 2010 as DSM ACC3087.

85. A cell line according to claim 78, which is hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086.

86. A cell line according to claim 78, which is hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085.

87. A cell line according to claim 78, which is hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082.

88. A cell line according to claim 78, which is hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083.

89. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

90. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

91. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

92. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

93. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

94. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6G7A12 deposited on August 25, 2010 as DSM ACC3087 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

95. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

96. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR

amplification of DNA of hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

97. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 49; SEQ ID NO: 56 and SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

98. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083 using

a. a pair of primers comprising a 5'-primer of SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

99. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 149 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, and 140 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 134, and 141-148, for amplification of a second binding domain.

100. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 169-174 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 124, 127, and 150-158 and a 3'-primer selected from the group consisting of SEQ ID NOs: 130, and 159-168, for amplification of a second binding domain.

101. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 178, 179 and 180 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 127, 139, 154, 155, and 175 and a 3'-primer selected from the group consisting of SEQ ID NOs: 128, 129, 147, 176, and 177, for amplification of a second binding domain.

102. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 188-192 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 124, 126, 181, 182 and 183 and a 3'-primer selected from the group consisting of SEQ ID NOs: 144, 145 and 184-187, for amplification of a second binding domain.

103. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 50 and 201-204 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 137, 151 and 193-197 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 141, 144, 166, 198, 199 and 200, for amplification of a second binding domain.

104. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 209-214, and 219-221 a 3'-primer of SEQ ID NO: 215, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 216, 217 and 218 and a 3'-primer of SEQ ID NOs: 208, for amplification of a second binding domain.

105. The antibody or a functional fragment thereof of any one of the preceding claims, wherein said antibody is an antibody of the IgG2a, IgG2b or the IgG3 isotype, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody.

106. A binding fragment or protein or an antibody according to any one of the preceding claims.

107. A cell line that produces the antibody of any one of claims 1-54, 56 or of any one of claims 89 – 105.

108. A cell line comprising the polynucleotide of claim 57 or 58.

109. A method of producing the antibody of any one of claims 1-54, 56 or of any one of claims 89 – 105, comprising the step of culturing the cell line of claim 106 in a suitable cultivation medium.

110. A method of producing an antibody according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , comprising the step of culturing the cell line of any one of claims 78-88 in a suitable cultivation medium.

111. The method of claim 109 or 110, further comprising the step of purifying the antibody from the cell line or cultivation medium.

112. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in the treatment of tauopathies, a group of tau protein associated diseases and disorders.

113. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in treating or alleviating the symptoms associated with tauopathies, a group of tau protein associated diseases and disorders.

114. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

115. A cell line according to claim 78, which is hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136.

116. A cell line according to claim 78, which is hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137.

117. A cell line according to claim 78, which is hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138.

118. A cell line according to claim 78, which is hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139.

119. A cell line according to claim 78, which is hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140.

120. A cell line according to claim 78, which is hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141.

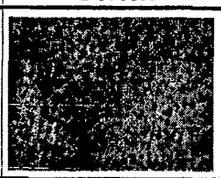
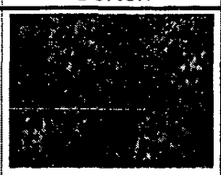
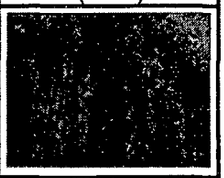
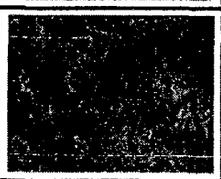
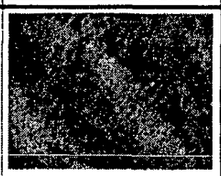
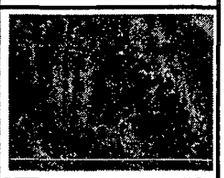
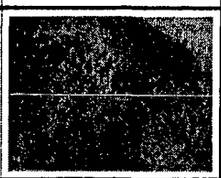
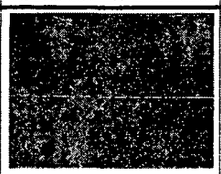
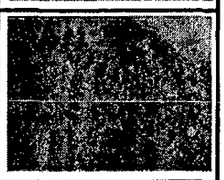
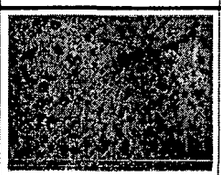
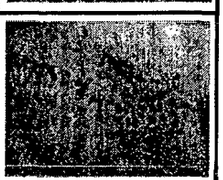
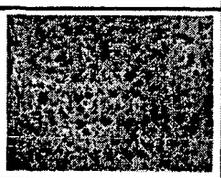
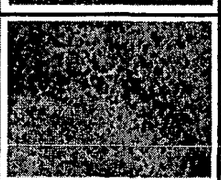
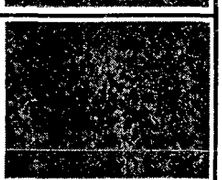
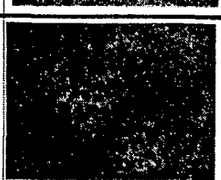
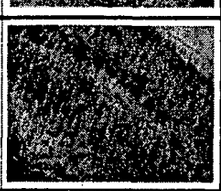
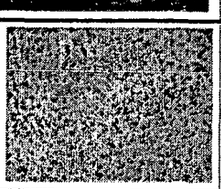
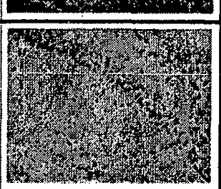
Clone	Cortex	Hippocampus (CA1)	Clone	Cortex	Hippocampus (CA1)
6C10F9 C12A11			6C10E5 E9C12		
6H1A11 C11			6H1G6E 6		
2B6A10 C11			2B6G7A 12		
3A8A12 G7			3A8E12 H8		
7C2(1) F10C10 D3			7C2(2) B9F11D 5		
Control AT100 antibody			***Control*** PG5 antibody		
Control No primary antibody					

FIGURE 1-1

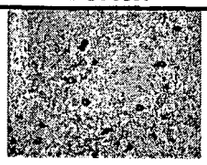
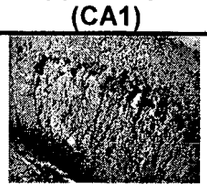
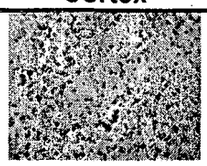
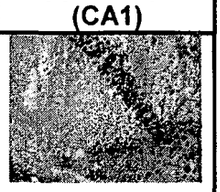
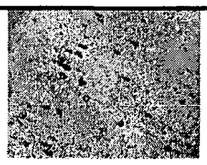
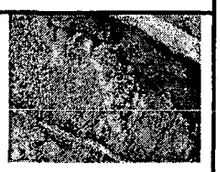
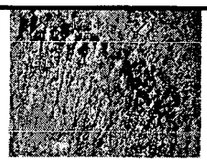
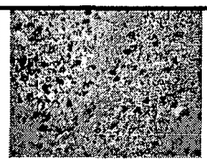
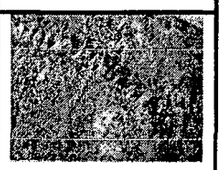
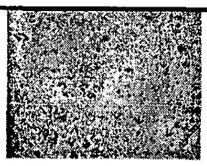
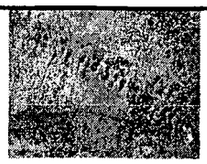
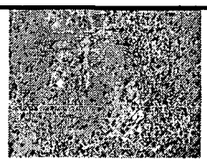
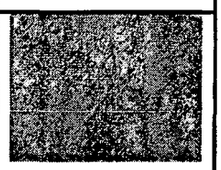
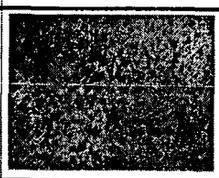
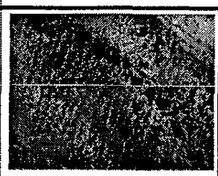
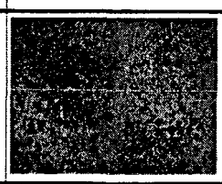
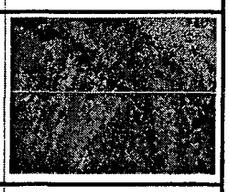
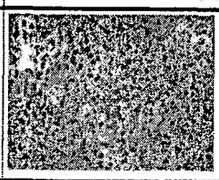
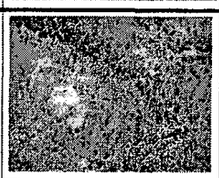
Clone	Cortex	Hippocampus (CA1)	Clone	Cortex	Hippocampus (CA1)
A4-2A1-18			A6-2G5-08		
A4-4A6-48			A6-2G5-30		
A4-2A1-40			A6-2G5-41		
A6-1D2-12			A4-4A6-18		
Control AT100 antibody			***Control*** PG5 antibody		
Control No primary antibody					

FIGURE 1-2

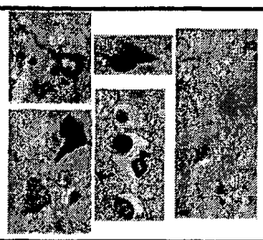
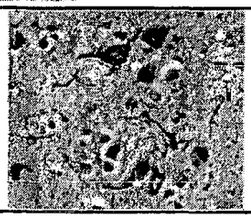
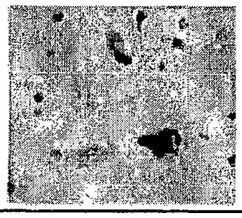
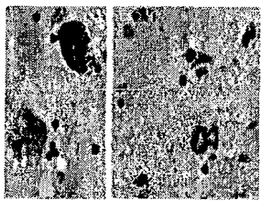
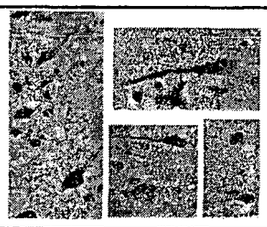
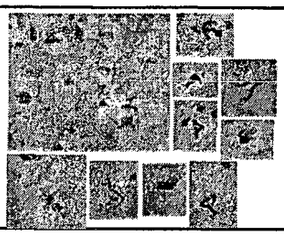
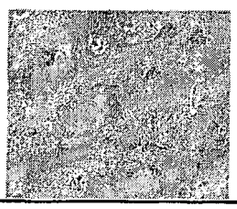
Diagnosis	Region	ACI-3A8-Ab1 TAUPIR staining
AD	Hippocampus	
FAD	Anterior hippocampus	
AGD	Anterior hippocampus	
PSP	Pallidum	
FTDP-17	Frontal cortex	
CBD	Frontal cortex	
Healthy control	Hippocampus	

FIGURE 2

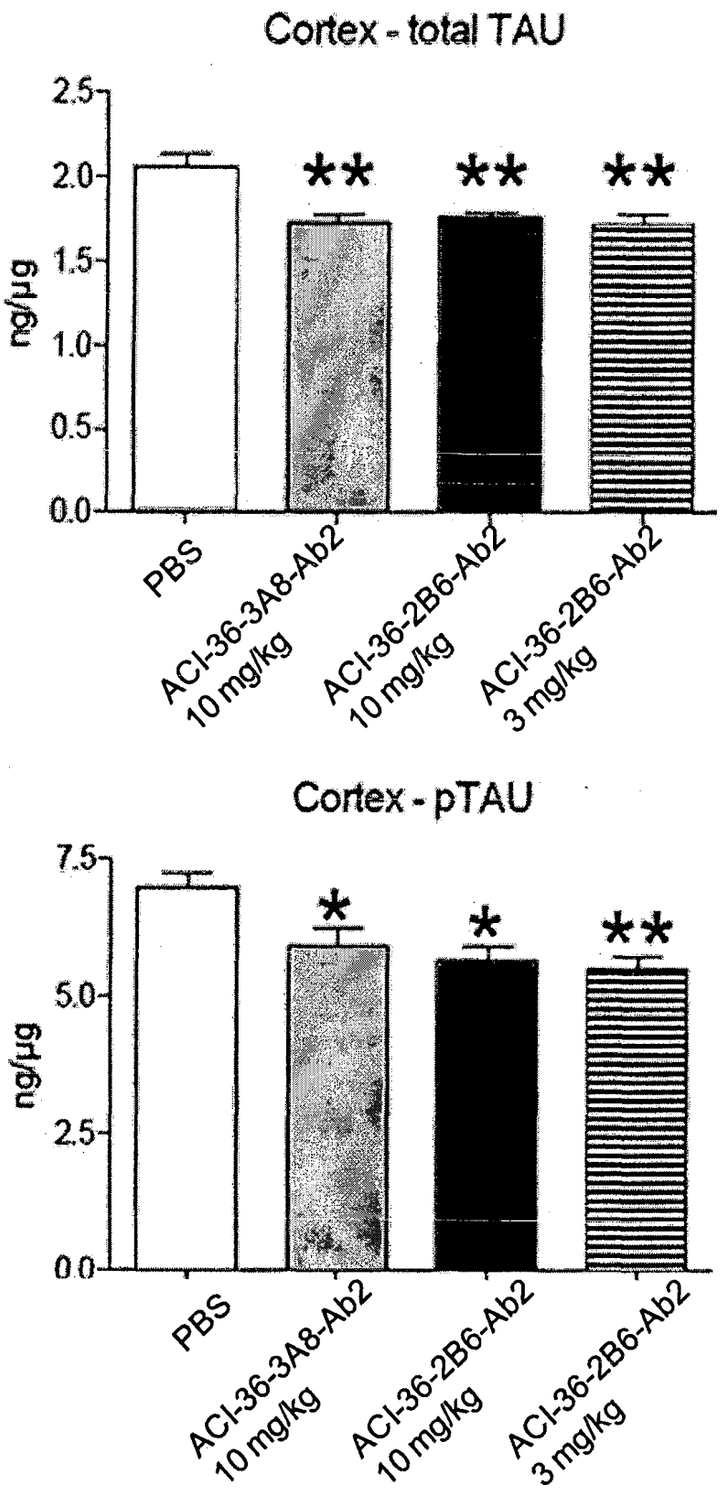

Cortex - total TAU
ng/mg
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg
Cortex - pTAU
ng/mg
7.5
5.0
2.5
0.0
*
*
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg

FIGURE 3

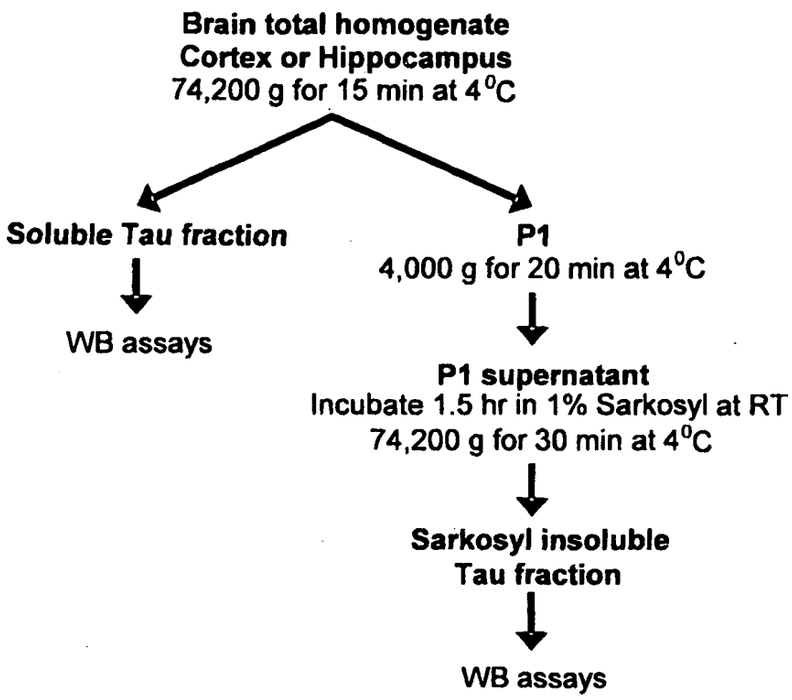


FIGURE 4-1

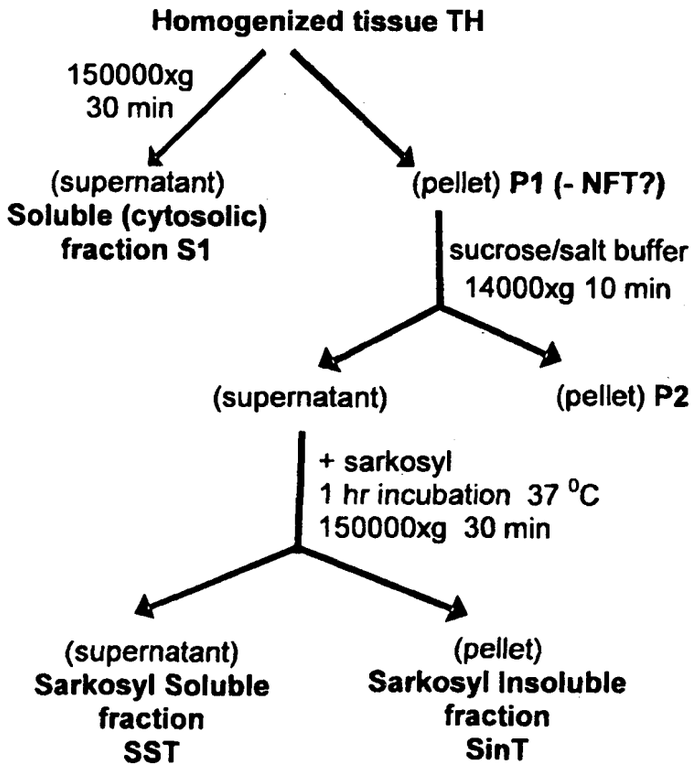


FIGURE 4-2

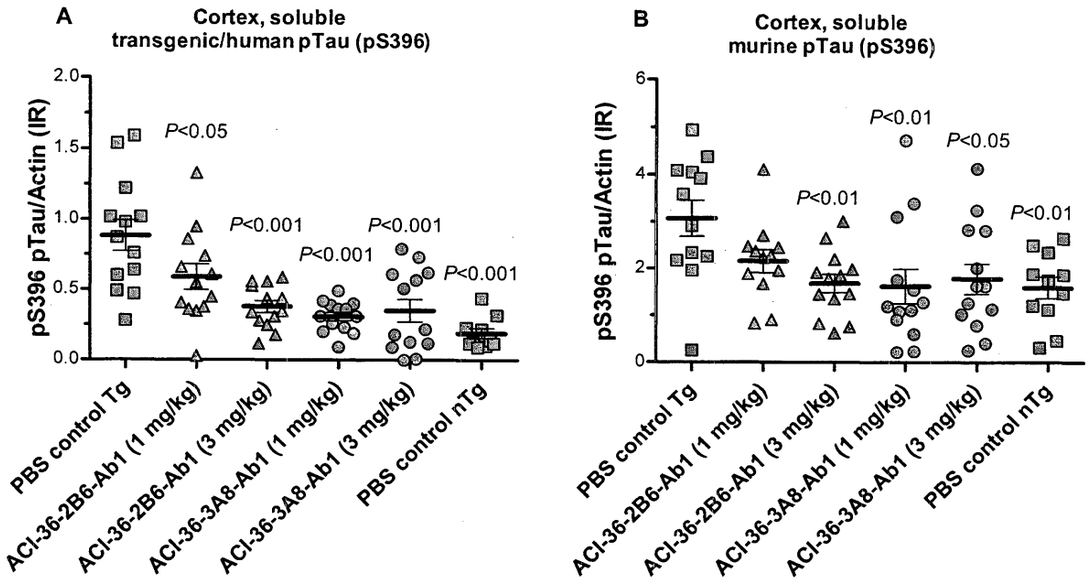
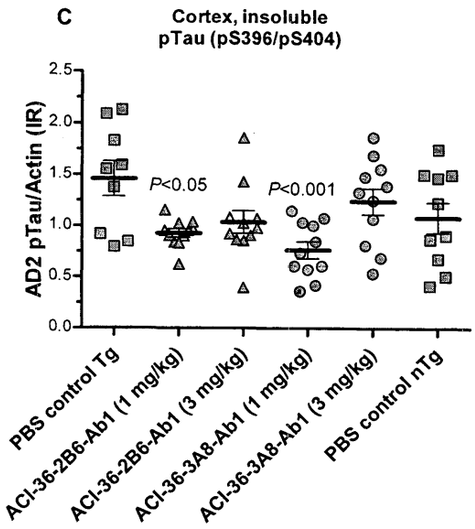

A
Cortex, soluble
transgenic/human pTau (pS396)
pS396 pTau/Actin (IR)
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
B
Cortex, soluble
murine pTau (pS396)
pS396 pTau/Actin (IR)
6
4
2
0
P<0.01
P<0.01
P<0.05
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-1


C
Cortex, insoluble
pTau (pS396/pS404)
AD2 pTau/Actin (IR)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

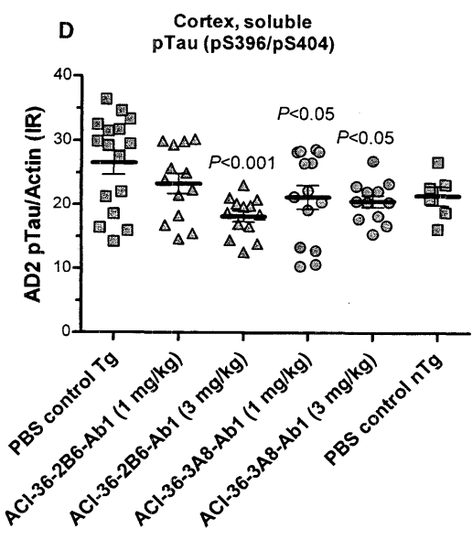
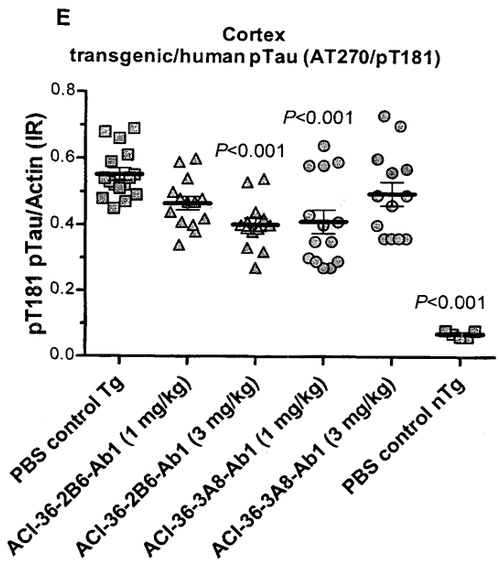

D
Cortex, soluble
pTau (pS396/pS404)
AD2 pTau/Actin (IR)
40
30
20
10
0
P<0.05
P<0.001
P<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-2


E
Cortex
transgenic/human pTau (AT270/pT181)
pT181 pTau/Actin (IR)
0.8
0.6
0.4
0.2
0.0
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

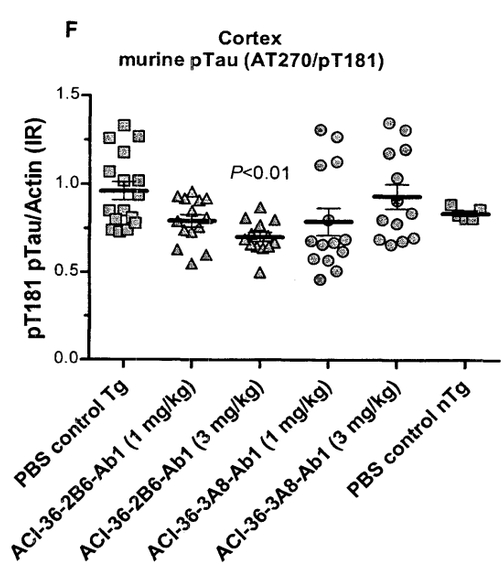

F
Cortex
murine pTau (AT270/pT181)
pT181 pTau/Actin (IR)
1.5
1.0
0.5
0.0
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-3

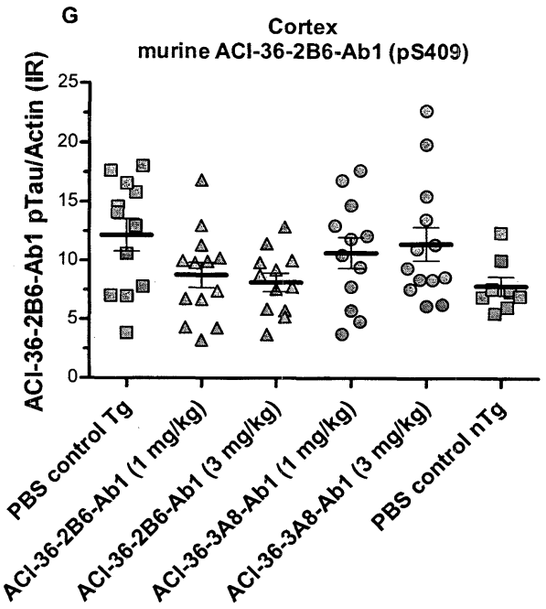

G
Cortex
murine ACI-36-2B6-Ab1 (pS409)
ACI-36-2B6-Ab1 pTau/Actin (IR)
25
20
15
10
5
0
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-4

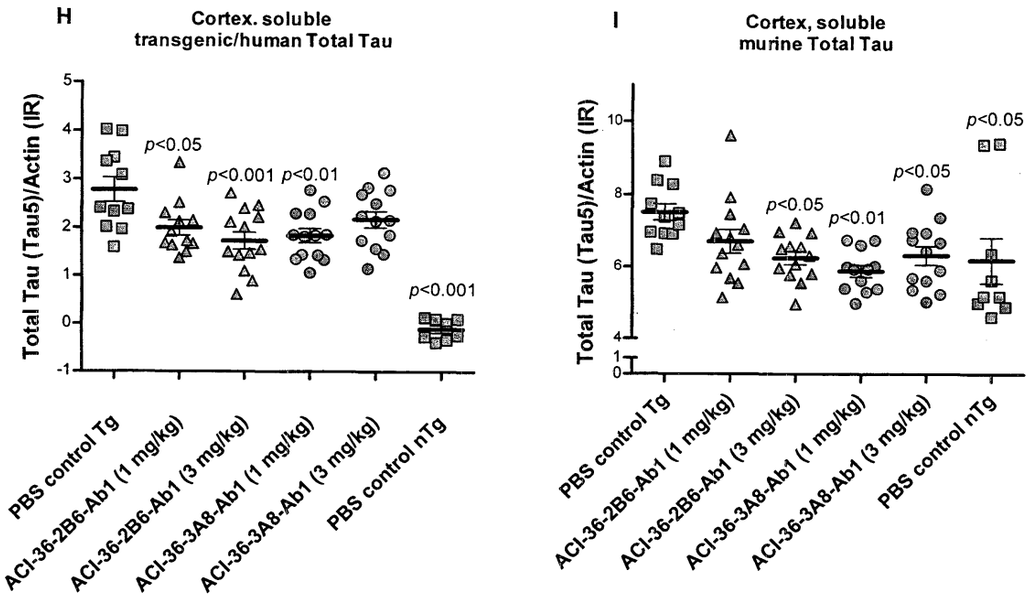

H
Cortex. soluble
transgenic/human Total Tau
Total Tau (Tau5)/Actin (IR)
p<0.05
p<0.001
p<0.01
p<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
I
Cortex, soluble
murine Total Tau
Total Tau (Tau5)/Actin (IR)
p<0.05
p<0.01
p<0.05
p<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-5

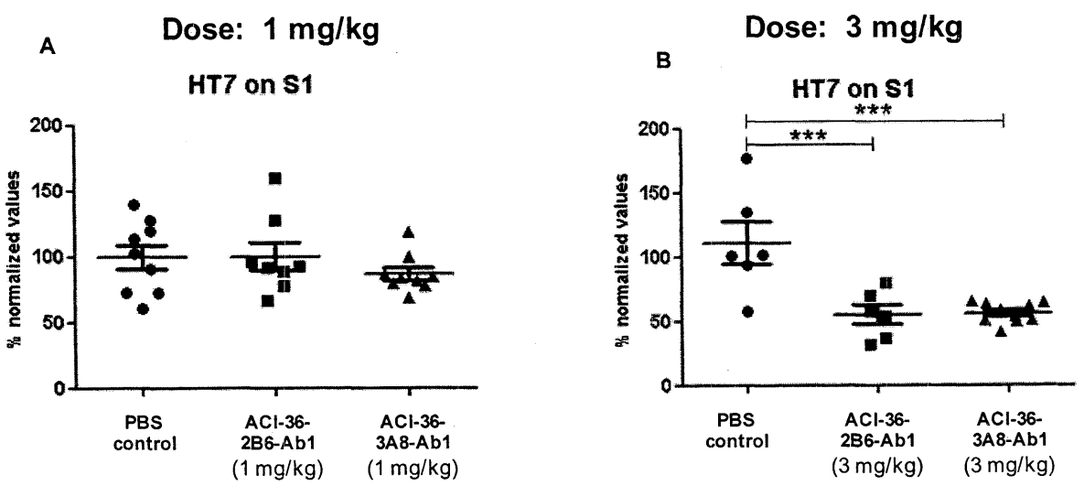

Cortex, soluble fraction (S1)
transgenic/human total Tau (HT7)
A
Dose: 1 mg/kg
HT7 on S1
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
B
Dose: 3 mg/kg
HT7 on S1
***
***
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-1

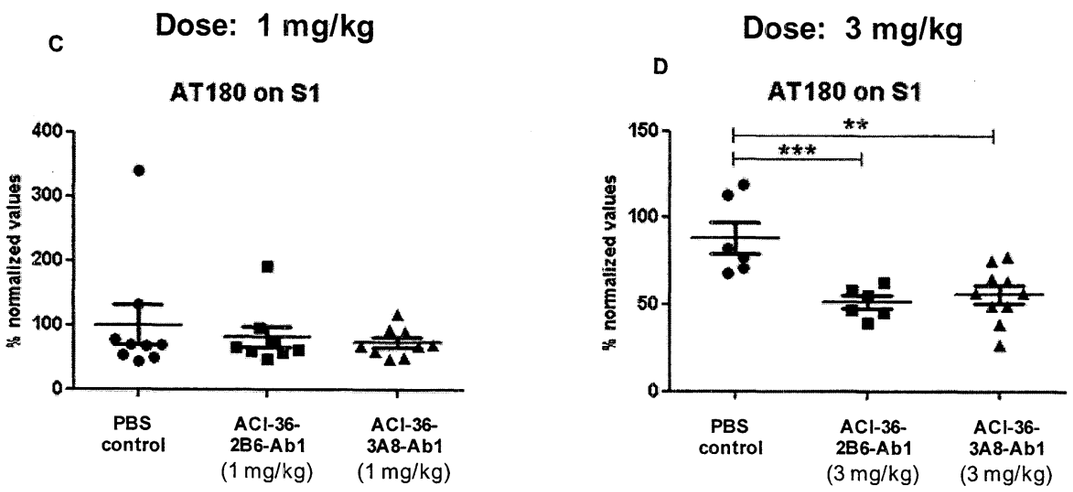

Cortex, soluble fraction (S1)
transgenic/human pTau (pT231/AT180)
C
Dose: 1 mg/kg
AT180 on S1
% normalized values
400
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
D
Dose: 3 mg/kg
AT180 on S1
% normalized values
150
100
50
0
**
***
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-2

Cortex, soluble fraction (S1) transgenic/human pTau (pS202/AT8)

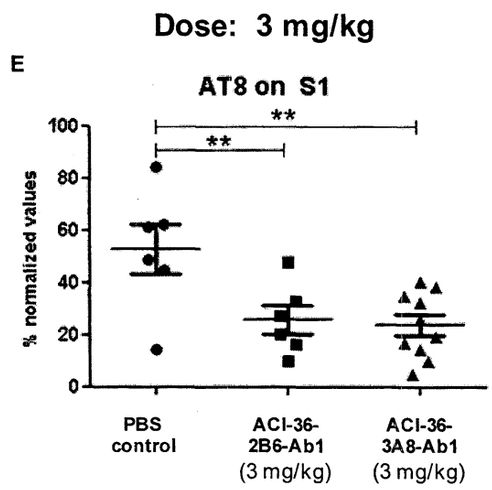


FIGURE 6-3

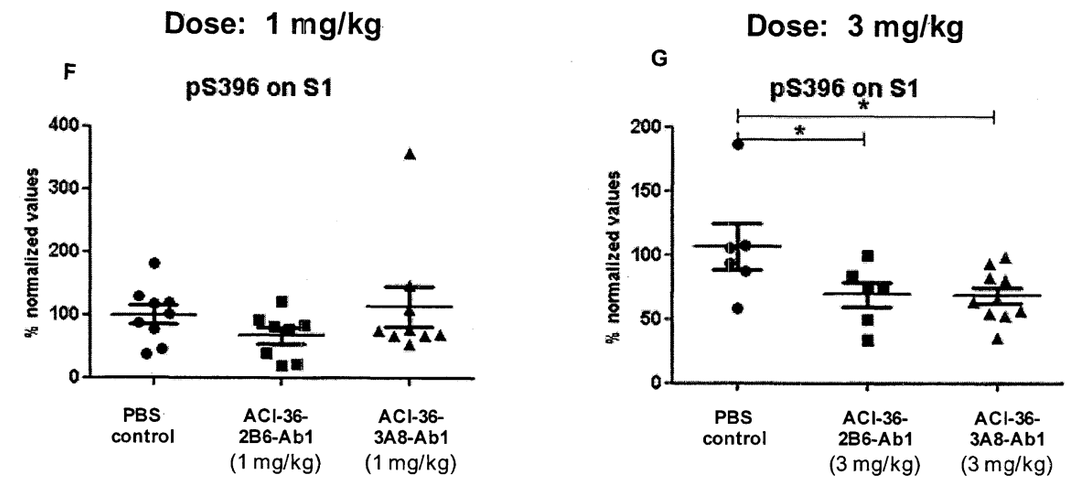

Cortex, soluble fraction (S1)
transgenic/human pTau (pS396)
Dose: 1 mg/kg
F
pS396 on S1
% normalized values
400
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
G
pS396 on S1
*
*
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-4

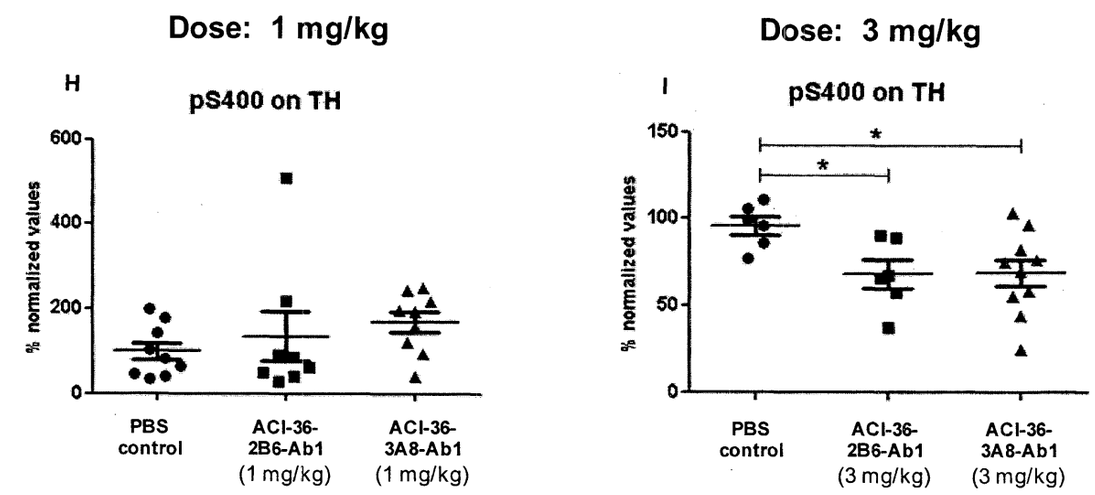

Cortex, total homogenate
transgenic/human pTau (pS400)
Dose: 1 mg/kg
H
pS400 on TH
% normalized values
600
400
200
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
I
pS400 on TH
% normalized values
150
100
50
0
*
*
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-5

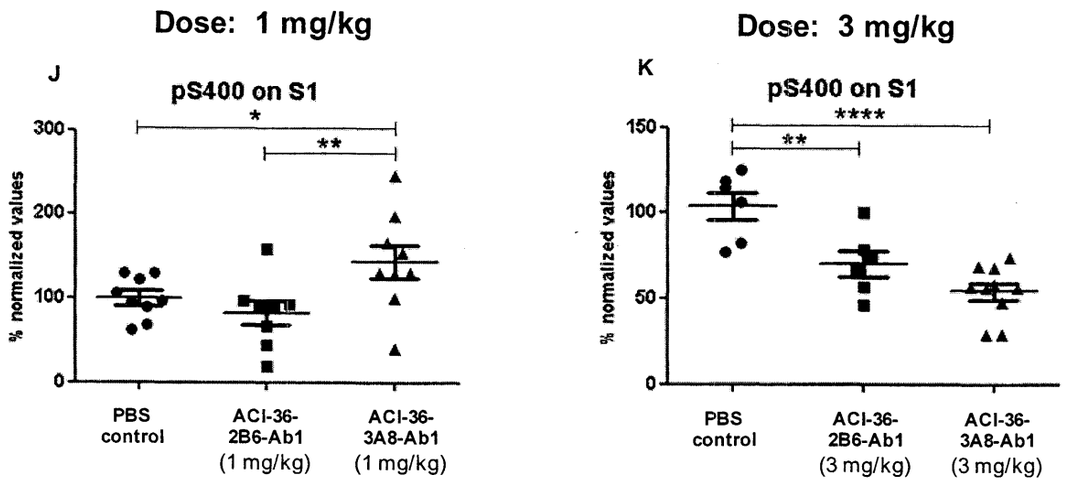

Cortex, soluble fraction (S1)
transgenic/human pTau (pS400)
Dose: 1 mg/kg
J
pS400 on S1
% normalized values
300
200
100
0
*
**
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
K
pS400 on S1
% normalized values
150
100
50
0
****
**
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-6

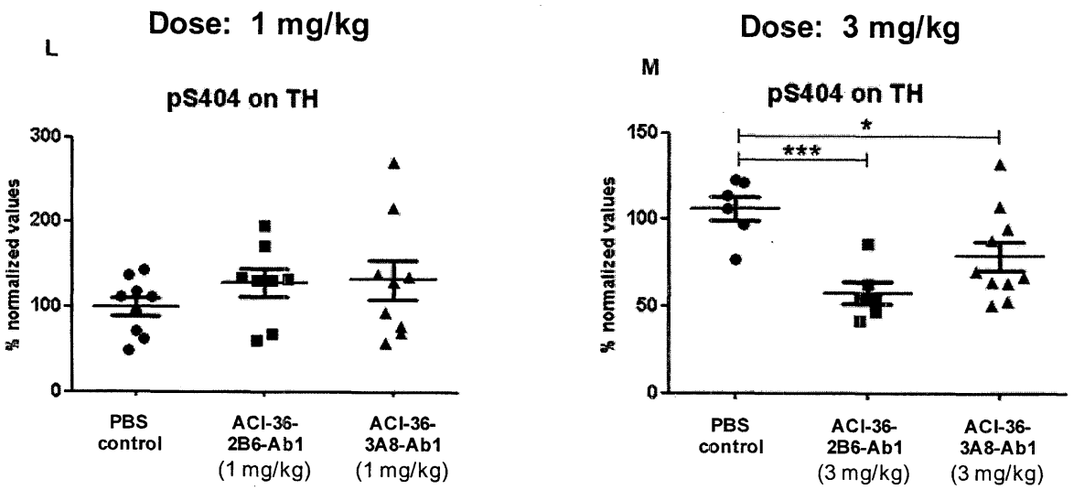

Cortex, total homogenate
transgenic/human pTau (pS404)
Dose: 1 mg/kg
L
pS404 on TH
% normalized values
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
M
pS404 on TH
% normalized values
150
100
50
0
*
***
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-7

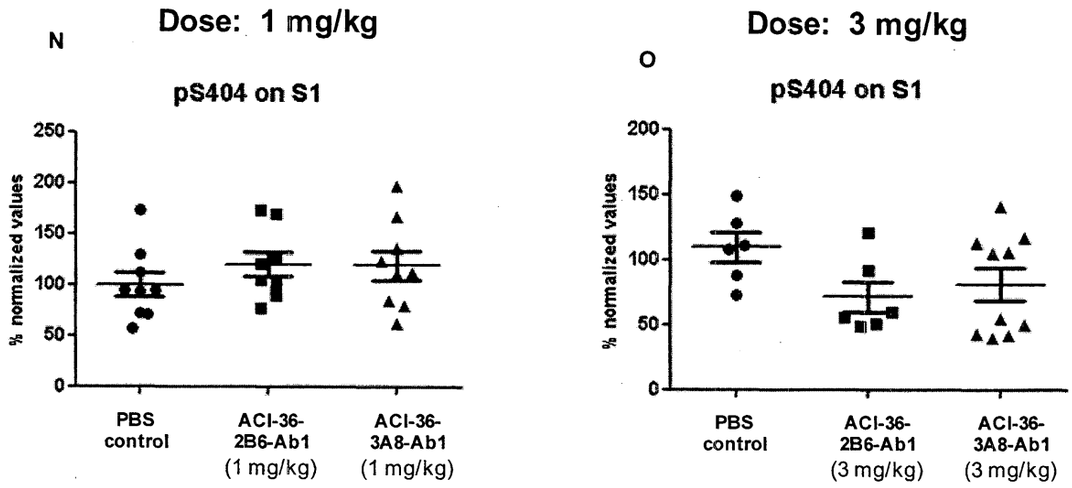

Cortex, soluble fraction (S1)
transgenic/human pTau (pS404)
N
Dose: 1 mg/kg
pS404 on S1
% normalized values
250
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
O
Dose: 3 mg/kg
pS404 on S1
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-8

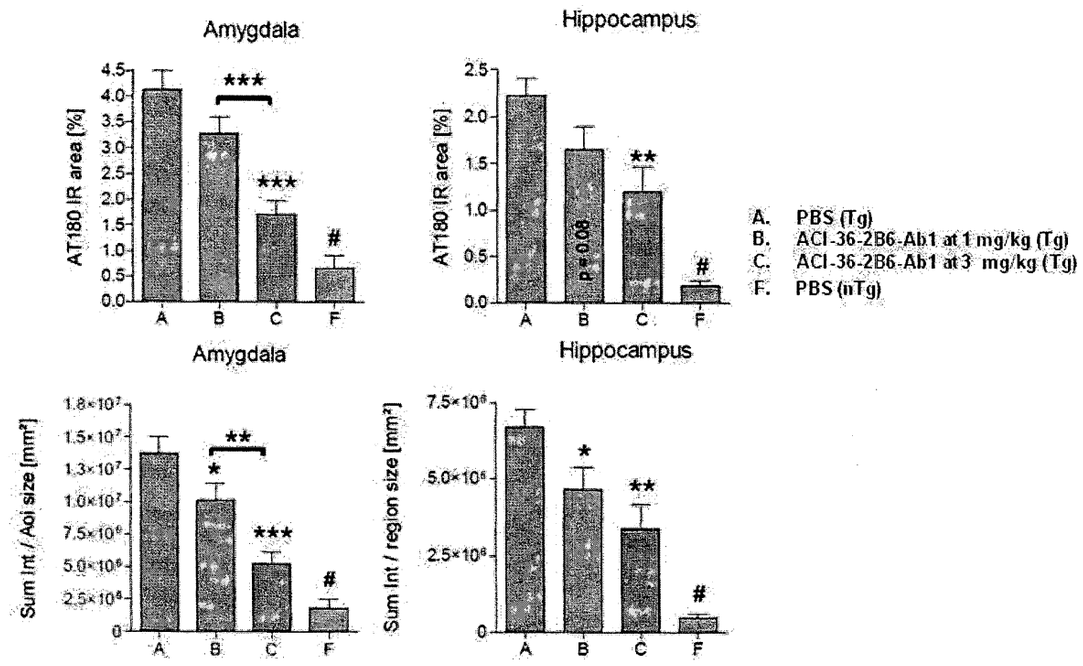

Amygdala
Hippocampus
AT180 IR area [%]
***
***
#
**
p = 0.08
#
A
B
C
F
A. PBS (Tg)
B. ACI-36-2B6-Ab1 at 1 mg/kg (Tg)
C. ACI-36-2B6-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)
Amygdala
Hippocampus
Sum Int / Aoi size [mm²]
Sum Int / region size [mm²]
**
*
***
#
*
**
#

FIGURE 7

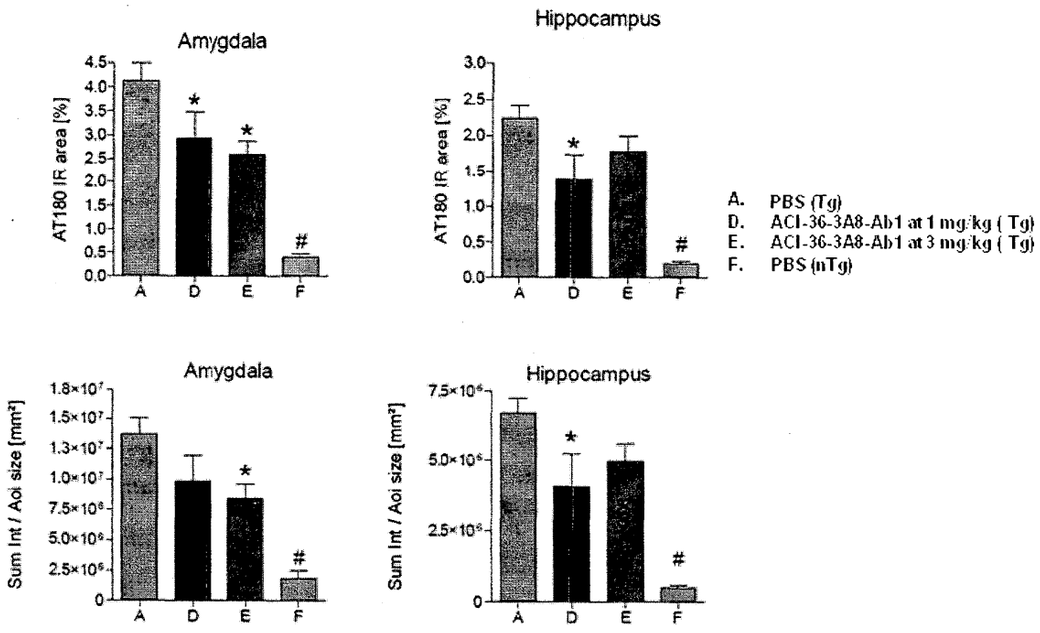
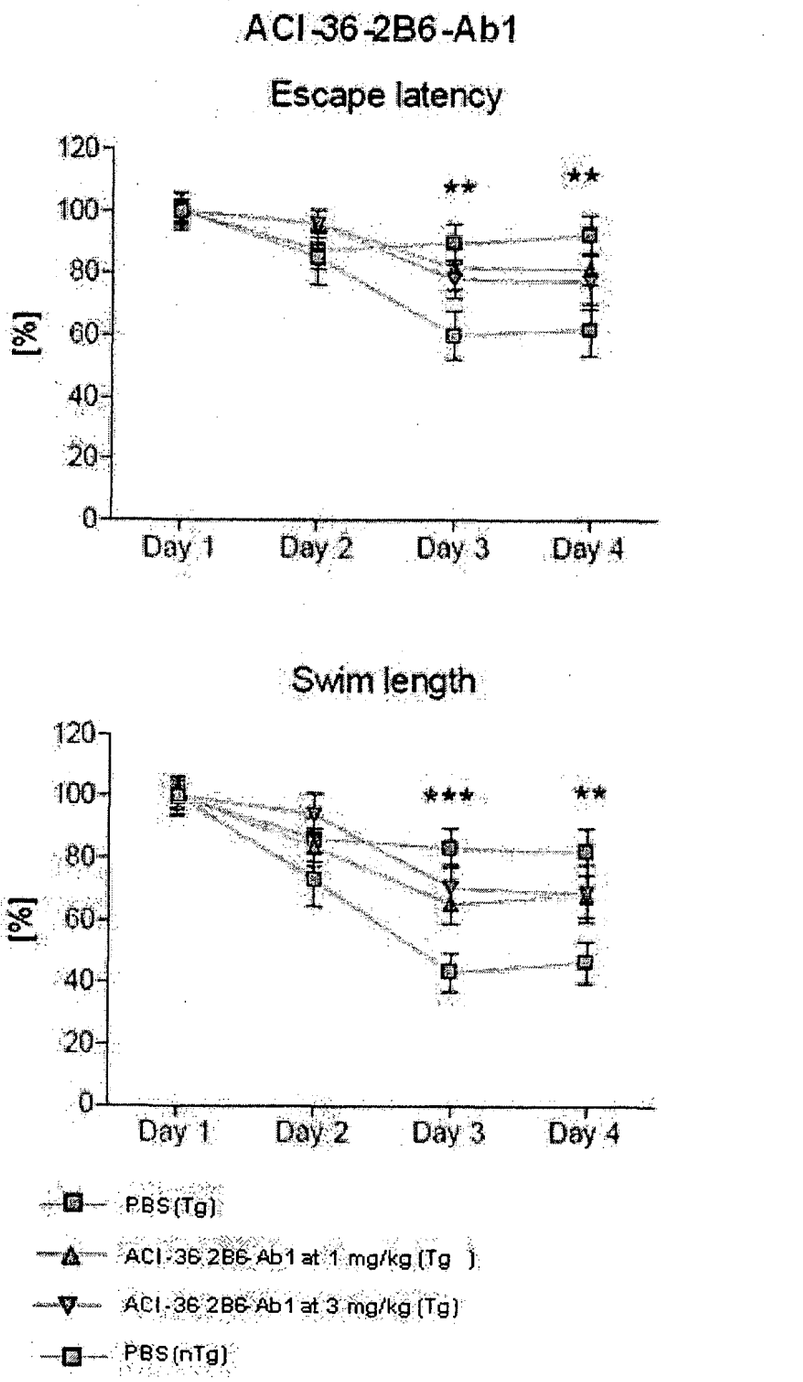
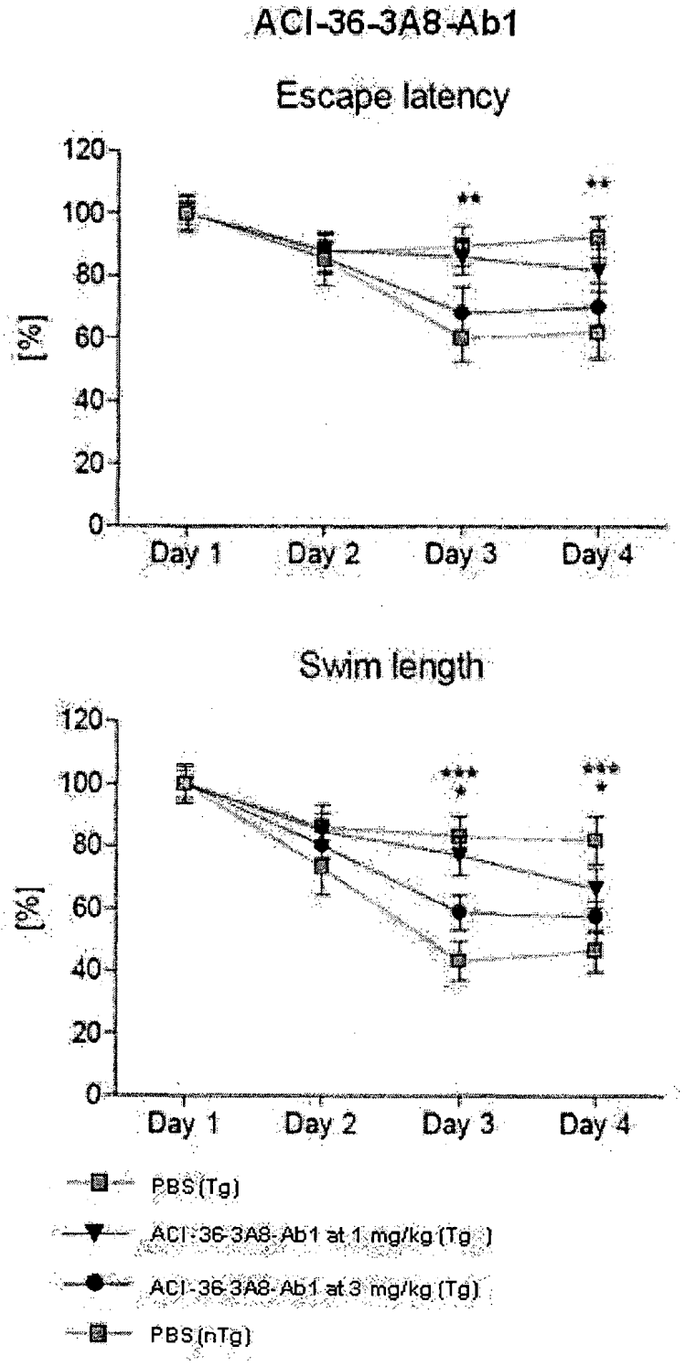

Amygdala
Hippocampus
AT180 IR area [%]
Sum Int / Aoi size [mm²]
A
D
E
F
*
#
A. PBS (Tg)
D. ACI-36-3A8-Ab1 at 1 mg/kg (Tg)
E. ACI-36-3A8-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)

FIGURE 8



	Escape latency			Swim length		
	Day 2	Day 3	Day 4	Day 2	Day 3	Day 4
A vs B	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs C	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.01

FIGURE 9



	Escape latency			Swim length		
	Day 2	Day 3	Day 4	Day 2	Day 3	Day 4
A vs D	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs E	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.001

FIGURE 10

COMPOSICIÓN FARMACÉUTICA

La presente invención se refiere a métodos y composiciones para el uso terapéutico y de diagnóstico en el tratamiento de enfermedades y trastornos que son causados por ovillos neurofibrilares o están relacionados con ellos. En particular, la invención se refiere a anticuerpos, que específicamente reconocen y se unen a conformadores de proteína tau patológicos fosforilados y con métodos y composiciones que incluyen dichos anticuerpos para el uso terapéutico y diagnóstico en el tratamiento de tauopatías entre las que encuentra la enfermedad de Alzheimer (EA).

Los ovillos neurofibrilares y los hilos de neurópilo (HN) son las características neuropatológicas distintivas principales de la enfermedad de Alzheimer (EA). Están compuestos por la proteína tau asociada a microtúbulos que ha sufrido modificaciones postraduccionales, que incluyen la fosforilación, la desamidación y la isomerización en residuos de asparaginilo o aspartilo. Se originan por el agregado de proteína tau hiperfosforilada y sus conformadores. La EA comparte esta patología con muchas tauopatías neurodegenerativas, en especial, con tipos específicos de demencia frontotemporal (DFT).

La proteína tau es una proteína altamente soluble, "naturalmente no plegada" que se une con avidez a los microtúbulos (MT) para promover su unión y estabilidad. Los MT son de suma importancia para la integridad citoesquelética de las neuronas, y por lo tanto, para la formación y funcionamiento adecuados de los circuitos neuronales, por ende, para el aprendizaje y la memoria. La unión de la proteína tau con el MT es controlada por fosforilación y desfosforilación dinámicas, según lo demuestran en especial las células *in vitro* y no neuronales. Debido a la gran cantidad de sitios de fosforilación posibles (>80), la contribución exacta de cada uno y la identidad de las quinasas responsables continúan en gran medida sin definirse *in vivo*.

En un cerebro con EA, la patología tau se desarrolla con posterioridad a una patología amiloide, y por lo tanto es probable que en respuesta a ella, lo que constituye la esencia de la hipótesis de la cascada amiloide. Esto se basa en los estudios en pacientes con EA y síndrome de Down y son indicados en ellos, y son corroborados por estudios en ratones transgénicos con patología tau y amiloide combinadas ((Lewis et al., 2001; Oddo et al., 2004; Ribe et al., 2005; Muyllaert et al, 2006; 2008; Terwel et al, 2008).

El preciso momento oportuno de ambas patologías en pacientes humanos con EA así como también los mecanismos que unen patología amiloide y tau continúan siendo desconocidos, pero se propone que involucran una activación de vías de señalización neuronal que actúan en GSK3 y cdk5 o a través de ellas como las "tau quinasas" principales (revisado por Muyllaert et al, 2006, 2008).

La hipótesis de que la tauopatía no es un efecto colateral inocente sino un ejecutor patológico principal en EA se basa en las observaciones genéticas, patológicas y experimentales sólidas que se corroboran entre sí por completo:

- en los casos de EA genética de aparición precoz que se deben a mutaciones en el precursor de la proteína amiloide (PPA) o presenilina, la causa patogénica obligada es la acumulación amiloide, pero invariablemente la patología comprende tauopatía colateral, idéntica a la de los casos de EA esporádica de aparición tardía;
- la gravedad en la disfunción cognitiva y en la demencia se corresponde con la tauopatía, no con la patología amiloide, ejemplificado últimamente a través de varios estudios clínicos de fase 1 y 2 que incluyen estudios por imágenes PIB–PET para amiloide e identifican muchos "falsos positivos"; individuos cognitivamente normales con una alta carga de amiloide cerebral;
- en DFT genético, la tauopatía es provocada por tau mutante y causa neurodegeneración en forma directa, sin patología amiloide;

REIVINDICACIONES

1. Un anticuerpo, o una de sus partes funcionales que reconoce y se une específicamente con un fosfo–epítope en la proteína tau de mamífero o en uno de sus fragmentos, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con la proteína tau soluble e insoluble y modula los niveles tau solubles e insolubles.

2. El anticuerpo de acuerdo con la reivindicación 1 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en el cerebro de un mamífero.

3. El anticuerpo de acuerdo con la reivindicación 2 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en la corteza cerebral y/o el hipocampo.

4. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total.

5. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau fosforilada soluble.

6. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de filamentos helicoidales en pares que contienen proteína tau hiperfosforilada.

7. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total, proteína tau fosforilada soluble y filamentos helicoidales pTau en pares.

8. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho mamífero es un humano.

9. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 10 nM.

10. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 5 nM, al menos 2 nM; al menos 1 nM, al menos 500 pM, al menos 300 pM, al menos 200 pM, al menos 100 pM.

11. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de tasa de asociación de 10^4 $M^{-1}s^{-1}$ o más; de 10^5 $M^{-1}s^{-1}$ o más, de 10^6 $M^{-1}s^{-1}$ o más.

12. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 4 nM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

13. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 3 nM y una constante de tasa de asociación de 10^6 $M^{-1}s^{-1}$ o más.

14. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de

disociación de al menos 2 nM y una constante de tasa de asociación de 10^4 $M^{-1}s^{-1}$ o más.

15. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 1 nM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

16. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 200 pM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

17. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 100 pM y una constante de tasa de asociación de 10^6 $M^{-1}s^{-1}$ o más.

18. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope seleccionado del grupo que consiste en Tau aa 15–20 que comprende una Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende una Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende una Ser fosforilada en la posición 409 (pS409); Tau aa 208–218 que comprende una Thr fosforilada en la posición 212 (pT212) y una Ser fosforilada en la posición (pS214); Tau aa 393–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 394–400 que comprende una Ser fosforilada en la posición 396 (pS396),

Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404), y Tau aa 393–400 que comprende una Ser fosforilada en la posición 396 (pS396).

19. El anticuerpo de acuerdo con la reivindicación 12 ó 13 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 15–20 con una Tyr fosforilada en la posición 18 (Y18).

20. El anticuerpo de acuerdo con la reivindicación 14 ó 15 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 405–412 con una Ser fosforilada en la posición 409 (pS409).

21. El anticuerpo de acuerdo con la reivindicación 16 ó 17 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 405–411 con una Ser fosforilada en la posición 409 (pS409).

22. El anticuerpo de acuerdo con la reivindicación 12 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396).

23. El anticuerpo de acuerdo con la reivindicación 12 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404).

24. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene, en particular en secuencia, un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, o una secuencia de aminoácido al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al

menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, o una secuencia de aminoácido al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, o una secuencia de aminoácido al menos 60%, en particular al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, 70, 78, 89, 98, o una secuencia de aminoácido al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, 115, o una secuencia de aminoácido al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, 72, 80, 91, 100, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

25. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 70% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 90% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 60% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 80% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 60% idéntica.

26. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 85% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 80% idéntica; y/o a dominio de anticuerpo que contiene un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 95% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 85% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 85% idéntica.

27. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que

contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 95% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica

28. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión (dominio de anticuerpo) que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

29. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 98% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y

un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

30. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 98% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 98% idéntica;. y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 98% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

31. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20.

32. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, o una secuencia de aminoácido al menos 76% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, o una secuencia de aminoácido al menos 66% idéntica, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, a DR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, o una secuencia de aminoácido al menos 88% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, o una secuencia de aminoácido al menos 66% idéntica.

33. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 24, o una secuencia de aminoácido al menos 88% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 25, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 26, o una secuencia de aminoácido al menos 66% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, o una secuencia de aminoácido al menos 88% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, o una secuencia de aminoácido al menos 66% idéntica

34. El anticuerpo de acuerdo con la reivindicación 33 o uno de sus fragmentos funcionales, que comprende un primer dominio de unión en donde el CDR1 tiene la secuencia de aminoácidos mostrada en SEQ ID NO: 27, o una secuencia de aminoácido al menos 88% idéntica.

35. El anticuerpo de acuerdo con la reivindicación 33 o uno de sus fragmentos funcionales, que comprende un primer dominio de unión en donde el

CDR1 tiene la secuencia de aminoácidos mostrada en SEQ ID NO: 28, o una secuencia de aminoácido al menos 88% idéntica.

36. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 29, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 30, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 31, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 15, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 16, o una secuencia de aminoácido al menos 94% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 17, o una secuencia de aminoácido al menos 36% idéntica.

37. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 32, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 34, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 19, o una secuencia de aminoácido al menos 95% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 20, o una secuencia de aminoácido al menos 63% idéntica.

38. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 73, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 74, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 75, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en

particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 70, o una secuencia de aminoácido al menos 95%, en particular 98%, en particular 99% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 71, o una secuencia de aminoácido al menos 94%, 95%, 96%, 97%, 98%, o 99% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 72, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

39. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 81, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 82, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 83, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 78, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 79, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 80, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en

particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

40. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 93, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 94, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 95, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 89, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 90, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 91, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

41. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 101, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 102, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 103, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos

93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 98, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 99, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 100, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

42. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 106, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 107, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 108, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 89, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 115, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 91, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en

particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

43. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 85% idéntica.

44. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 91% idéntica.

45. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión (dominio de anticuerpo) que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 91% idéntica.

46. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 69, 77, 116/92, 97, 105, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica, y/o un segundo dominio de unión que contiene la secuencia de

aminoácidos mostrada en SEQ ID NO: 68, 76, 88, 96, 104, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

47. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6 o SEQ ID NO: 7, o una secuencia de aminoácido al menos 90% y 94%, respectivamente, idénticas; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, o una secuencia de aminoácido al menos 91% idéntica.

48. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 8, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 2, o una secuencia de aminoácido al menos 90% idéntica.

49. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión, que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 9, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 3, o una secuencia de aminoácido al menos 90% idéntica.

50. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 10, o una secuencia de aminoácido al menos 99% idéntica; y/o un segundo dominio de unión que contiene

la secuencia de aminoácidos mostrada en SEQ ID NO: 4, o una secuencia de aminoácido al menos 89% idéntica.

51. El péptido de unión de cualquiera de las reivindicaciones precedentes que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 11, o una secuencia de aminoácido al menos 98% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 5, o una secuencia de aminoácido al menos 87% idéntica.

52. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende

a. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1; o

b. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 7 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1; o

c. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 8 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 2; o

d. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 9 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 3; o

e. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 10 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 4; o

f. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 11 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 5; o

g. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 69 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 68; o

h. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 77 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 76; o

i. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 116 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 88; o

j. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 92 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 88; o

k. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 97 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6; o

l. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 105 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 104.

53. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que es un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo completamente humano o uno de sus fragmentos funcionales.

54. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que es del isotipo IgG2b, IgG2a o IgG3.

55. Un péptido o proteína de unión del anticuerpo de acuerdo con cualquiera de las reivindicaciones 1–54.

56. El péptido o proteína de unión o el anticuerpo o uno de sus fragmentos funcionales de acuerdo con cualquiera de las reivindicaciones precedentes, en donde

dicho péptido de union, anticuerpo de proteína o fragmento de anticuerpo reconoce y específicamente se une con un fosfo–epítope en el mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular a un fosfo–epítope en la proteína Tau agregada, pero no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados.

57. Un polinucleótido que codifica el anticuerpo cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales.

58. El polinucleótido de acuerdo con la reivindicación 57 que comprende una molécula de ácido nucleico seleccionada del grupo que consiste en

a. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que codifica un polipéptido que comprende la secuencia de aminoácidos tal como se indica en SEQ ID NOs: 35–45;

b. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 85% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45, SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

c. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 90% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117;

d. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 95% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

e. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 98% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

f. una molécula de ácido nucleico que comprende una secuencia de nucleótidos cuya cadena complementaria hibrida en la molécula de ácidos nucleicos de cualquiera de a) – f);

g. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que se desvía de la secuencia de nucleótidos definida en cualquiera de a) – g) por degeneración del código genético:

en donde dicha molécula de ácido nucleico tal como se define en cualquiera de a) – g) reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular a un confórmero de proteína tau patológica, pero no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho anticuerpo tiene una alta afinidad de unión con la proteína tau agregada, y modula los niveles de tau soluble e insoluble.

59. Una composición farmacéutica que comprende un péptido o una proteína de unión, un anticuerpo o uno de sus fragmentos funcionales o un polinucleótido de acuerdo con cualquiera de las reivindicaciones anteriores, o una combinación de ellos, en una cantidad terapéuticamente efectiva junto con un portador farmacéuticamente aceptable.

60. La composición farmacéutica de acuerdo con la reivindicación 59, que comprende al menos dos péptidos o proteínas de union, anticuerpos o sus fragmentos funcionales o polinucleótidos de acuerdo con cualquiera de las reivindicaciones precedentes, que reconoce y se une con dos diferentes fosfo–epítopes en la proteína tau de mamífero.

61. La composición farmacéutica de acuerdo con la reivindicación 59 ó 60, que comprende un anticuerpo adicional o uno de sus fragmentos funcionales que reconoce y se une con una proteína o péptido amiloidogénico diferente.

62. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, un polinucleótido o una composición farmacéutica de acuerdo con

cualquiera de las reivindicaciones anteriores, o una combinación de ellos, para su uso en terapia, en particular, para su uso en el tratamiento de trastornos neurodegenerativos como ser tauopatías en un mamífero, en particular en un ser humano que necesita de dicho tratamiento.

63. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, o la composición farmacéutica de acuerdo con la reivindicación anterior, pasa su uso en el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano que sufre de una deficiencia como esta.

64. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior, donde el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano, lleva al detenimiento en el avance de las deficiencias cognitivas.

65. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior, donde el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano, lleva al aumento de la retención, en particular a una restauración completa de la capacidad de memoria cognitiva en el sujeto tratado.

66. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior para su uso en el tratamiento de enfermedades y trastornos que son causados por la formación de lesiones neurofibrilares o están asociados con ella, patología cerebral predominante en tauopatía que comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de

Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica.

67. El anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior para su uso en el tratamiento de enfermedad de Alzheimer.

68. Un método para el tratamiento de una enfermedad o trastorno neurodegenerativo como ser una tauopatía que comprende la administración a un animal, en particular a un mamífero, pero en especial a un ser humano, que sufre de una enfermedad o trastorno neurodegenerativo como este, de un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, un polinucleótido o una composición farmacéutica de acuerdo una cualquiera de las reivindicaciones anteriores, o una combinación de ellas.

69. El método de acuerdo con la reivindicación anterior para el tratamiento de enfermedades y trastornos que son causados por la formación de lesiones neurofibrilares o están asociados con ella, patología cerebral predominante en tauopatía que comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea

taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica.

70. Un método para inducir una respuesta inmune pasiva en un animal, en particular en un mamífero o ser humano, que sufre de una enfermedad o trastorno neurodegenerativo como ser una tauopatía mediante la administración a dicho animal o ser humano de un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones anteriores, o una combinación de ellas.

71. Un método de diagnosis de una enfermedad, trastorno o condición asociados con la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un anticuerpo o uno de sus fragmentos activos a un epítope de la proteína tau en una muestra o in situ que incluye las etapas de

a. poner en contacto la muestra o una parte del cuerpo específica o área corporal sospechada de contener la proteína tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con la proteína tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área específica del cuerpo.

72. Un método de diagnosis de una predisposición a una enfermedad, trastorno o condición asociado a la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un anticuerpo o uno de sus fragmentos activos a un epítope de la proteína tau en una muestra o in situ que incluye las etapas de

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico a un valor de control normal,

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente padece o está en riesgo de desarrollar una enfermedad o condición asociados con la proteína Tau.

73. Un método de control de una enfermedad residual mínima en un paciente después del tratamiento con un anticuerpo o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones precedentes, que comprende:

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico a un valor de control normal,

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente todavía padece de una enfermedad residual mínima.

74. Un método para predecir la respuesta de un paciente en tratamiento con un anticuerpo o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones precedentes que comprende

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico antes y después del inicio del tratamiento,

en donde una disminución de la cantidad de dicho agregado indica que dicho paciente tiene un alto potencial de responder al tratamiento.

75. Kits de ensayo para la detección y diagnosis de enfermedades, trastornos o condiciones asociadas con la proteína Tau que comprenden un anticuerpo de acuerdo con cualquier reivindicación precedente.

76. Kit de ensayo de acuerdo con la reivindicación precedente que comprende un recipiente que porta uno o varios anticuerpos o uno de sus fragmentos funcionales de acuerdo con la presente invención e instrucciones de uso de los anticuerpos a los fines de unirse con el antígeno tau para formar un complejo inmunológico y detectar la formación del complejo inmunológico de modo que la presencia o la ausencia del complejo inmunológico se correlacione con la presencia o ausencia del antígeno tau.

77. Un epítope seleccionado del grupo que consiste en Tau aa 15–20 que comprende Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende Ser fosforilada en la posición 409 (pS409); y Tau aa 208–218 que comprende Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición (pS214), Tau aa 393–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 394–400 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404), y Tau aa 393–400 que comprende una Ser fosforilada en la posición 396 (pS396).

78. Una línea celular que produce un anticuerpo de acuerdo con cualquier reivindicación precedente.

79. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079.

80. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081.

81. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080.

82. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088.

83. Una línea celular de acuerdo con la reivindicación 77, que es la línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084.

84. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 2B6G7A12 depositada el 10 de marzo de 2010 como DSM ACC3087.

85. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086.

86. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085.

87. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082.

88. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083.

89. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

90. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081 usando

a. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

91. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de

hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

92. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

93. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y SEQ ID NO: 52 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

94. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 2B6G7A12 depositada el 25 de agosto de 2010 como DSM ACC3087 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y SEQ ID NO: 52 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

95. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086 usando

a_1. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

96. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085 usando

a_1. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

97. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082 usando

a. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 49; SEQ ID NO: 56 y SEQ ID NO: 57 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión;

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 55 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

98. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de

hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 57 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión;

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 55 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

99. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 149 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, y 140 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 131, 134 y 141–148, para la amplificación de un segundo dominio de unión.

100. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 51 y 169–174 y un cebador 3'

de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 124, 127 y 150–158 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 130 y 159–168, para la amplificación de un segundo dominio de unión.

101. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 178, 179 y 180 y un cebador 3' de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 121, 127, 139, 154, 155 y 175 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 128, 129, 147, 176 y 177, para la amplificación de un segundo dominio de unión.

102. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 51 y 188–192 y un cebador 3'

de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 120, 124, 126, 181, 182 y 183 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 144, 145 y 184–187, para la amplificación de un segundo dominio de unión.

103. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 50 y 201–204 y un cebador 3' de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 121, 137, 151 y 193–197 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 131, 141, 144, 166, 198, 199 y 200, para la amplificación de un segundo dominio de unión.

104. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 209–214, y 219–221 un

cebador 3' de SEQ ID NO: 215, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 216, 217 y 218 y un cebador 3' de SEQ ID NOs: 208, para la amplificación de un segundo dominio de unión.

105. El anticuerpo o uno de sus fragmentos funcionales de acuerdo con cualquiera de las reivindicaciones precedentes, en donde dicho anticuerpo es un anticuerpo del isotipo IgG2a, IgG2b o IgG3, un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo completamente humano.

106. Un fragmento de union o proteína o un anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes.

107. Una línea celular que produce el anticuerpo de acuerdo con cualquiera de las reivindicaciones 1–54, 56 o cualquiera de las reivindicaciones 89 – 105.

108. Una línea celular que comprende el polinucleótido de acuerdo con la reivindicación 57 ó 58.

109. Un método para producir el anticuerpo de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o con una cualquiera de las reivindicaciones 89 a 105, que comprende el paso de cultivo de la línea celular de acuerdo con la reivindicación 106 en un medio de cultivo adecuado.

110. Un método para producir un anticuerpo de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, que comprende el paso de cultivo de la línea celular de acuerdo con cualquiera de las reivindicaciones 78–88 en un medio de cultivo adecuado.

111. El método de acuerdo con la reivindicación 100 ó 110, que comprende, además, el paso de purificación del anticuerpo a partir de la línea celular o medio de cultivo.

112. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, con una cualquiera de las reivindicaciones 88 a 99, en la preparación de un medicamento para su uso en el tratamiento de tauopatías, un grupo de enfermedades y trastornos asociados con la proteína tau.

113. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, en la preparación de un medicamento para su uso en el tratamiento o mitigación de los síntomas asociados con tauopatías, un grupo de enfermedades y trastornos asociados con la proteína tau.

114. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, en la preparación de un medicamento para su uso en la retención o aumento de la capacidad de memoria cognitiva en un mamífero que sufre de una tauopatía.

115. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136.

116. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137.

117. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138.

118. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139.

119. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140.

120. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141.

p.p.de. AC IMMUNE S.A. * K.U. LEUVEN RESEARCH & DEVELOPMENT

RESUMEN

La presente invención se refiere a métodos y composiciones para el uso terapéutico y de diagnóstico en el tratamiento de enfermedades y trastornos que son causados por ovillos neurofibrilares o están asociados con ellos. En particular, la invención se refiere a anticuerpos, que específicamente reconocen y se unen a conformadores de proteína tau patológicos fosforilados y con métodos y composiciones que incluyen dichos anticuerpos para el uso terapéutico y diagnóstico en el tratamiento de tauopatías entre las que encuentra la enfermedad de Alzheimer (EA).

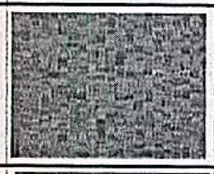
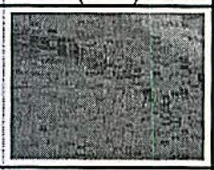
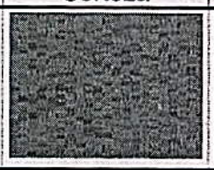
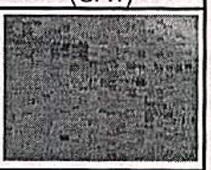
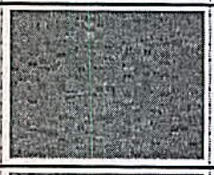
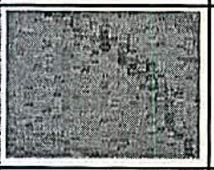
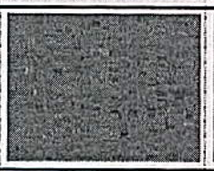
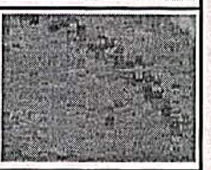
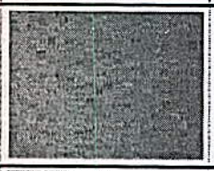
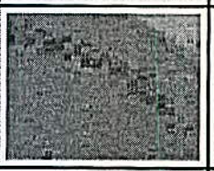
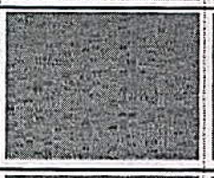
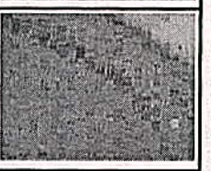
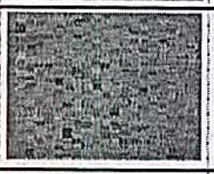
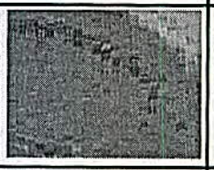
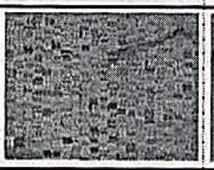
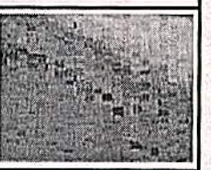
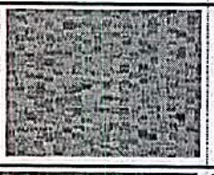
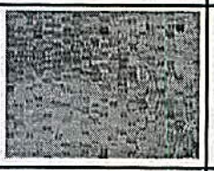
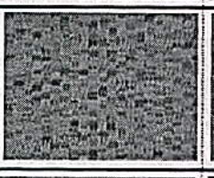
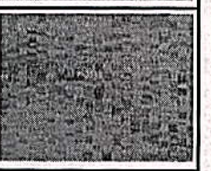
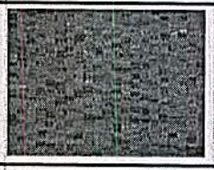
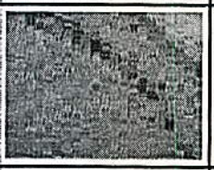
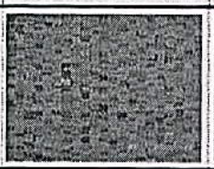
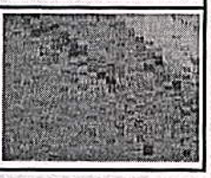
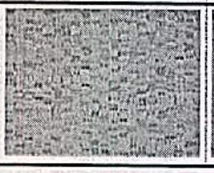
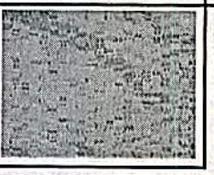
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6H1A11 C11			6H1G6E 6		
2B6A10 C11			2B6G7A 12		
3A8A12 G7			3A8E12 H8		
7C2(1) F10C10 D3			7C2(2) B9F11D 5		
Control AT100 anticuerpo			***Control*** PG5 anticuerpo		
Control Ningún anticuerpo primario					

FIGURA 1-1

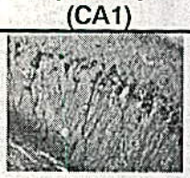
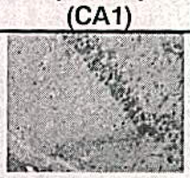
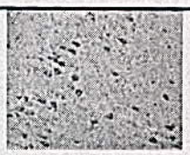
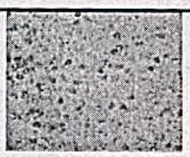
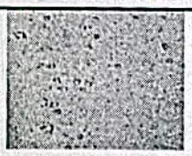
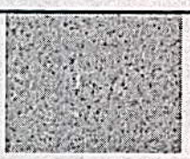
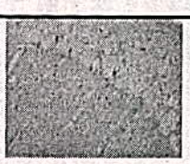
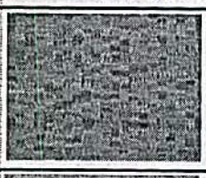
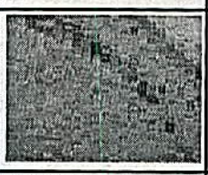
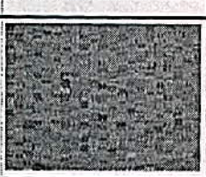
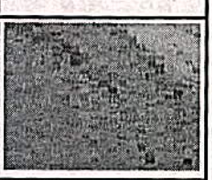
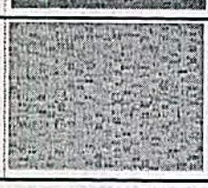
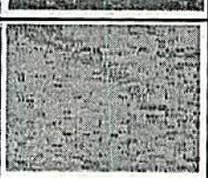
Clon	Corteza	Hipocampo (CA1)	Clon	Corteza	Hipocampo (CA1)
A4-2A1-18			A6-2G5-08		
A4-4A6-48			A6-2G5-30		
A4-2A1-40			A6-2G5-41		
A6-1D2-12			A4-4A6-18		
Control AT100 anticuerpo			***Control*** PG5 anticuerpo		
Control Sin anticuerpo primario					

FIGURE 1-2

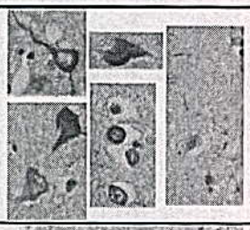
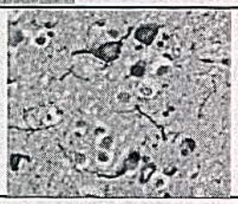
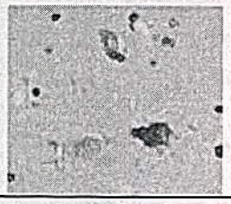
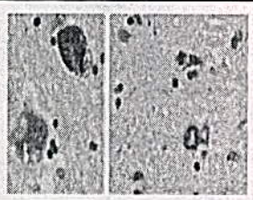
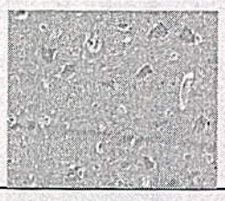
Diagnosis	Región	Tinción TAUPIR de ACI-3A8-Ab1
AD	Hipocampo	
FAD	Hipocampo anterior	
AGD	Hipocampo anterior	
PSP	Pallidum	
FTDP-17	Corteza frontal	
CBD	Corteza frontal	
Control Sano	Hipocampo	

FIGURA 2

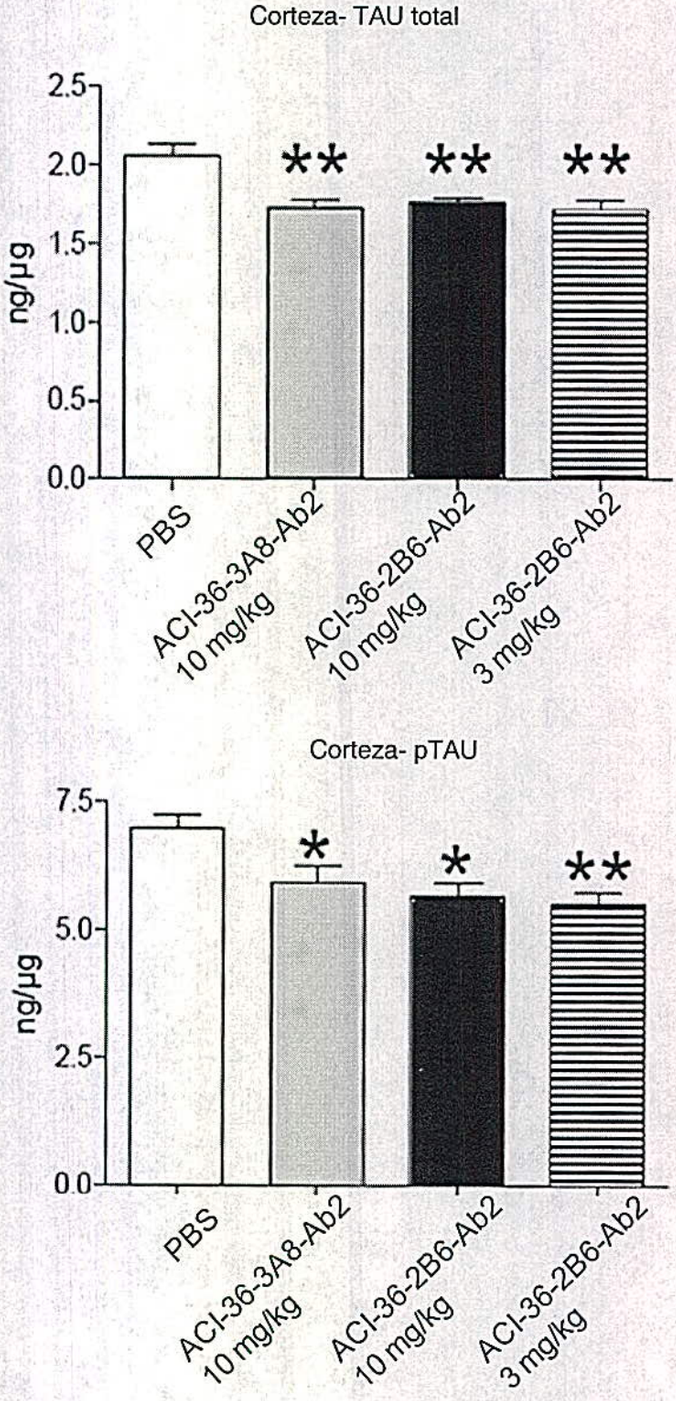

Corteza- TAU total
ng/µg
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg
Corteza- pTAU
ng/µg
7.5
5.0
2.5
0.0
*
*
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg

FIGURA 3

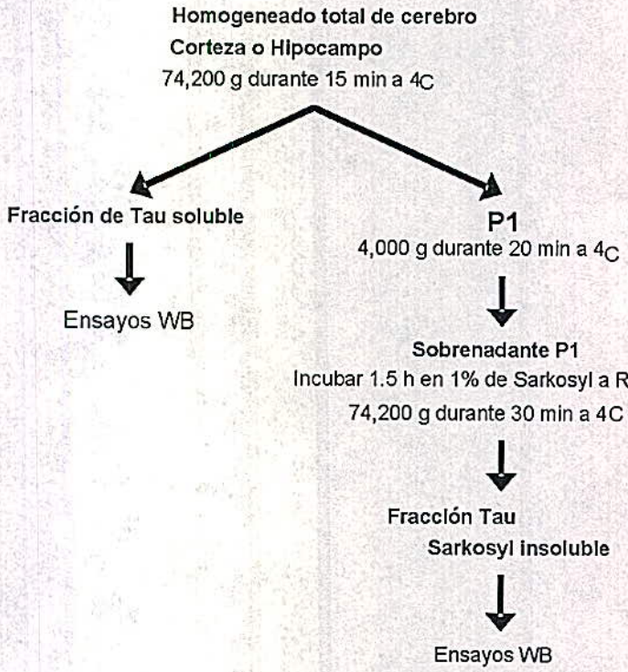


FIGURA 4.1

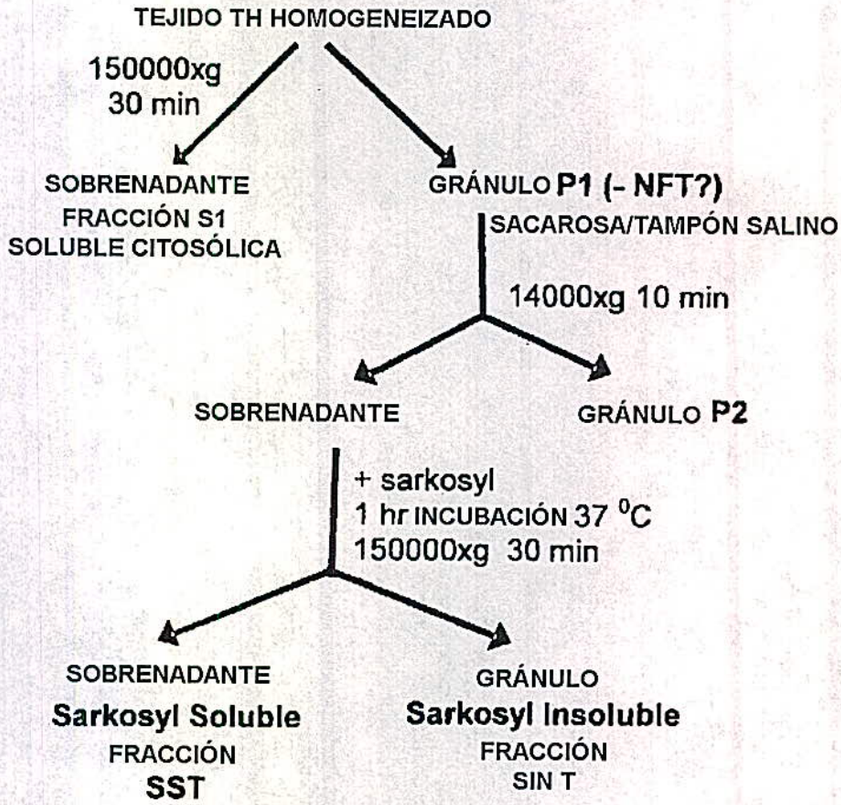


FIGURA 4.2

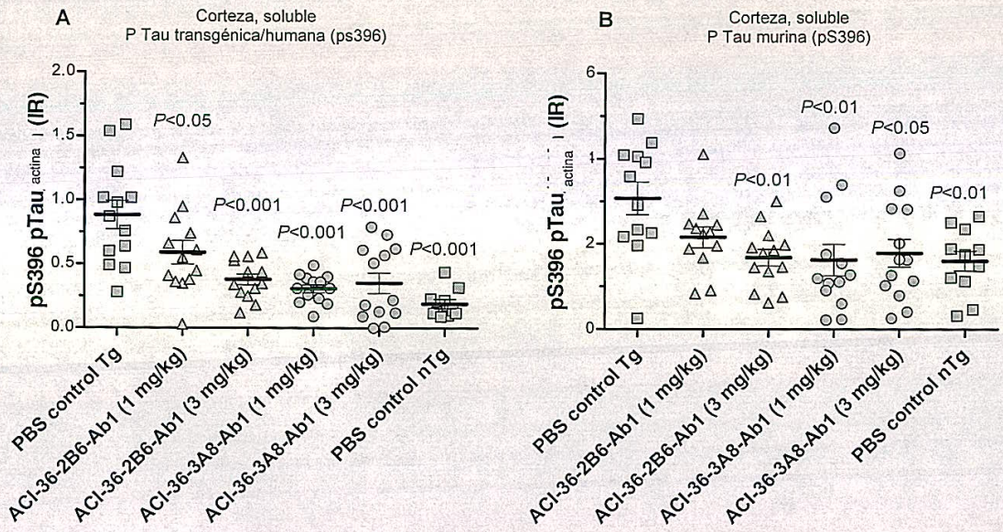

A
Corteza, soluble
P Tau transgénica/humana (ps396)
pS396 pTau actina (IR)
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
B
Corteza, soluble
P Tau murina (pS396)
pS396 pTau actina (IR)
6
4
2
0
P<0.01
P<0.01
P<0.05
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-1

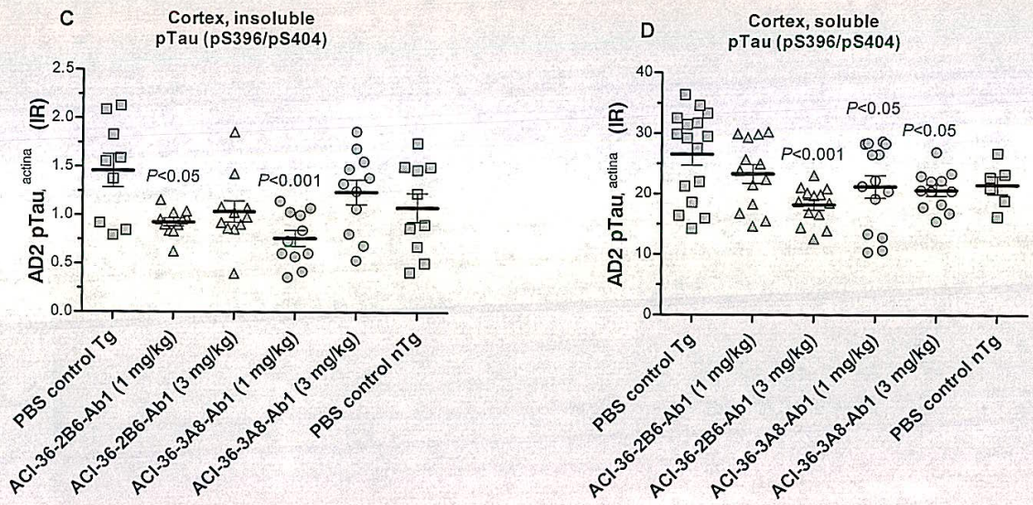


FIGURA 5-2

137

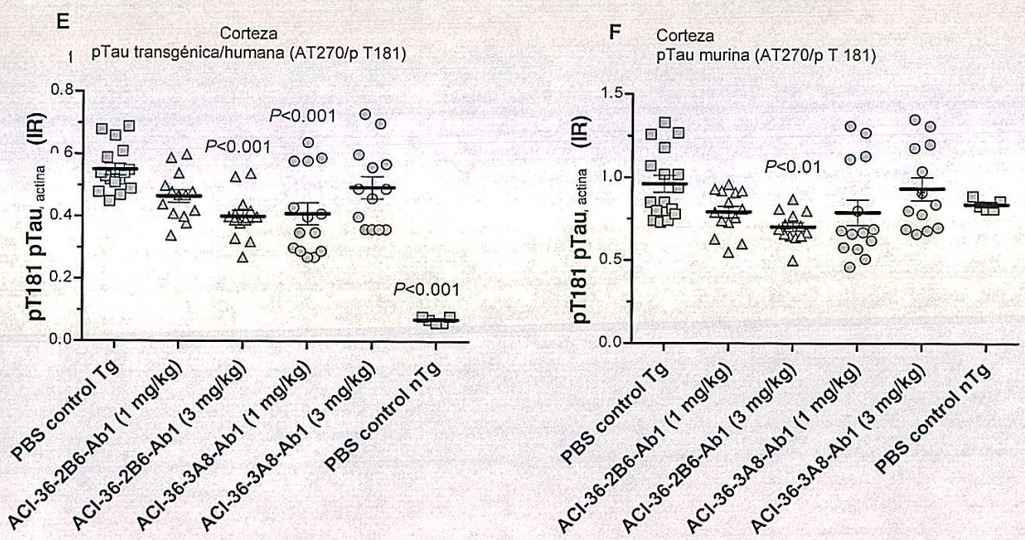

E
Corteza
pTau transgénica/humana (AT270/p T181)
pT181 pTau, actina (IR)
0.8
0.6
0.4
0.2
0.0
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
F
Corteza
pTau murina (AT270/p T 181)
pT181 pTau, actina (IR)
1.5
1.0
0.5
0.0
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-3

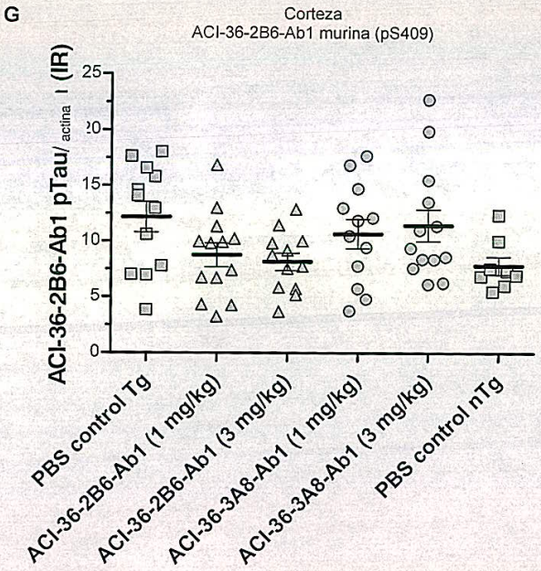

G
Corteza
ACI-36-2B6-Ab1 murina (pS409)
ACI-36-2B6-Ab1 pTau/actina I (IR)
25
20
15
10
5
0
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-4

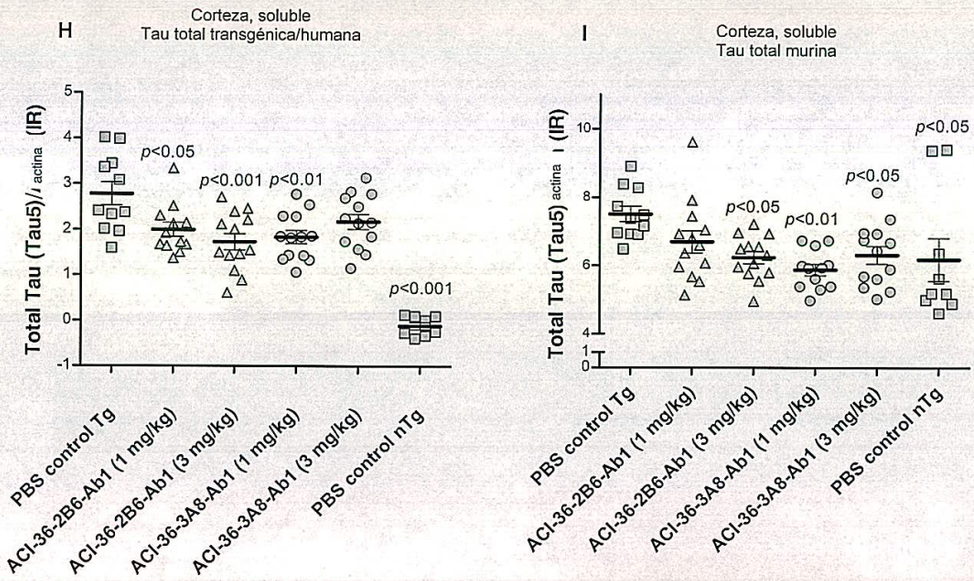

H
Corteza, soluble
Tau total transgénica/humana
Total Tau (Tau5)/ actina (IR)
p<0.05
p<0.001
p<0.01
p<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
I
Corteza, soluble
Tau total murina
Total Tau (Tau5) actina I (IR)
p<0.05
p<0.05
p<0.01
p<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-5

Corteza, fracción soluble (S1)
Tau total transgénica/humana (HT7)

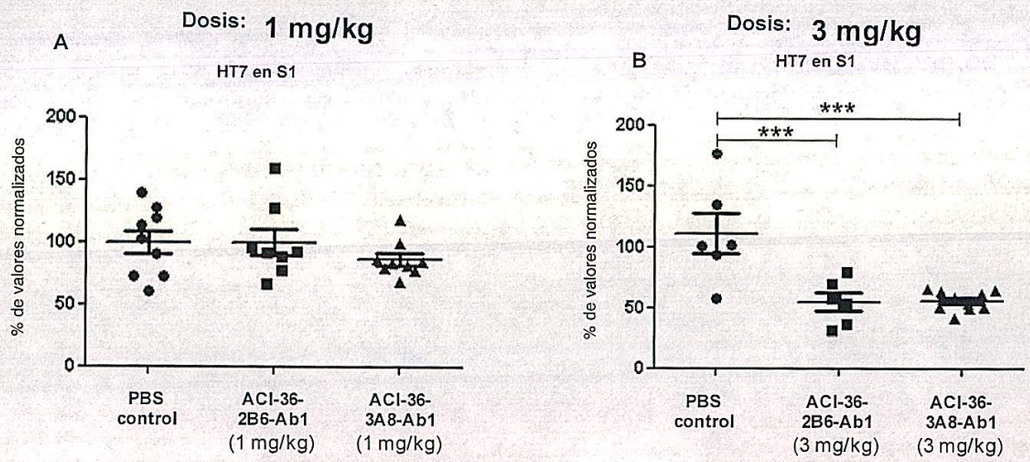
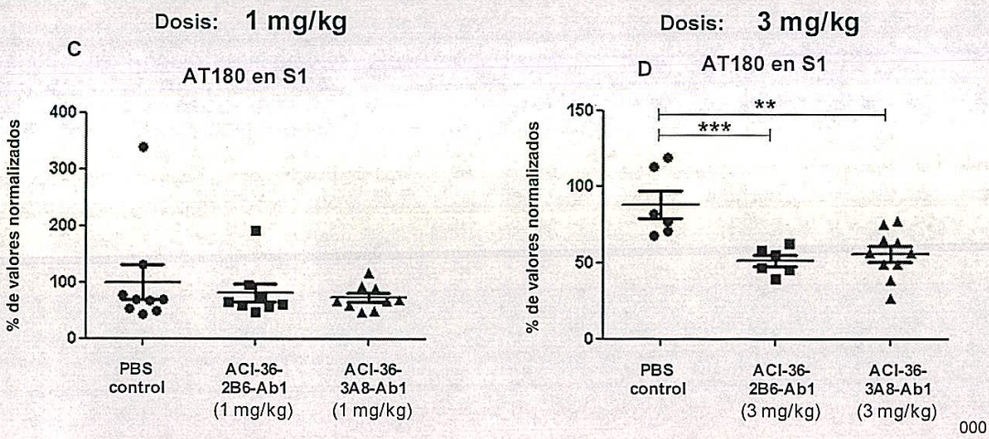


FIGURA 6-1

141

Corteza, fracción soluble (S1)
pTau transgénica/humana (pT231/AT180)



000

FIGURA 6-2

142

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS202/AT8)

Dosis: 3 mg/kg

E

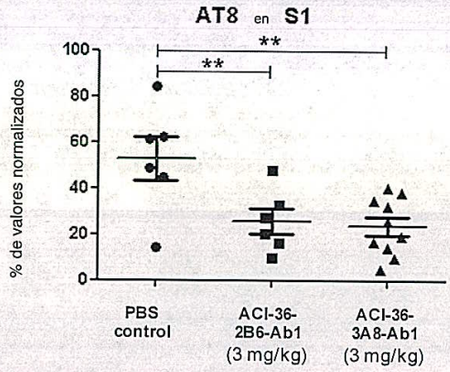


FIGURA 6-3

143

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS396)

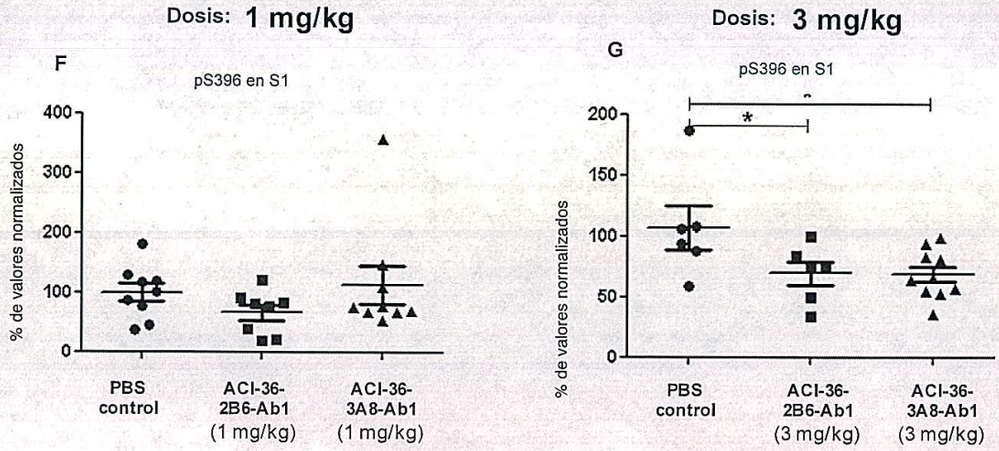


FIGURA 6-4

144

Corteza, homogenado total
pTau transgénica/humana (pS400)

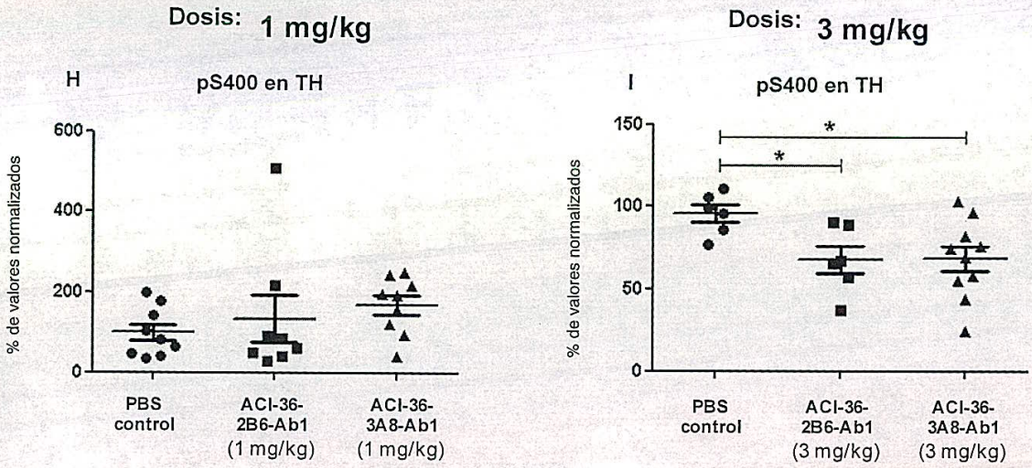


FIGURA 6-5

145

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS400)

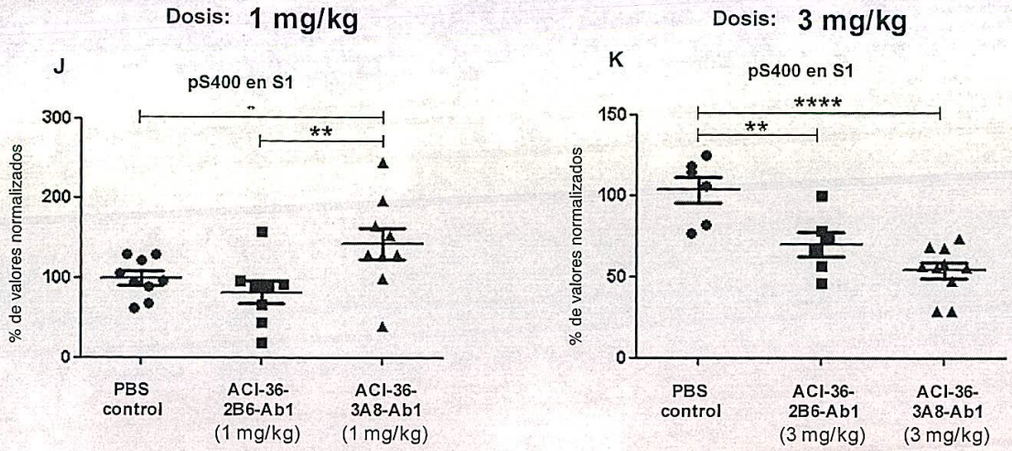


FIGURA 6-6

946

Corteza, homogenado total
pTau transgénica/humana (pS404)

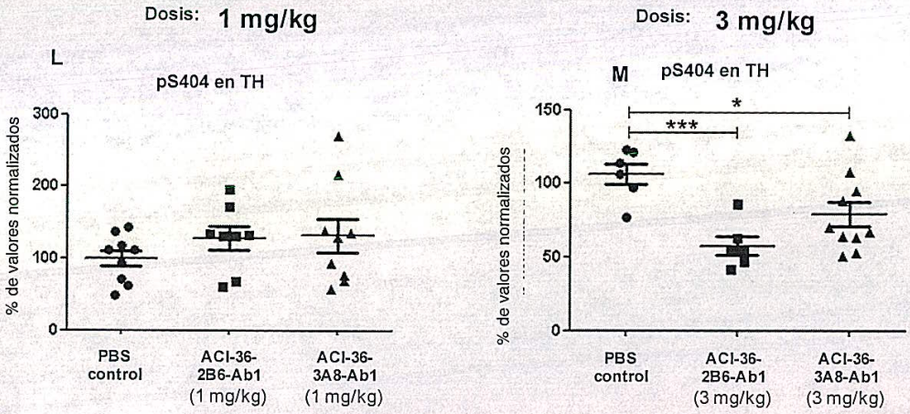


FIGURA 6-7

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS404)

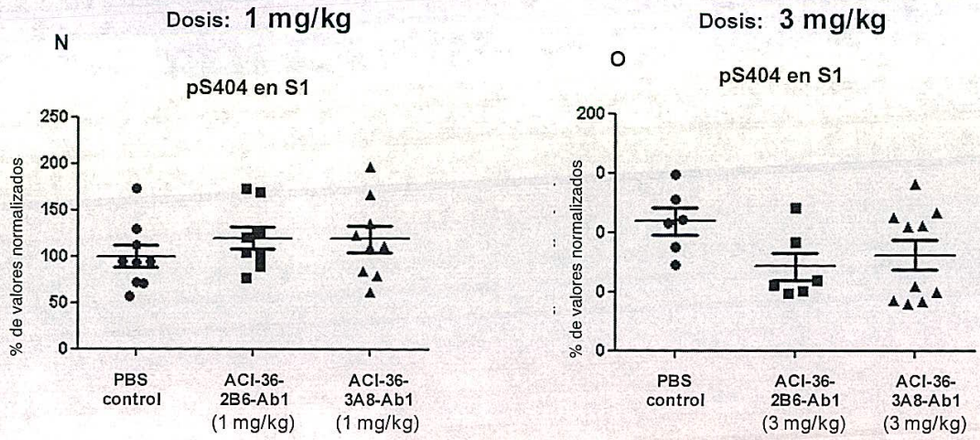


FIGURA 6-8

148

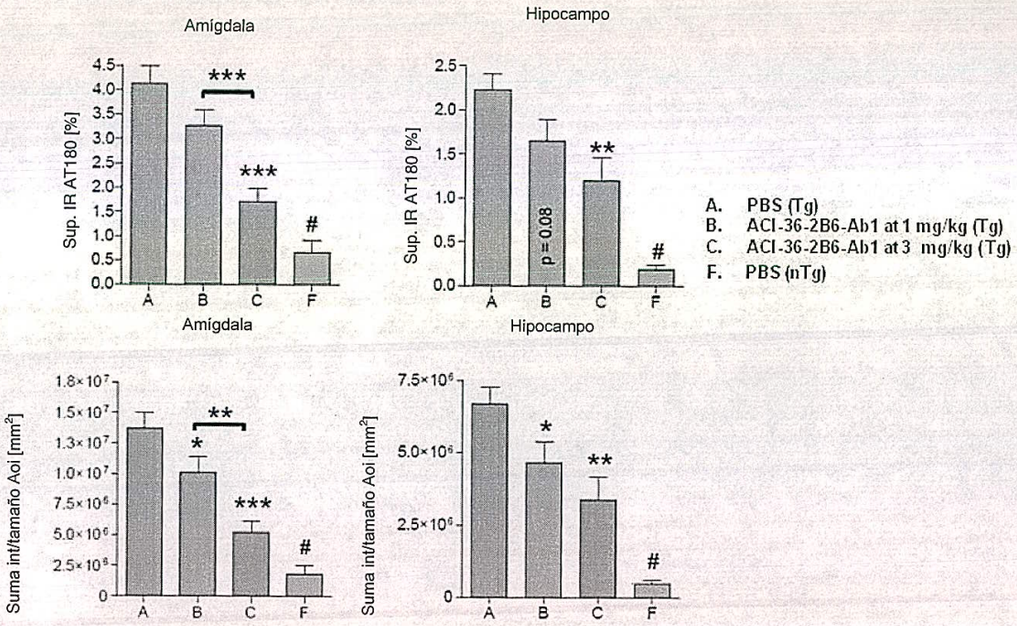
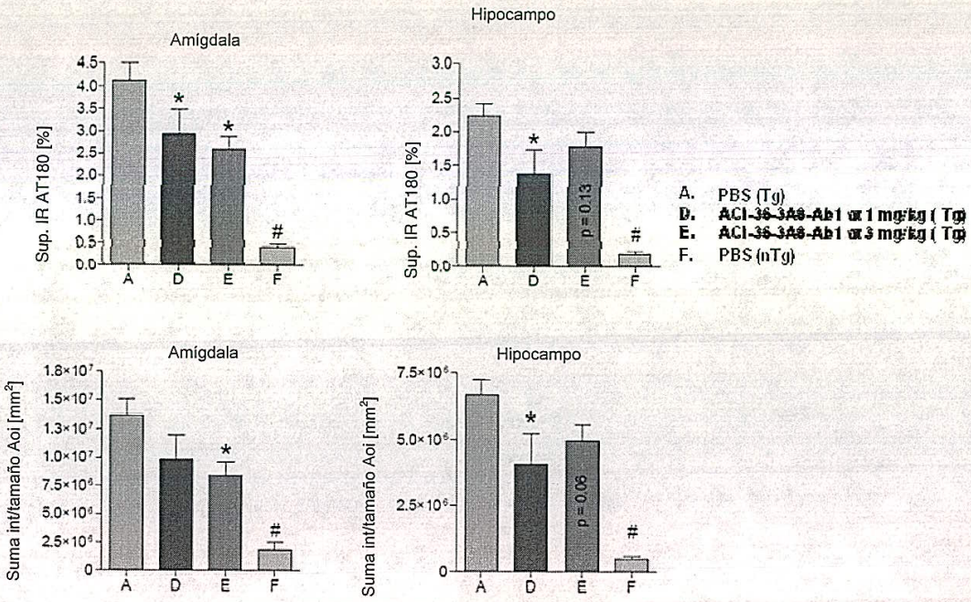

Amígdala
Hipocampo
Sup. IR AT180 [%]
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
***
#
**
p = 0.08
#
A
B
C
F
Amígdala
Hipocampo
A. PBS (Tg)
B. ACI-36-2B6-Ab1 at 1 mg/kg (Tg)
C. ACI-36-2B6-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)
Suma int/tamaño Aoi [mm²]
1.8×10⁷
1.5×10⁷
1.3×10⁷
1.0×10⁷
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
**
*
***
#
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
*
**
#

FIGURA 7


Amígdala
Hipocampo
Sup. IR AT180 [%]
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
#
*
p = 0.13
#
A
D
E
F
A. PBS (Tg)
D. ACI-35-3A8-Ab1 ox 1 mg/kg (Tg)
E. ACI-35-3A8-Ab1 ox 3 mg/kg (Tg)
F. PBS (nTg)

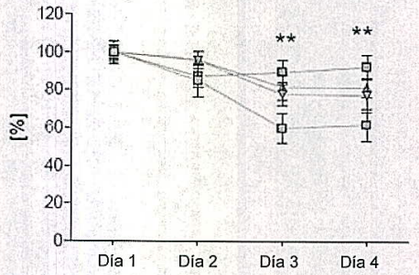
Amígdala
Hipocampo
Suma int/tamaño Aoi [mm²]
1.8×10⁷
1.5×10⁷
1.3×10⁷
1.0×10⁷
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
7.5×10⁶
5.0×10⁶
2.5×10⁶
0
*
*
#
p = 0.06
#
A
D
E
F

FIGURA 8

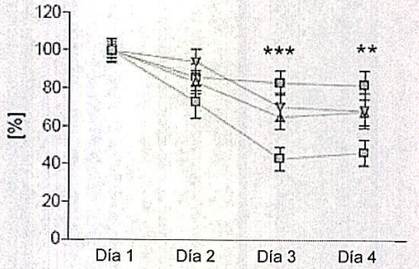
281

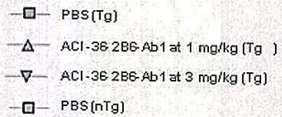
ACI-36-2B6-Ab1

Latencia de escape



Long. Flot.

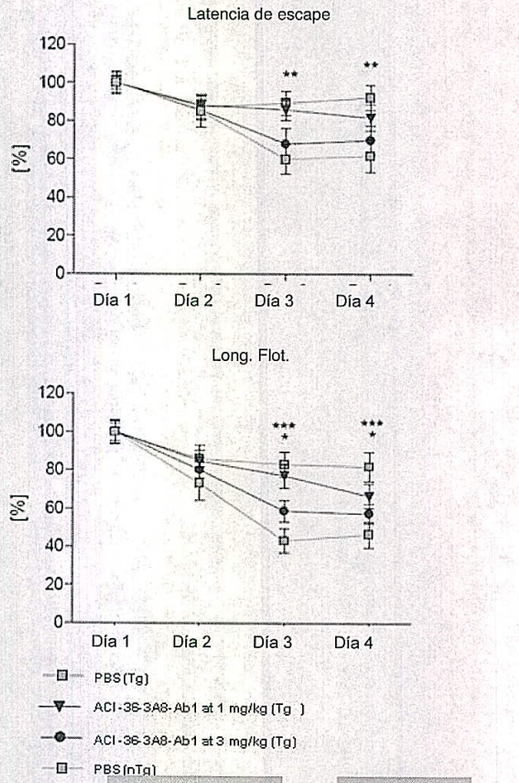




	Latencia de escape			Long. Flot.		
	Día 2	Día 3	Día 4	Día 2	Día 3	Día 4
A vs B	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs C	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.01

FIGURA 9

ACI-36-3A8-Ab1



	Latencia de escape			Long. Flot.		
	Día 2	Día 3	Día 4	Día 2	Día 3	Día 4
A vs D	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs E	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.001

FIGURA 10

Industria y Comercio
SUPERINTENDENCIA

PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA
RELACIÓN DE ANEXOS

Dependencia:

Fecha: ____/____/____/ AAAA MM DD

Recorrido:

Tipo de Radicación: Entrada: ☐ Salida: ☐ Traslado: ☐ Convenio: ☐

Planilla:

Radicador:

ID	No. Radicación	Consecutivo	Item
1	13-89514-0		
2			
3			
4			
5			
6			
7			
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9			
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14			
15			
16			
17			

Item	Unidad de Conservación
1	Bolsa
2	Caja
3	Libro
4	Sobre
5	Tomo
6	Otro
Item	*Soporte Documental*
7	CD
8	Disquete
9	DVD
10	Folleto
11	Foto
12	Periódico
13	Plano
14	Publicación Periódica
15	USB
10	Otro

Observaciones:

folio 153 Sobre manila con co

CASTELLANOS & CO LTDA
ABOGADOS
Calle 94 A No. 7 A-09
Bogotá, D.C. – COLOMBIA

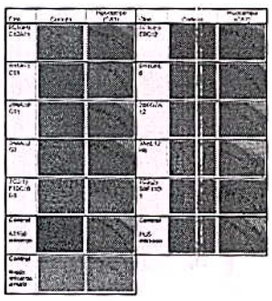


FIGURA 1-1

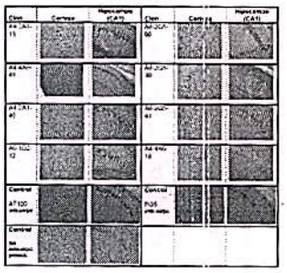


FIGURE 1-2

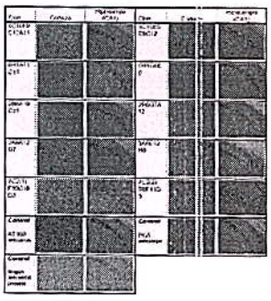


FIGURA 1-1

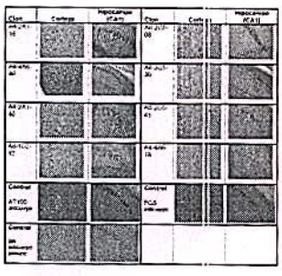


FIGURE 1-2





Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
TARJETA ARCHIVO PROPIETARIOS

PATENTE DE INVENCION ___ PCT __X__ MODELO DE UTILIDAD ___ DISEÑO INDUSTRIAL ___

(21) N° de solicitud: (22) Fecha de solicitud:
(51) Clasificación Internacional :

(71)Solicitante(s) **AC IMMUNE S.A.; KATHOLIEKE UNIVERSITEIT LEUVEN**

(72) Inventor(es) **PFEIFER, Andrea; MUHS, Andreas; VAN LEUVEN, Fred; PIHLGREN, Maria; ADOLFSSON, Oskar**

(74) Apoderado: **DRA. MARGARITA CASTELLANOS ABONDANO**

(30) Prioridad	(31)	No. Prioridad	32)	Fecha	(33)	País
		10186810.7		07/10/2010		EP
		11174248.2		15/07/2011		EP

(85) Fecha inicio fase nacional:
(86) Datos relativos a la presentación de la solicitud PCT
Fecha de presentación de la solicitud: **07/10/2011**
No. de solicitud **PCT/EP2011/067604**

(87) Publicación internacional
Fecha: **12/04/2012**
N° publicación: **WO 2012/045882 A3**

(54) Titulo: **COMPOSICION FARMACEUTICA**

Industria y Comercio
SUPERINTENDENCIA

EXTRACTO PARA PUBLICACIÓN

___ Patente de Invención ___ Patente modelo de utilidad _X_ PCT

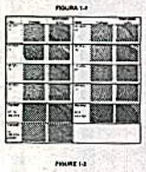
(21) No. De solicitud	(51) int. Cl:
(22) Fecha de solicitud	(71) Solicitante (s) **AC IMMUNE S.A.** **KATHOLIEKE UNIVERSITEIT LEUVEN**
(30) Prioridad (31) No. Prioridad **10186810.7** **11174248.2** (32) Fecha **07/10/2010** **15/07/2011** (33) País **EP, EP**	(72) Interventor (es) **PFEIFER, Andrea** **MUHS, Andreas** **VAN LEUVEN, Fred** **PIHLGREN, Maria** **ADOLFSSON, Oskar**
	(74) Apoderado (s) **DRA. MARGARITA CASTELLANOS ABONDANO**
(54) Título **COMPOSICION FARMACEUTICA**	
Las casillas identificadas con 85, 86 y 87 sólo aplican para PCT	
(85) Fecha límite inicio Fase Nacional (86) Datos relativos a la presentación de la solicitud PCT Fecha de presentación de la solicitud **07/10/2011** No. de solicitud **PCT/EP2011/067604**	(87) Publicación internacional Fecha **12/04/2012** No. Publicación **WO2012/045882 A3**

Resumen reivindicación (es):

1. Un anticuerpo, o una de sus partes funcionales que reconoce y se une especificamente con un fosfo–epítope en la proteína tau de mamifero o en uno de sus fragmentos, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con la proteína tau soluble e insoluble y modula los niveles tau solubles e insolubles.
2. El anticuerpo de acuerdo con la reivindicación 1 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en el cerebro de un mamífero.
3. El anticuerpo de acuerdo con la reivindicación 2 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en la corteza cerebral y/o el hipocampo.
4. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total.
5. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau fosforilada soluble.
6. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de filamentos helicoidales en pares que contienen proteína tau hiperfosforilada.
7. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total, proteína tau fosforilada soluble y filamentos helicoidales pTau en pares.
8. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho mamífero es un humano.
9. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 10 nM.
10. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 5 nM, al menos 2 nM; al menos 1 nM, al menos 500 pM, al menos 300 pM, al menos 200 pM, al menos 100 pM.
11. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de tasa de asociación de 10^4 $M^{-1}s^{-1}$ o más; de 10^5 $M^{-1}s^{-1}$ o más, de 10^6 $M^{-1}s^{-1}$ o más.

...





Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-089514- -00000-0000
Fecha: 2013-04-05 14:18:14 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 156

PATENTE DE INVENCIÓN _____ **MODELO DE UTILIDAD** _____ **Art 33 Decisión 486/00**

Indicación que se solicita una patente.
Datos de identificación del solicitante o de la persona que presenta la solicitud
Descripción de la invención
Dibujos de ser estos pertinentes
Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)

Completa _____ Incompleta _____

PATENTE DE INVENCIÓN PCT _X_ **MODELO DE UTILIDAD PCT** ___ **Art.33 Decisión 486/00, Circular Única**

X Indicación que se solicita una PCT
X Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)
X Dibujos de ser estos pertinentes
X Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)

Completa X Incompleta _____

DISEÑO INDUSTRIAL _____ **(Art. 119 Decisión 486/00)**

Indicación que se solicita Diseño industrial
Datos de identificación del solicitante o de la persona que presenta la solicitud
Representación gráfica y fotográfica del Diseño industrial o muestra del material que incorpora el diseño

Comprobante de pago de las tasas establecidas

Completa _____ Incompleta _____

ESQUEMA DE TRAZADO _____ **(Art. 92 Decisión 486/00)**

Indicación que se solicita un esquema de trazado

Datos de identificación del solicitante o de la persona que presenta la solicitud

Representación gráfica de un esquema de trazado

Comprobante de pago de las tasas establecidas

Completa _____ Incompleta _____

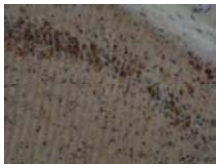
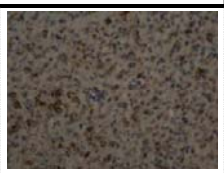
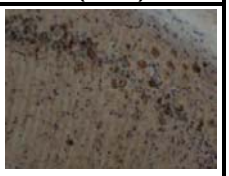
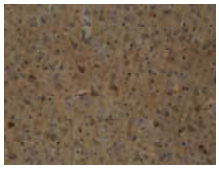
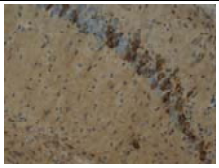
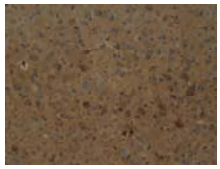
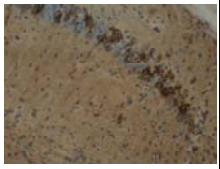
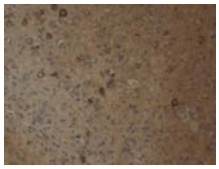
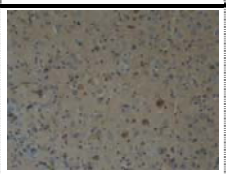
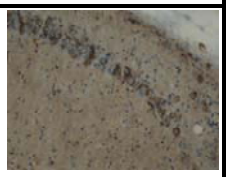
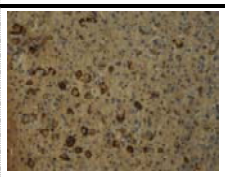
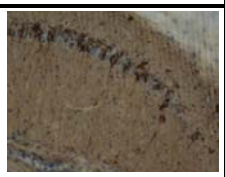
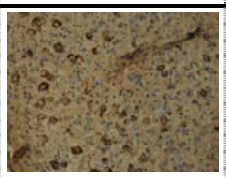
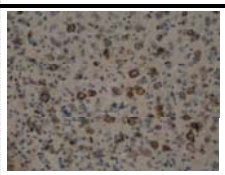
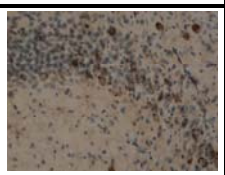
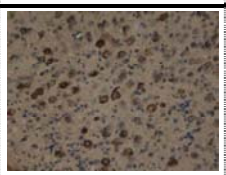
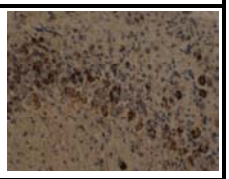
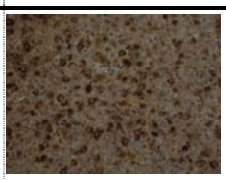
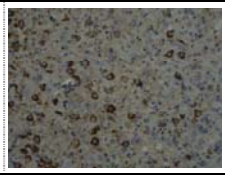
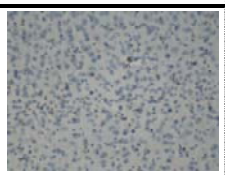
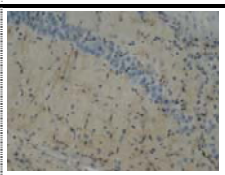
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6C10F9 C12A11			6C10E5 E9C12		
6H1A11 C11			6H1G6E 6		
2B6A10 C11			2B6G7A 12		
3A8A12 G7			3A8E12 H8		
7C2(1) F10C10 D3			7C2(2) B9F11D 5		
Control AT100 anticuerpo			***Control*** PG5 anticuerpo		
Control Ningún anticuerpo primario					

FIGURA 1-1

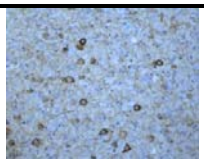
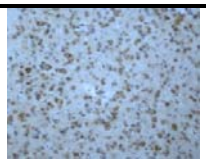
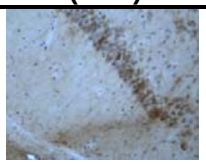
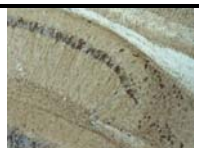
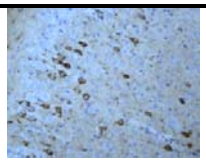
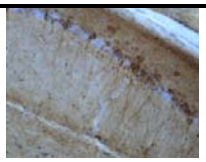
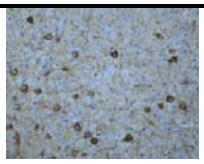
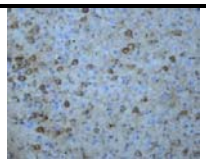
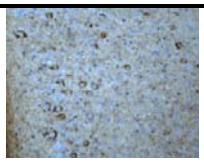
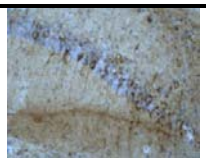
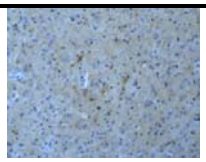
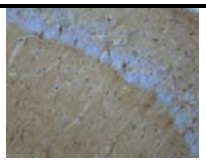
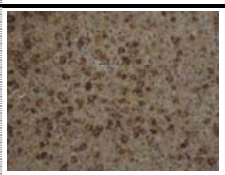
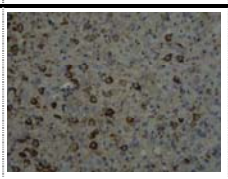
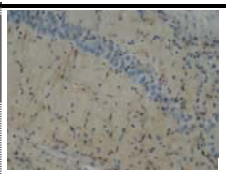
Clon	Corteza	Hipocampo (CA1)	Clon	Corteza	Hipocampo (CA1)
A4-2A1-18			A6-2G5-08		
A4-4A6-48			A6-2G5-30		
A4-2A1-40			A6-2G5-41		
A6-1D2-12			A4-4A6-18		
Control AT100 anticuerpo			***Control*** PG5 anticuerpo		
Control Sin anticuerpo primario					

FIGURE 1-2

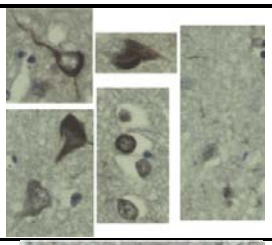
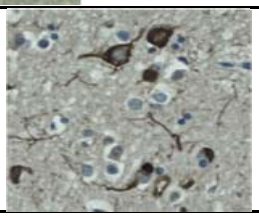
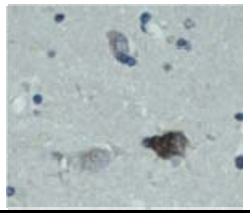
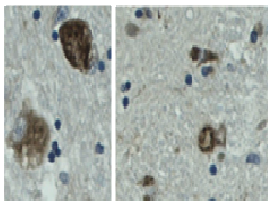
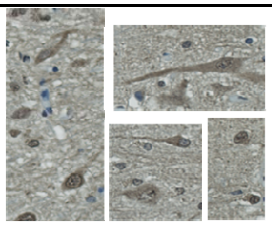
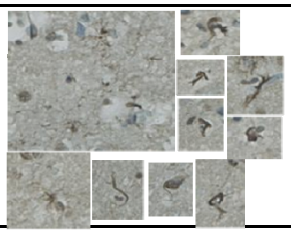
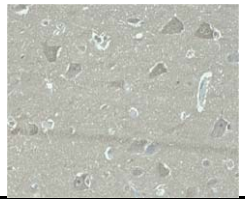
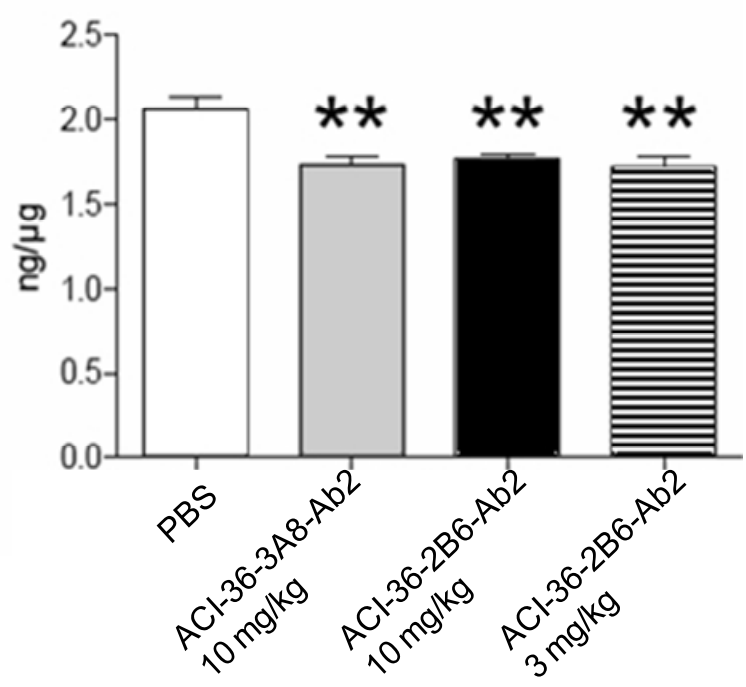
Diagnosis	Región	Tinción TAUPIR de ACI-3A8-Ab1
AD	Hipocampo	
FAD	Hipocampo anterior	
AGD	Hipocampo anterior	
PSP	Pallidum	
FTDP-17	Corteza frontal	
CBD	Corteza frontal	
Control Sano	Hipocampo	

FIGURA 2

Corteza- TAU total



Corteza- pTAU

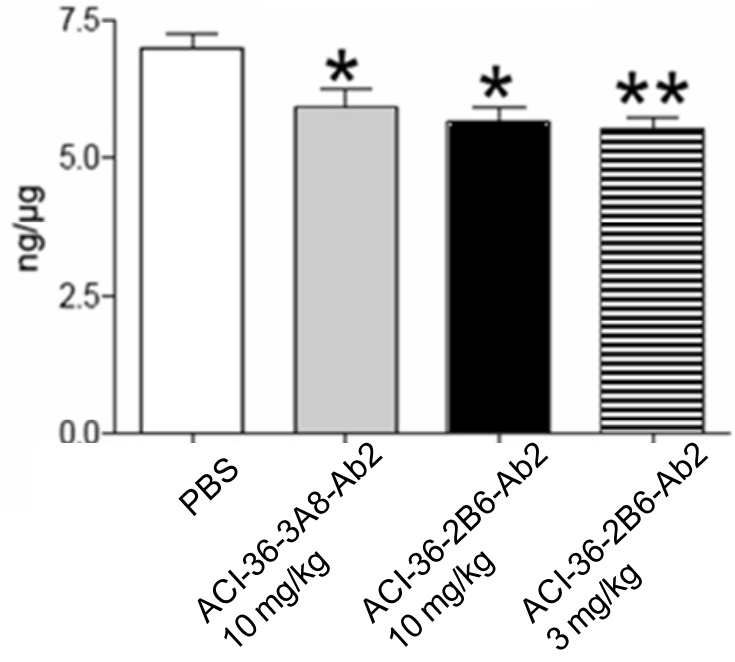


FIGURA 3

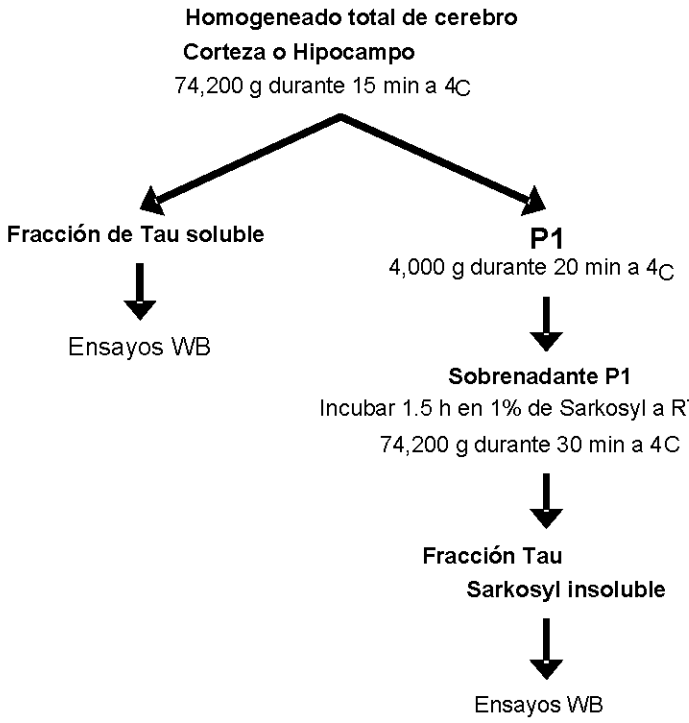


FIGURA 4.1

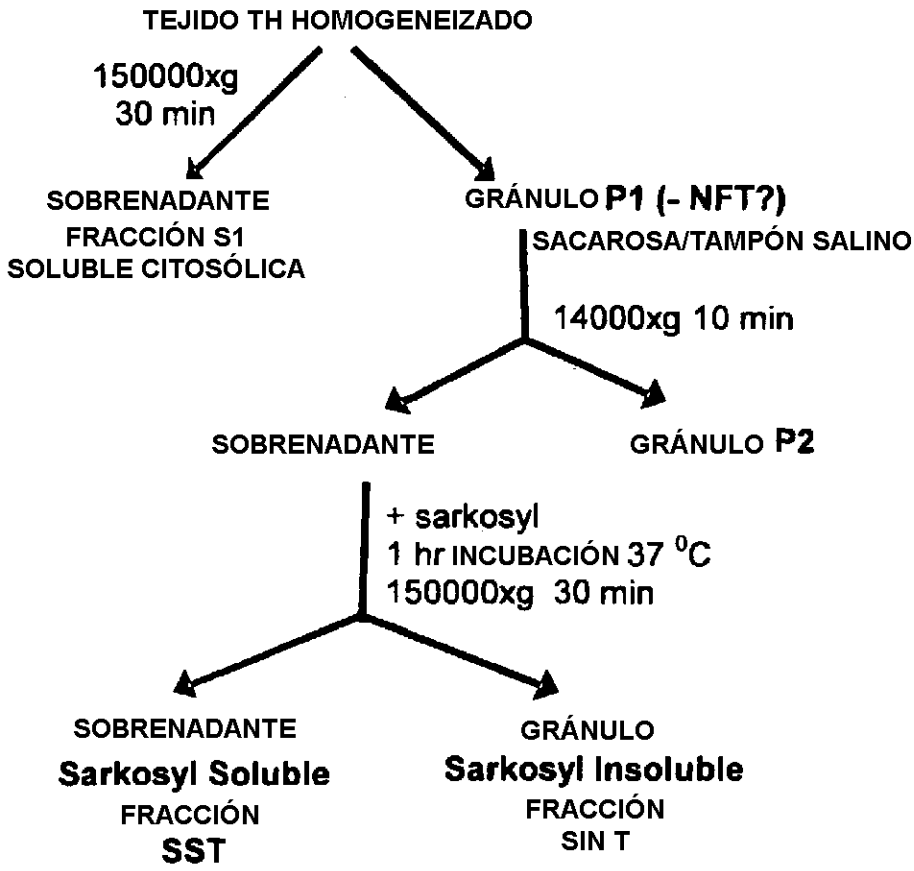


FIGURA 4.2

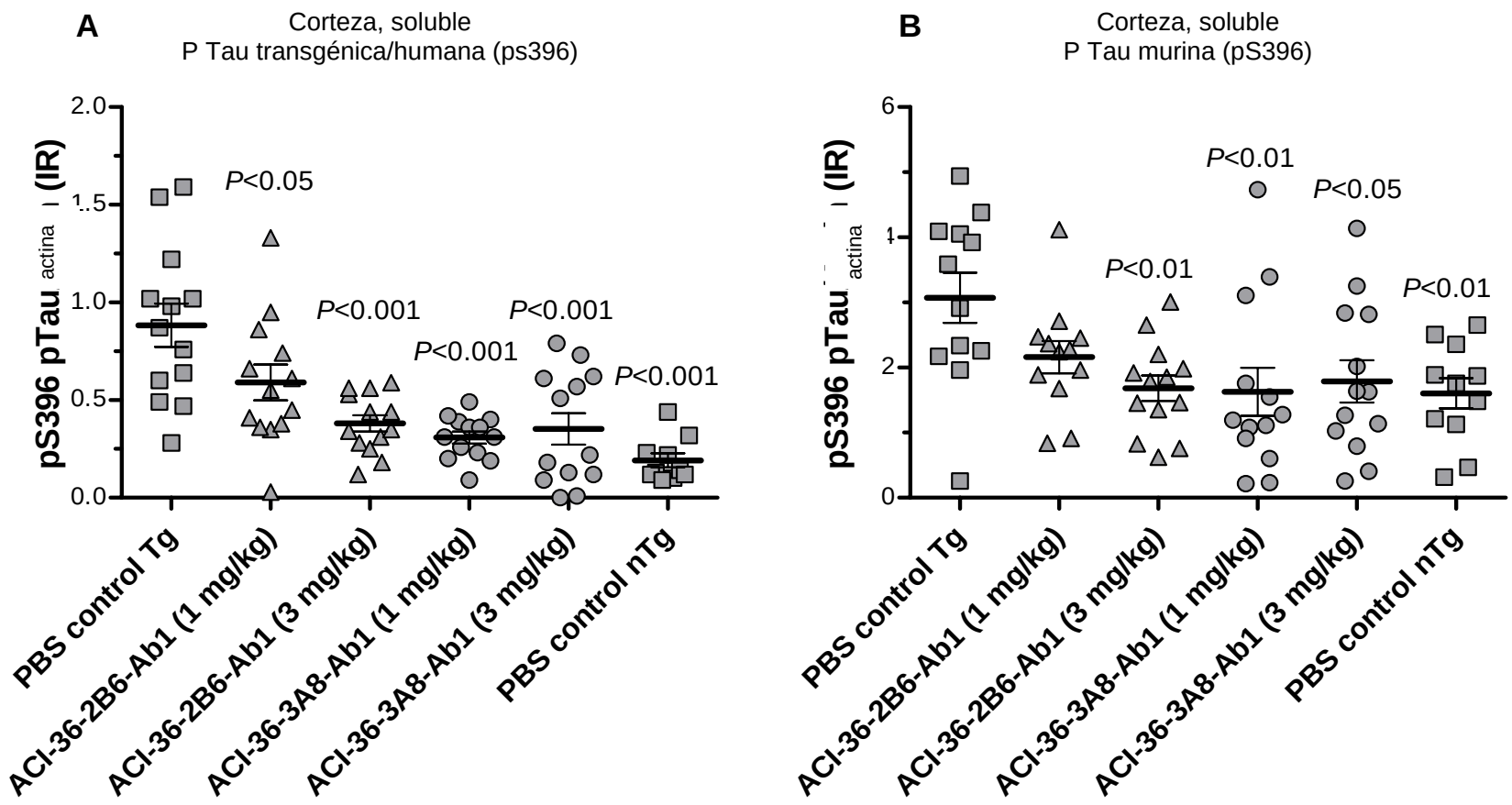

A
Corteza, soluble
P Tau transgénica/humana (ps396)
pS396 pTau actina (IR)
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
B
Corteza, soluble
P Tau murina (pS396)
pS396 pTau actina (IR)
6
4
2
0
P<0.01
P<0.01
P<0.05
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-1

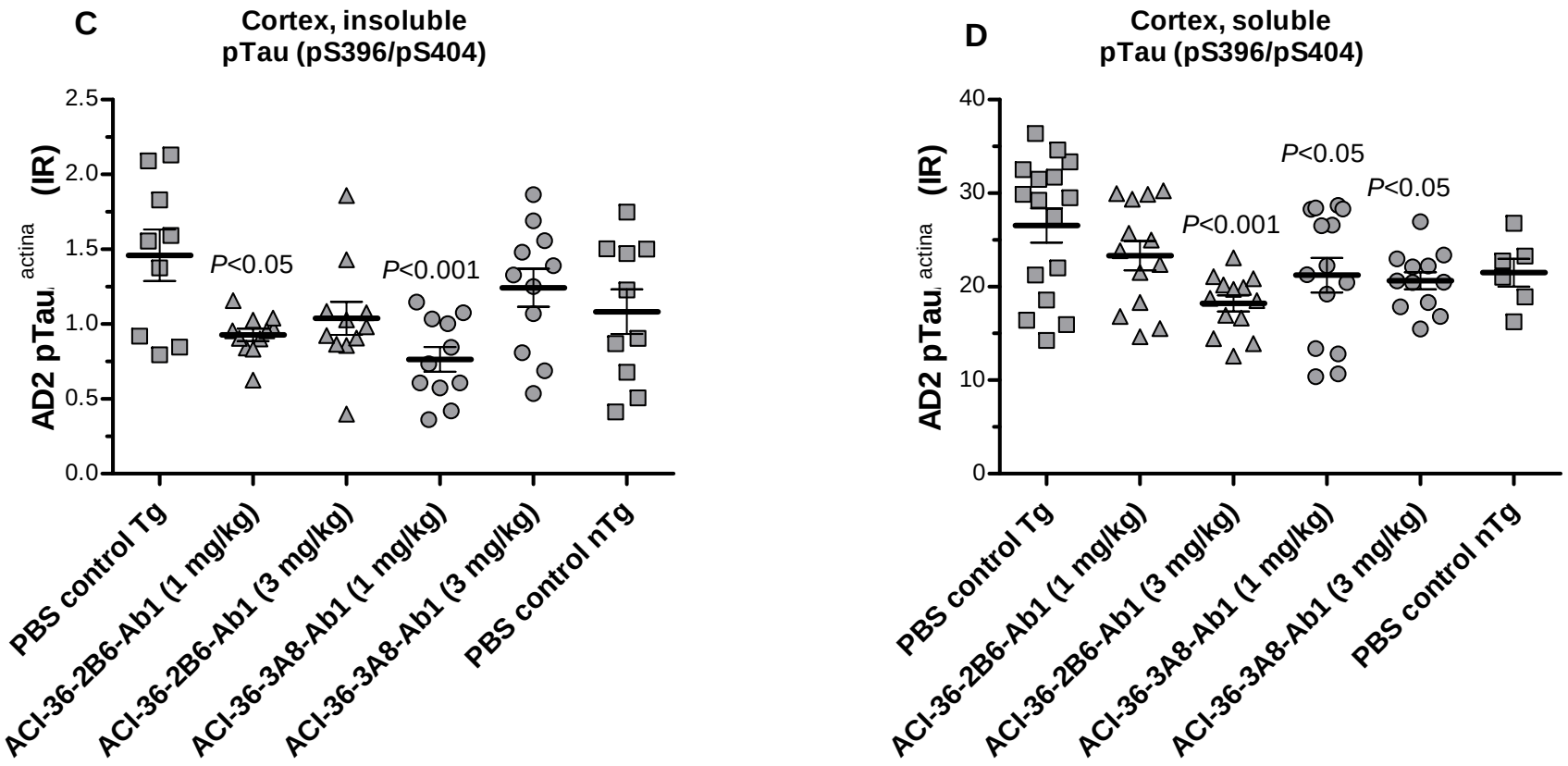

C
Cortex, insoluble
pTau (pS396/pS404)
AD2 pTau/actina (IR)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
D
Cortex, soluble
pTau (pS396/pS404)
AD2 pTau/actina (IR)
40
30
20
10
0
P<0.001
P<0.05
P<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-2

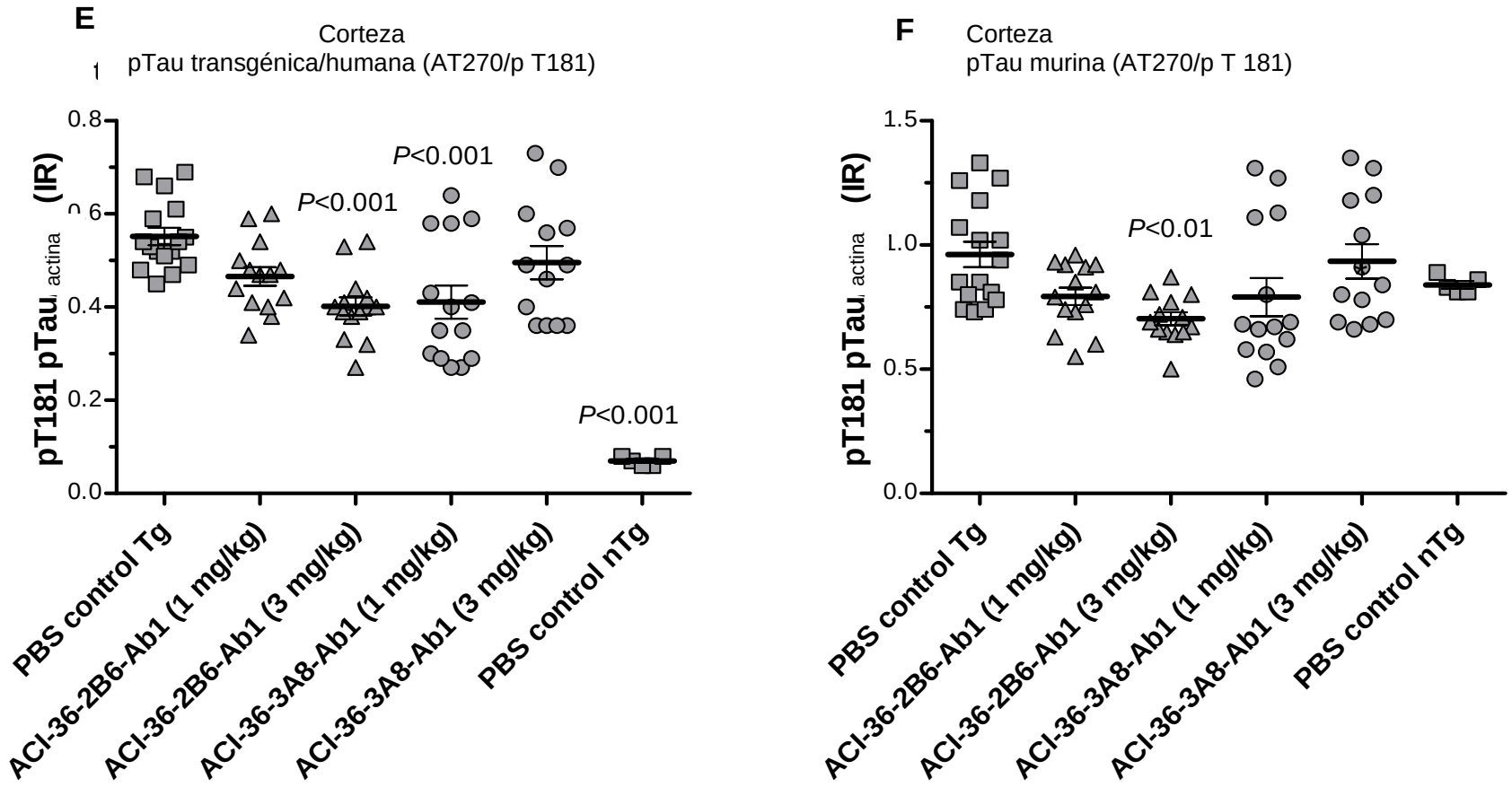

E
Corteza
pTau transgénica/humana (AT270/p T181)
pT181 pTau/actina (IR)
0.8
0.4
0.2
0.0
P<0.001
P<0.001
P<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
F
Corteza
pTau murina (AT270/p T 181)
pT181 pTau/actina (IR)
1.5
1.0
0.5
0.0
P<0.01
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-3

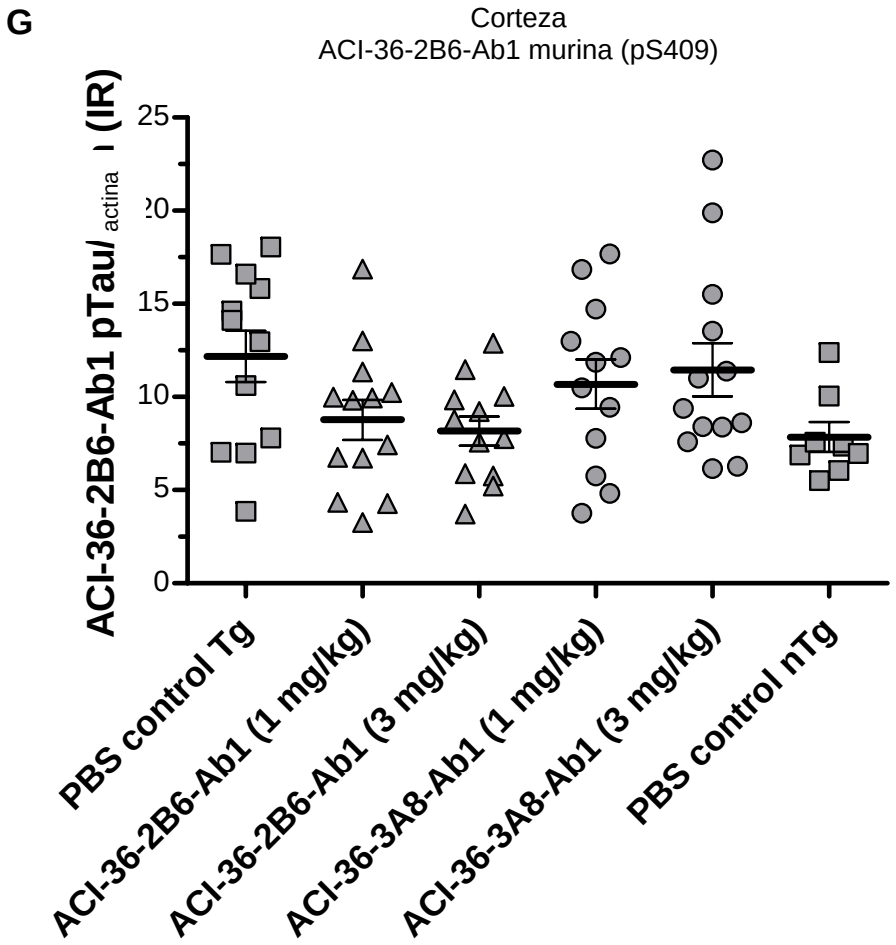

G
Corteza
ACI-36-2B6-Ab1 murina (pS409)
ACI-36-2B6-Ab1 pTau/actina (IR)
25
20
15
10
5
0
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-4

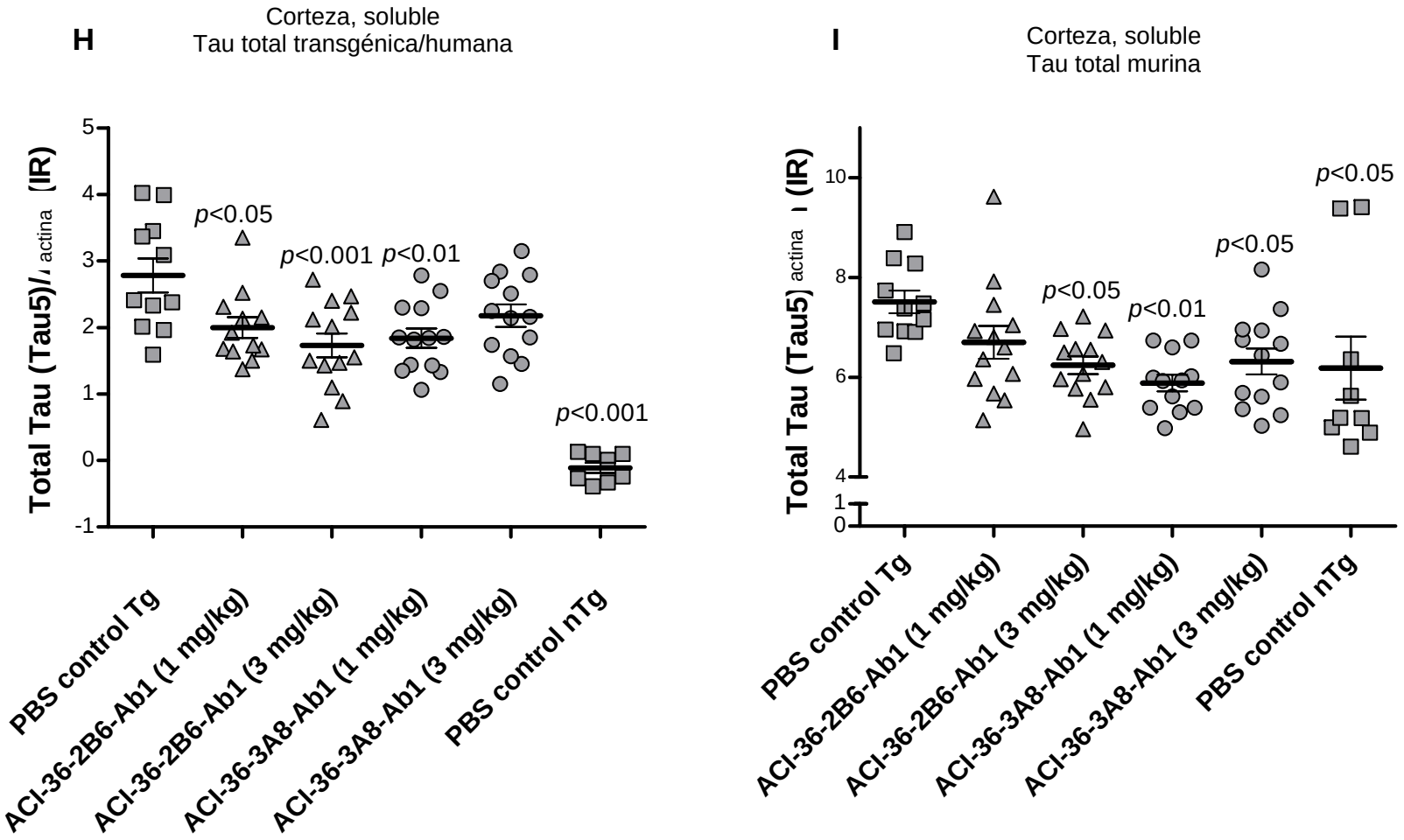

H
Corteza, soluble
Tau total transgénica/humana
Total Tau (Tau5)/ actina (IR)
5
4
3
2
1
0
-1
p<0.05
p<0.001
p<0.01
p<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
I
Corteza, soluble
Tau total murina
Total Tau (Tau5) actina (IR)
10
8
6
4
1
0
p<0.05
p<0.05
p<0.01
p<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURA 5-5

Corteza, fracción soluble (S1)
Tau total transgénica/humana (HT7)

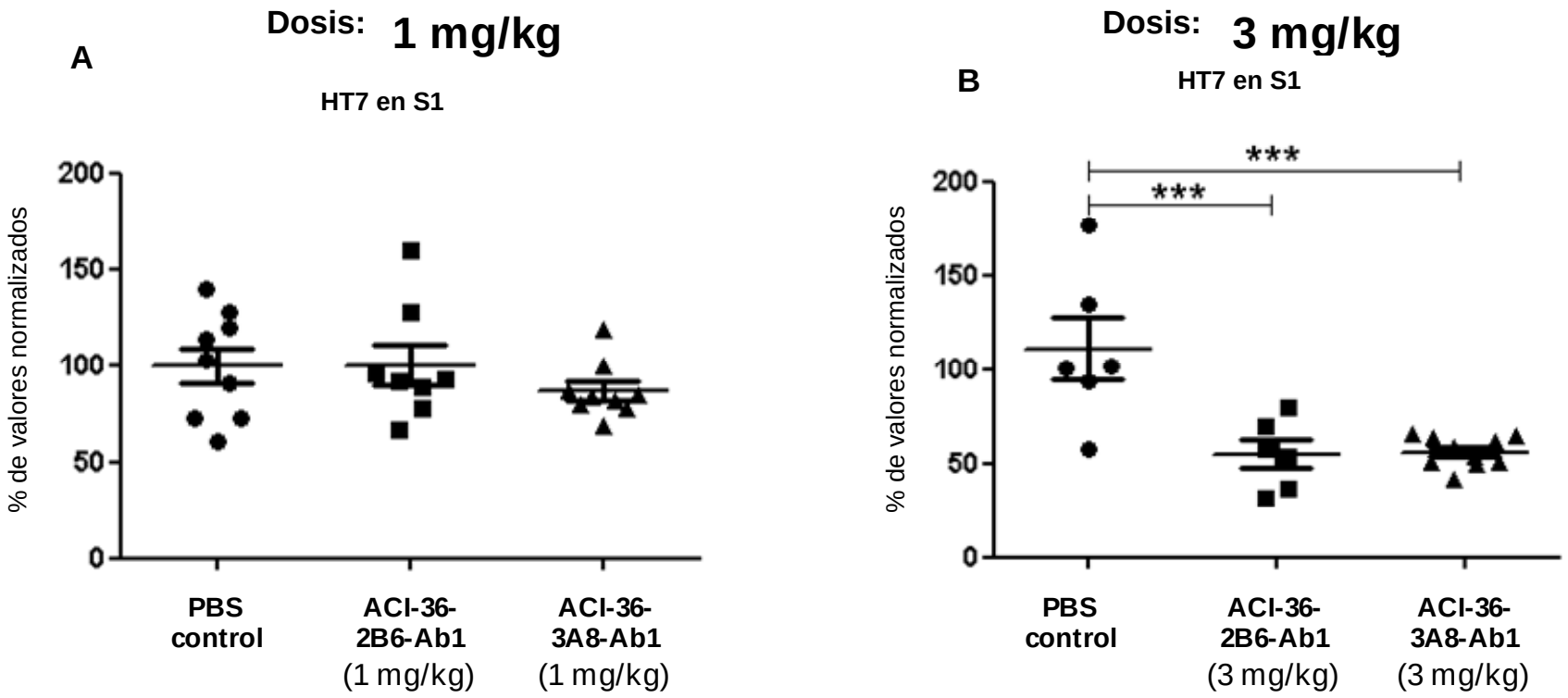
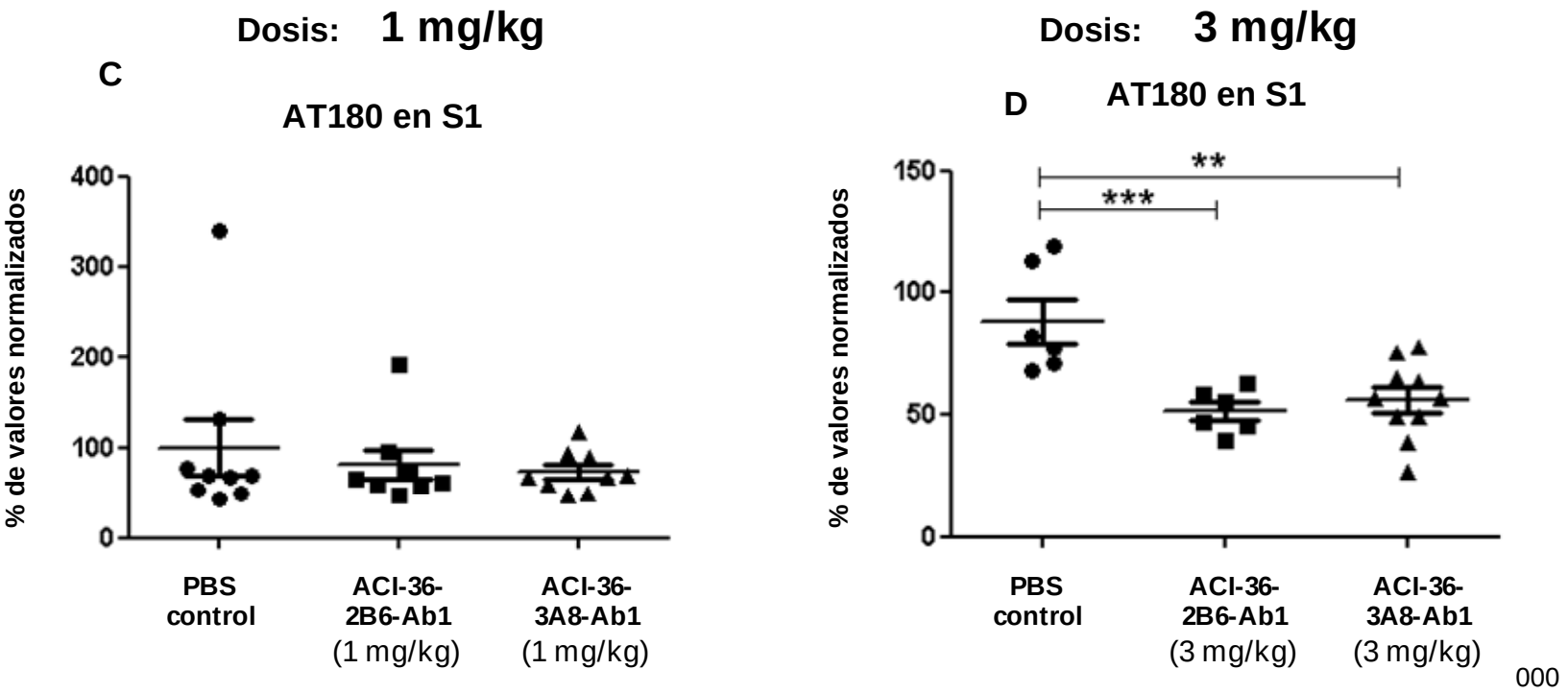


FIGURA 6-1

Corteza, fracción soluble (S1)
pTau transgénica/humana (pT231/AT180)



000

FIGURA 6-2

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS202/AT8)

Dosis: 3 mg/kg

E

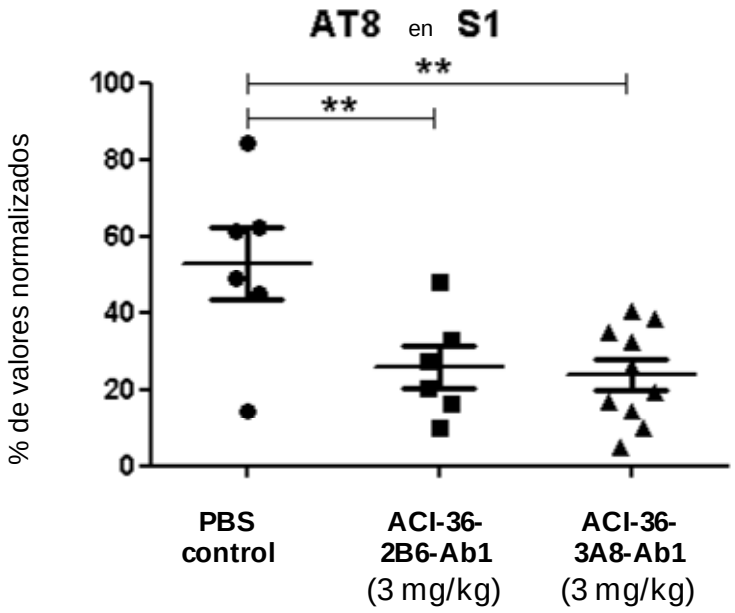


FIGURA 6-3

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS396)

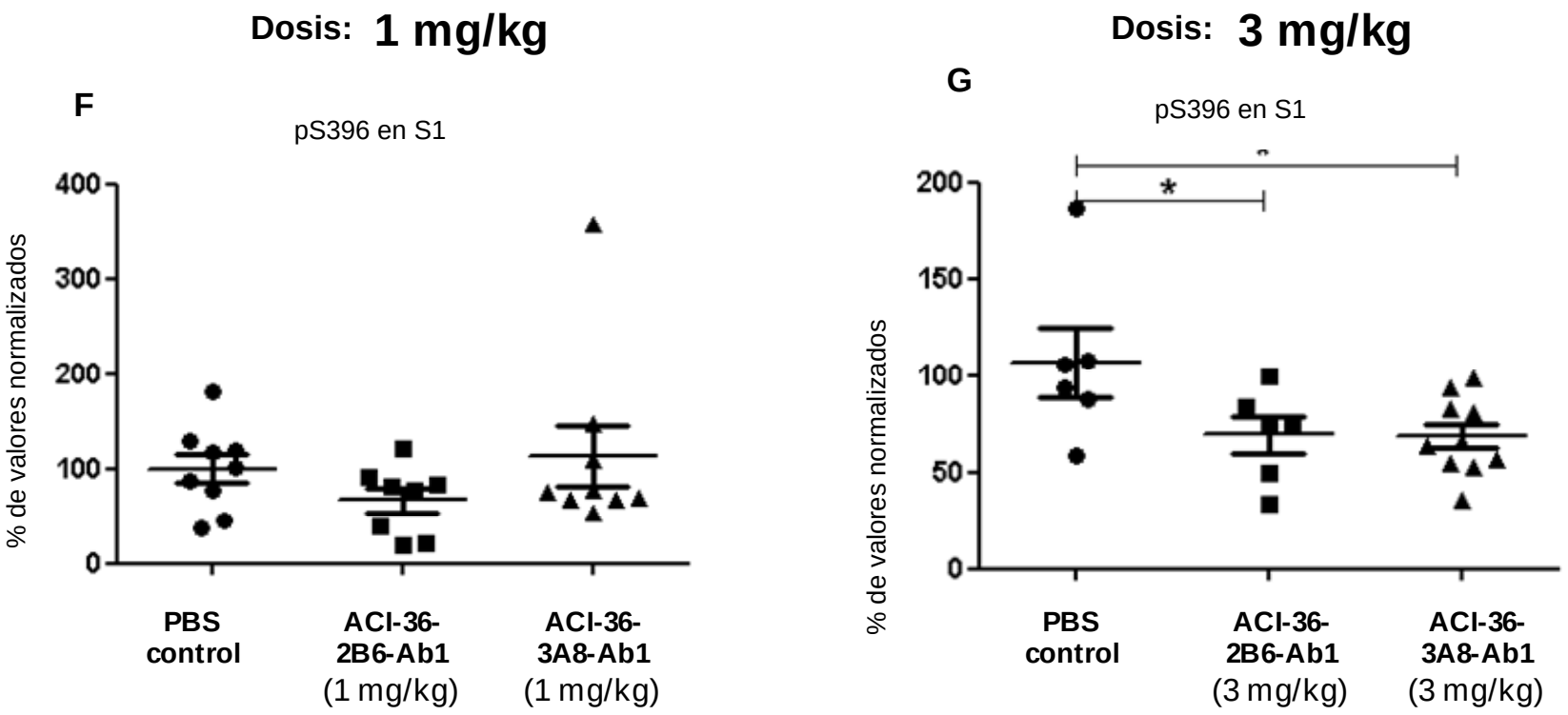


FIGURA 6-4

Corteza, homogenado total
pTau transgénica/humana (pS400)

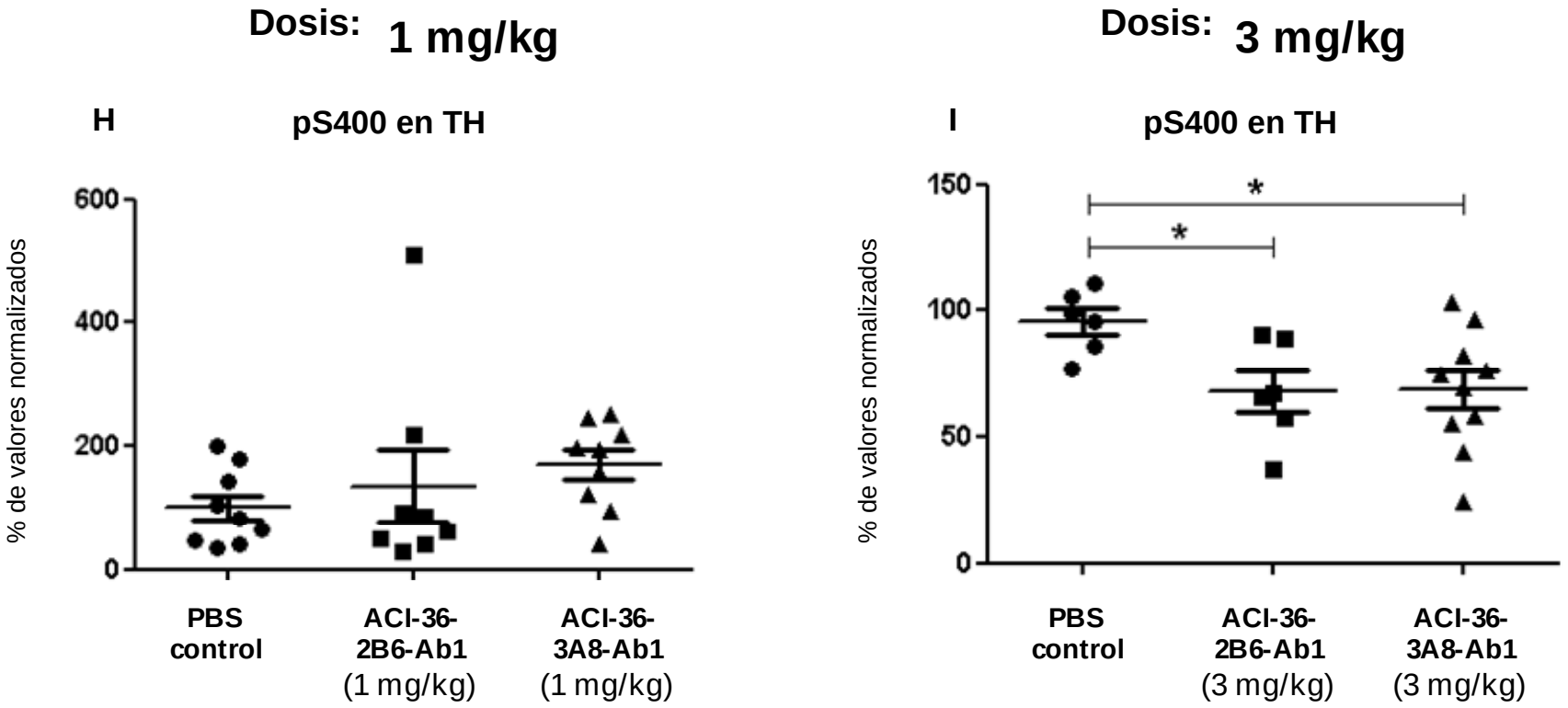


FIGURA 6-5

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS400)

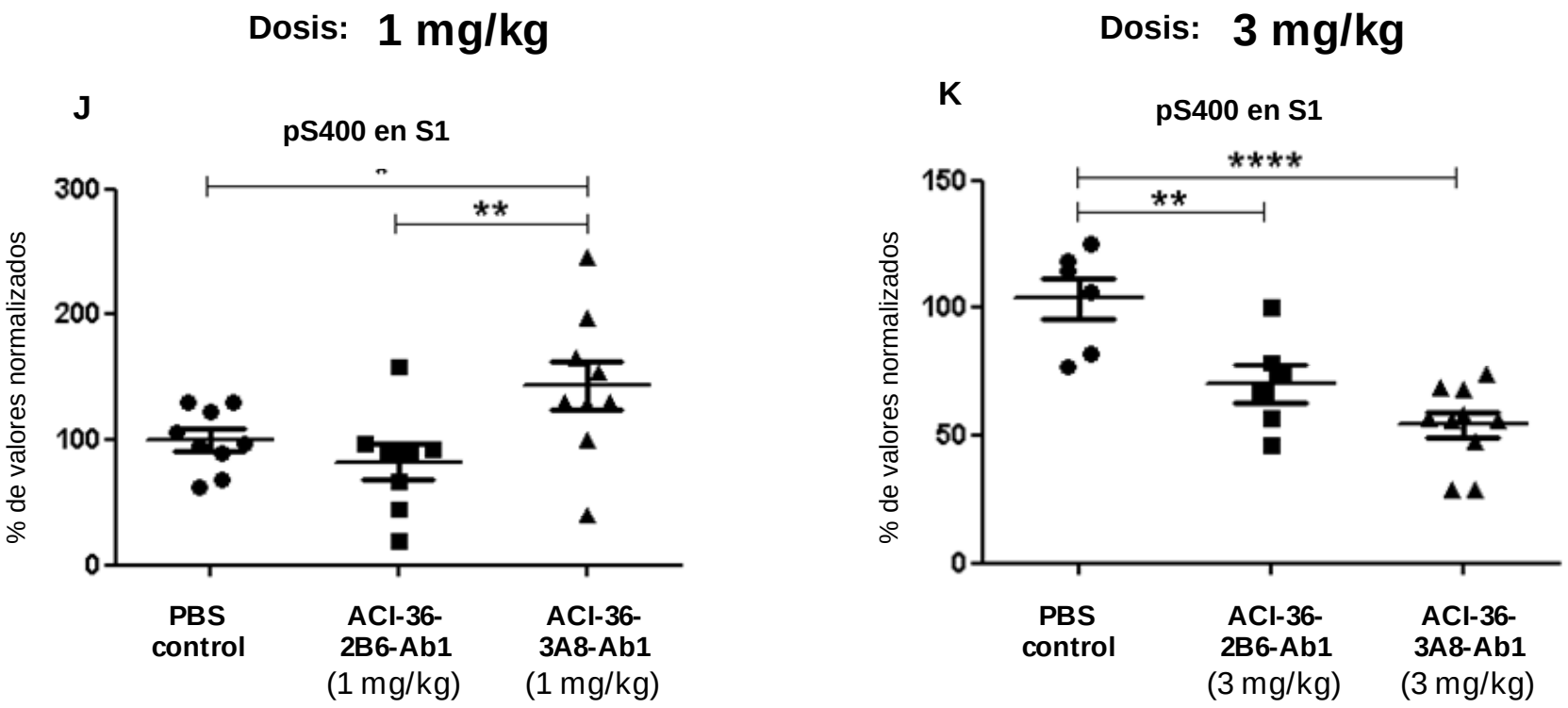


FIGURA 6-6

Corteza, homogenado total
pTau transgénica/humana (pS404)

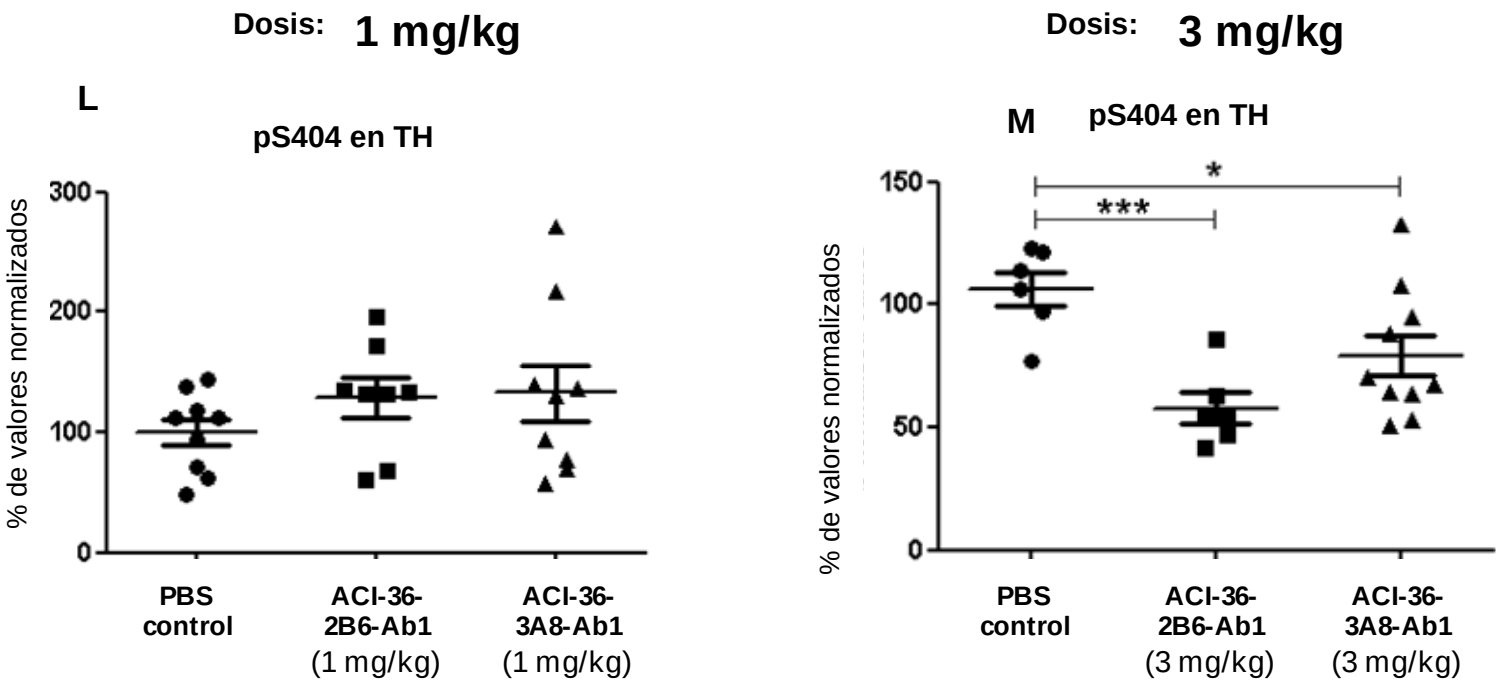


FIGURA 6-7

Corteza, fracción soluble (S1)
pTau transgénica/humana (pS404)

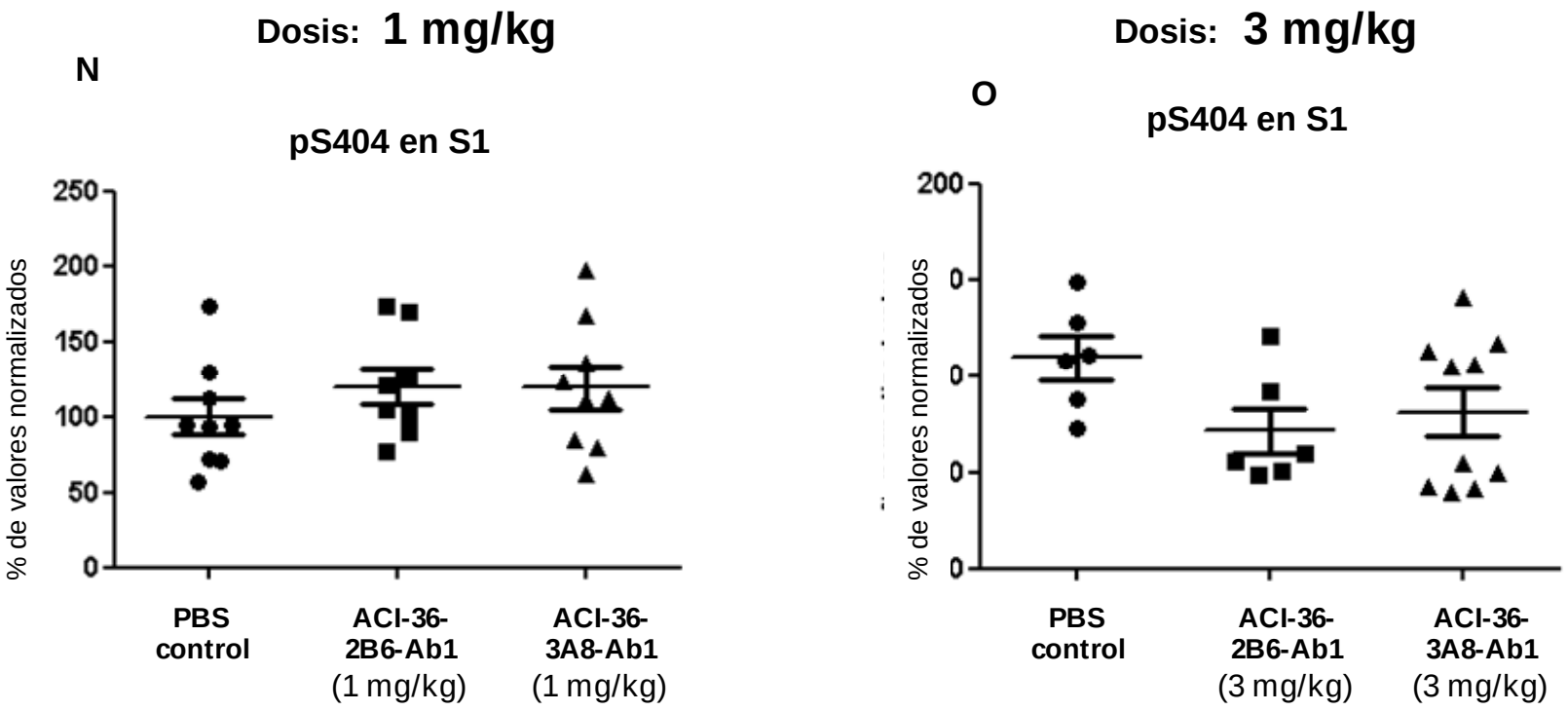


FIGURA 6-8

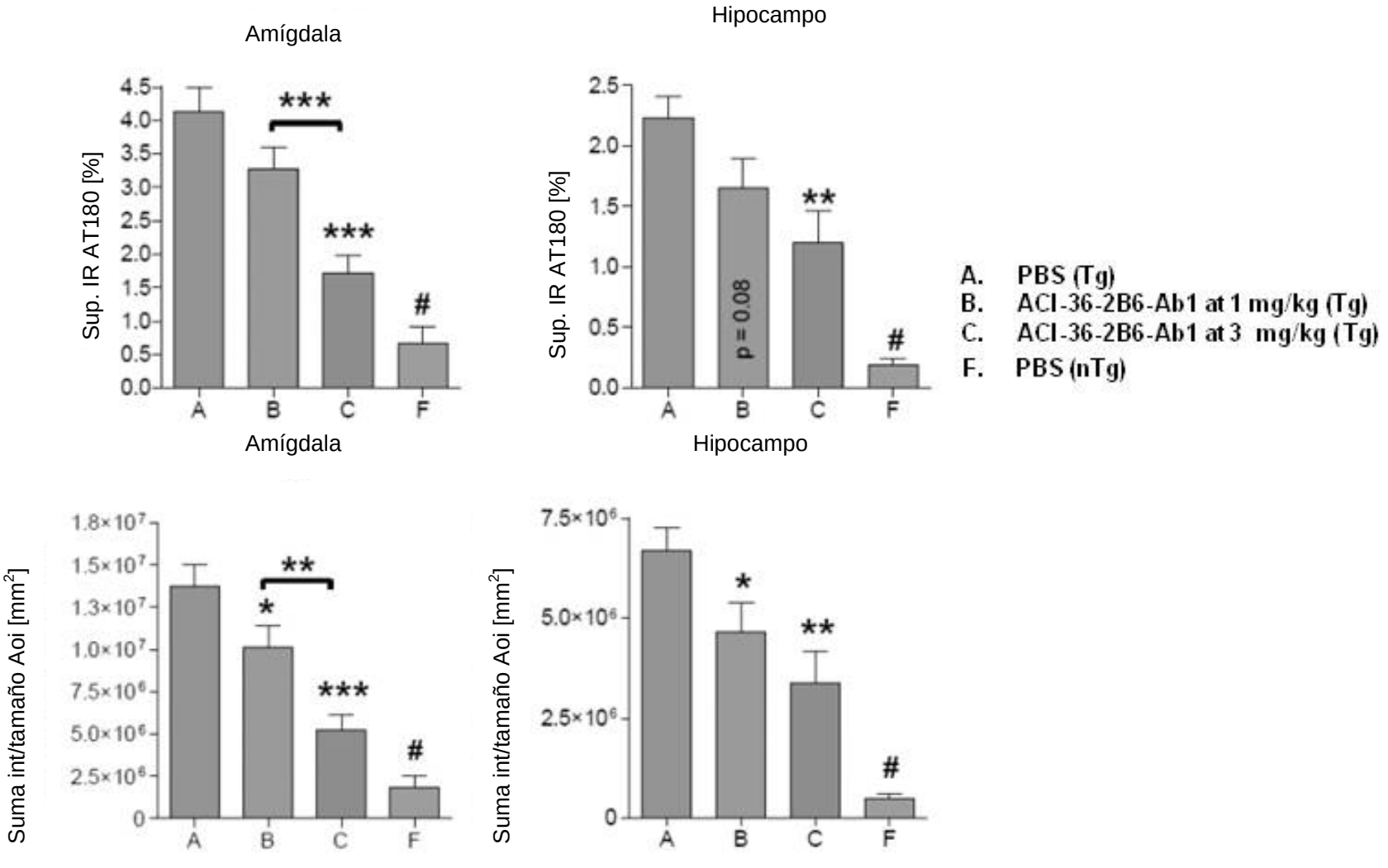

Amígdala
Hipocampo
Sup. IR AT180 [%]
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
***
#
**
p = 0.08
A
B
C
F
A. PBS (Tg)
B. ACI-36-2B6-Ab1 at 1 mg/kg (Tg)
C. ACI-36-2B6-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)
Amígdala
Hipocampo
Suma int/tamaño Aoi [mm2]
1.8×10^7
1.5×10^7
1.3×10^7
1.0×10^7
7.5×10^6
5.0×10^6
2.5×10^6
0
7.5×10^6
5.0×10^6
2.5×10^6
0
*
**
***
#

FIGURA 7

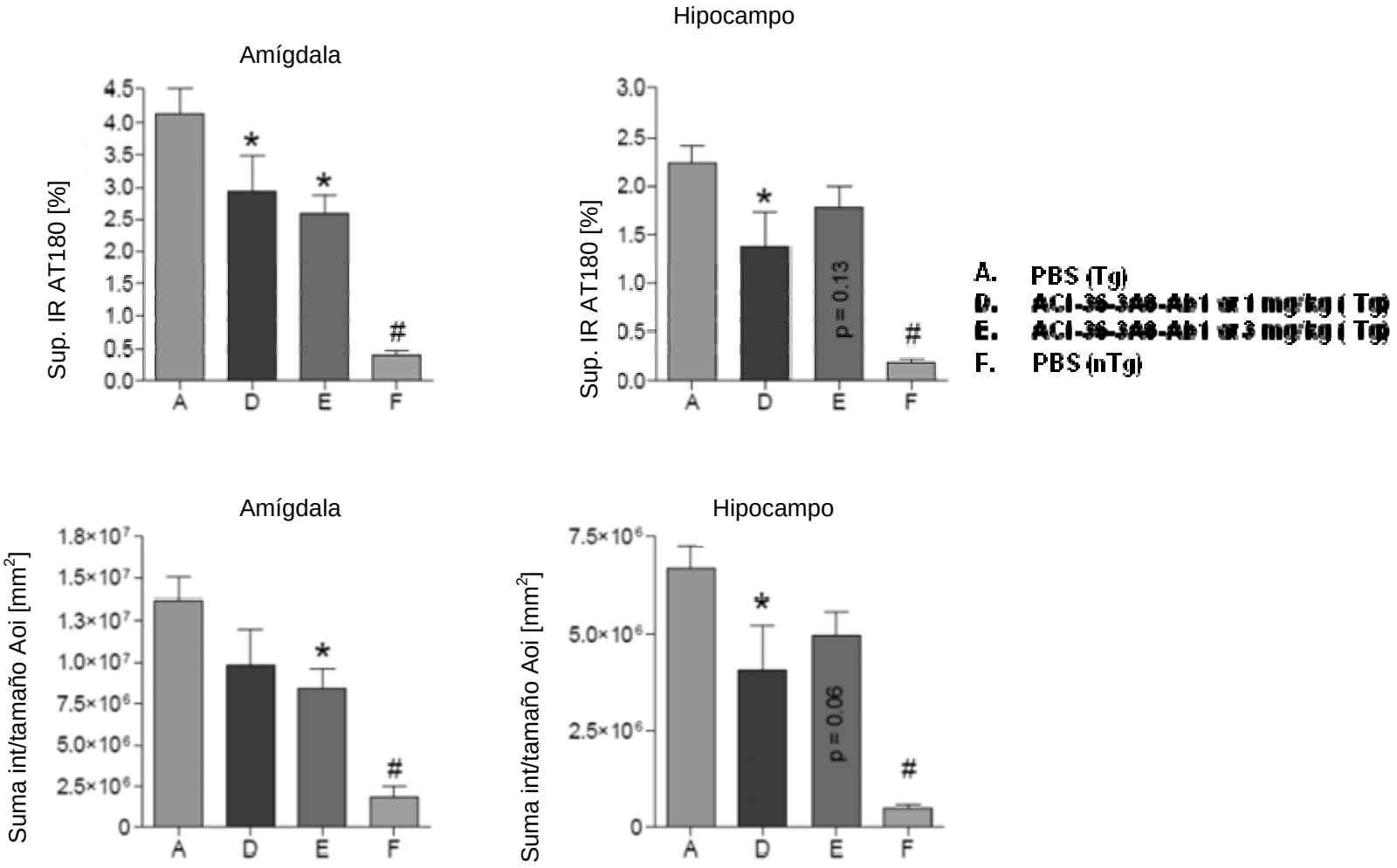
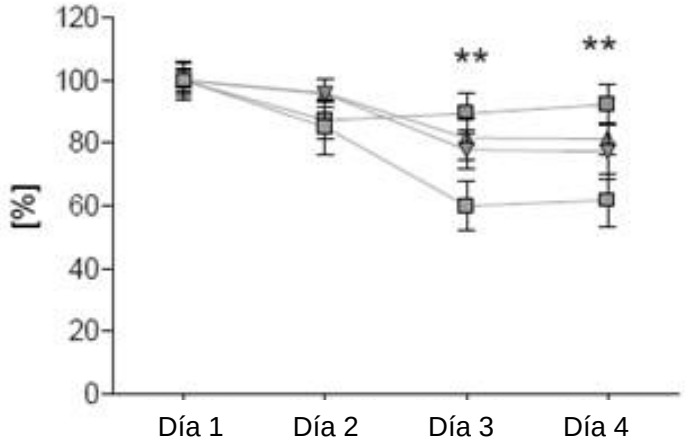

Amígdala
Sup. IR AT180 [%]
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
#
A
D
E
F
Hipocampo
Sup. IR AT180 [%]
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
p = 0.13
#
A
D
E
F
A. PBS (Tg)
D. ACI-35-3A8-Ab1 a 1 mg/kg (Tg)
E. ACI-35-3A8-Ab1 a 3 mg/kg (Tg)
F. PBS (nTg)
Amígdala
Suma int/tamaño Aoi [mm2]
1.8×10^7
1.5×10^7
1.3×10^7
1.0×10^7
7.5×10^6
5.0×10^6
2.5×10^6
0
*
#
A
D
E
F
Hipocampo
Suma int/tamaño Aoi [mm2]
7.5×10^6
5.0×10^6
2.5×10^6
0
*
p = 0.06
#
A
D
E
F

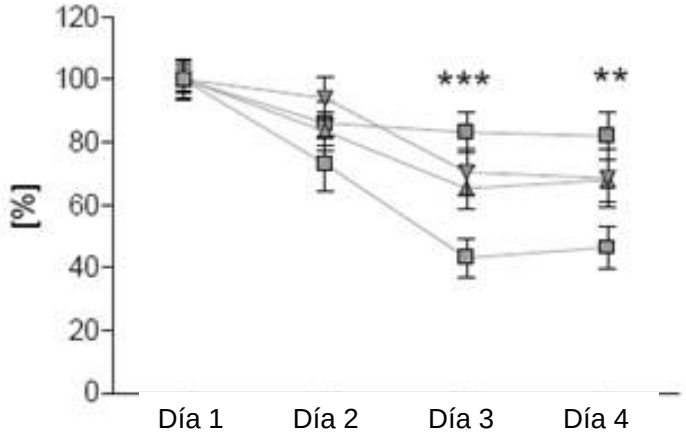
FIGURA 8

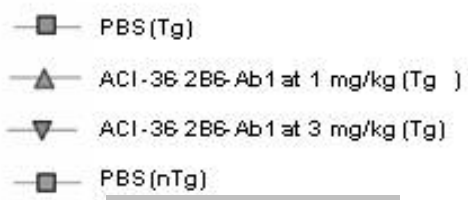
ACI-36-2B6-Ab1

Latencia de escape



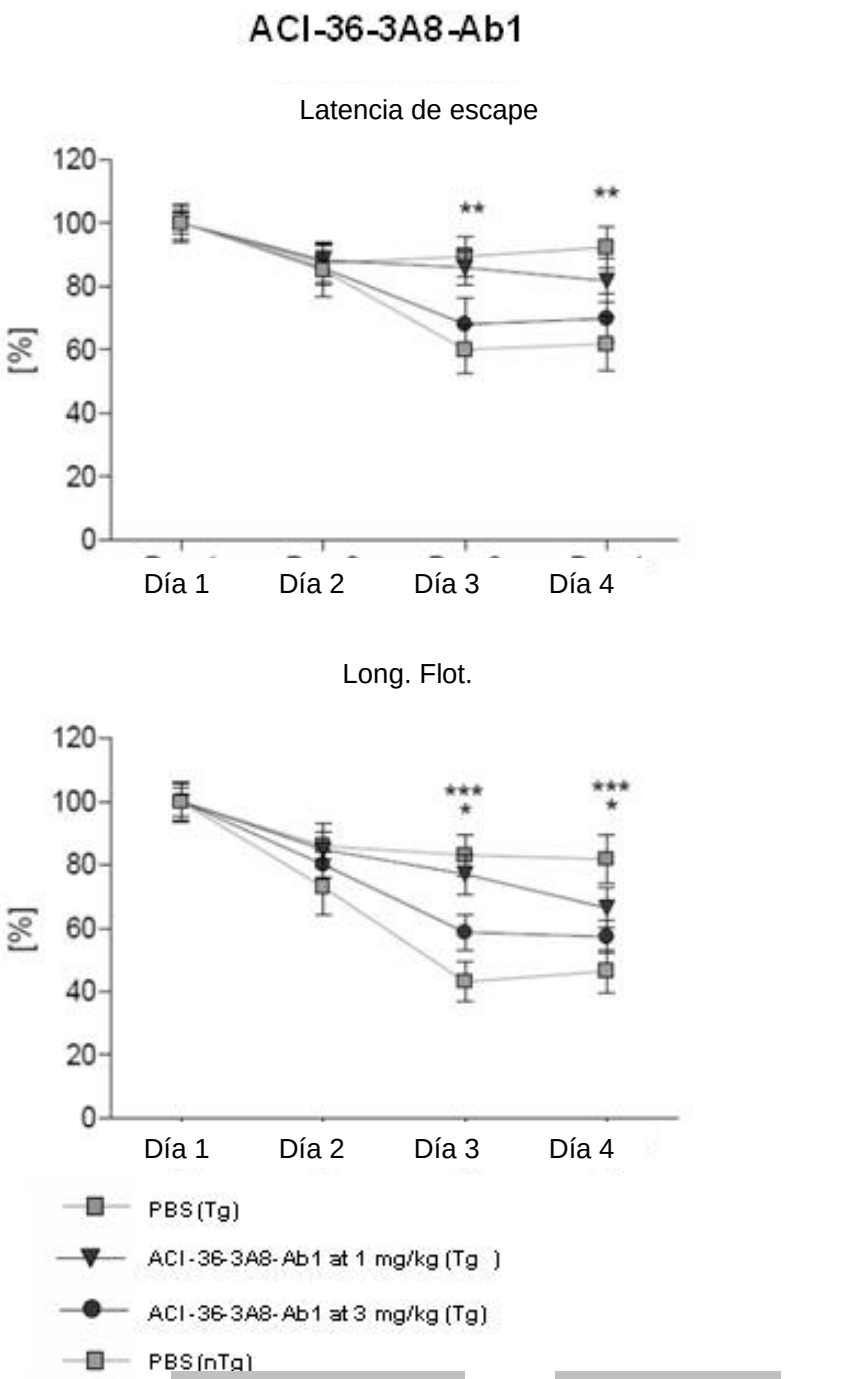
Long. Flot.





	Latencia de escape			Long. Flot.		
	Día 2	Día 3	Día 4	Día 2	Día 3	Día 4
A vs B	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs C	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.01

FIGURA 9



	Latencia de escape			Long. Flot.		
	Día 2	Día 3	Día 4	Día 2	Día 3	Día 4
A vs D	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs E	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.001

FIGURA 10

SEQUENCE LISTING

<110> AC Immune S.A.
K.U. Leuven Research & Development

<120> PHARMACEUTICAL COMPOSITION

<130> P2654 PCT/A BS

<150> EP 10 18 6810.7
<151> 2010-10-07
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35 40 45
Gly Asp Ile Asn Pro Asn Arg Gly Gly Thr Thr Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Ser Tyr Tyr Ala Val Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr
100 105 110
Val Ser Ser
115

<210> 2
<211> 115
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..115
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 2
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Thr
1   5   10   15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35   40   45
Gly Asp Ile Asn Pro Asn Arg Gly Gly Thr Thr Tyr Asn Gln Lys Phe
    50   55   60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85   90   95
Ala Ser Tyr Tyr Ala Val Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr
            100   105   110
Val Ser Ser
        115

<210> 3
<211> 115
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..115
<223> /mol_type="protein"
      /organism="Mus musculus"

<400> 3
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1   5   10   15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20   25   30
Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35   40   45
Gly Asp Ile Asn Pro Asn Arg Gly Gly Thr Thr Tyr Asn Gln Lys Phe
    50   55   60
Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85   90   95
Ala Ser Tyr Tyr Ala Val Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr
            100   105   110
Val Ser Ser
        115

<210> 4
<211> 120
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..120
<223> /mol_type="protein"

/organism="Mus musculus"

<400> 4
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1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Pro Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Glu Lys Gly Leu Glu Trp Val
35 40 45
Ala Tyr Ile Ser Ser Gly Ser Ser Thr Ile Tyr Tyr Gly Asp Thr Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Phe
65 70 75 80
Leu Gln Met Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg Arg Gly Gln Leu Arg Leu Arg Leu Phe Ala Tyr Trp Gly Gln
100 105 110
Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 5
<211> 119
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..119
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 5
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1 5 10 15
Ser Leu Arg Leu Tyr Cys Ala Ala Ser Gly Phe Thr Phe Thr Asp Tyr
20 25 30
Tyr Met Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Pro Glu Trp Leu
35 40 45
Ala Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Thr Ala
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Ile
65 70 75 80
Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr
85 90 95
Tyr Cys Val Lys Ala Leu Gly Arg Tyr Phe Asp Val Trp Gly Thr Gly
100 105 110
Thr Thr Val Thr Val Ser Ser
115

<210> 6
<211> 113
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..113

<223> /mol_type="protein"
/organism="Mus musculus"

<400> 6
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1 5 10 15
Gln Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Phe Asn Ser
20 25 30
Gly Asn Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Val Tyr Phe Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Ile Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Thr
65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr Phe Cys Gln Glu
85 90 95
His Tyr Thr Thr Pro Pro Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu
100 105 110
Lys

<210> 7
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 7
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Arg Leu Val His Ser
20 25 30
His Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
85 90 95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 8
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"

/organism="Mus musculus"

<400> 8
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
His Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
85 90 95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 9
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 9
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30
His Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
85 90 95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 10
<211> 107
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..107
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 10

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1               5                  10                  15
Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Ala
            20                  25                  30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Leu
                85                  90                  95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105
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<210> 11
<211> 113
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..113
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 11

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1               5                  10                  15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30
Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80
Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95
Tyr Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
            100                 105                 110
Lys
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<210> 12
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 12

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Gly Tyr Thr Phe Thr Asp Tyr Tyr Met Asn
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1 5 10

<210> 13
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 13
Asp Ile Asn Pro Asn Arg Gly Gly Thr Thr Tyr Asn Gln Lys Phe Lys
1 5 10 15
Gly

<210> 14
<211> 6
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..6
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 14
Tyr Tyr Ala Val Gly Tyr
1 5

<210> 15
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 15
Gly Phe Thr Phe Ser Asp Tyr Gly Met His
1 5 10

<210> 16
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17

<223> /mol_type="protein"
/organism="Mus musculus"

<400> 16
Tyr Ile Ser Ser Gly Ser Ser Thr Ile Tyr Tyr Gly Asp Thr Val Lys
1 5 10 15
Gly

<210> 17
<211> 11
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..11
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 17
Arg Gly Gln Leu Arg Leu Arg Leu Phe Ala Tyr
1 5 10

<210> 18
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 18
Gly Phe Thr Phe Thr Asp Tyr Tyr Met Ser
1 5 10

<210> 19
<211> 19
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..19
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 19
Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Thr Ala Ser
1 5 10 15
Val Lys Gly

<210> 20

<211> 8
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..8
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 20
Ala Leu Gly Arg Tyr Phe Asp Val
1 5

<210> 21
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 21
Lys Ser Ser Gln Ser Val Phe Asn Ser Gly Asn Gln Lys Asn Ser Leu
1 5 10 15
Ala

<210> 22
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 22
Phe Ala Ser Thr Arg Glu Ser
1 5

<210> 23
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 23
Gln Glu His Tyr Thr Thr Pro Pro Thr
1 5

<210> 24
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 24
Arg Ser Ser Gln Arg Leu Val His Ser His Gly Lys Thr Tyr Leu His
1 5 10 15

<210> 25
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 25
Lys Val Ser Asn Arg Phe Ser
1 5

<210> 26
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 26
Ser Gln Thr Ala His Phe Pro Tyr Thr
1 5

<210> 27
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16

<223> /mol_type="protein"
/organism="Mus musculus"

<400> 27
Arg Ser Ser Gln Ser Leu Val His Ser His Gly Lys Thr Tyr Leu His
1 5 10 15

<210> 28
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 28
Arg Ser Ser Gln Ser Leu Leu His Ser His Gly Asn Thr Tyr Leu His
1 5 10 15

<210> 29
<211> 11
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..11
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 29
Lys Ala Ser Gln Asp Val Ser Thr Ala Val Ala
1 5 10

<210> 30
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 30
Ser Ala Ser Tyr Arg Tyr Thr
1 5

<210> 31
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 31
Gln Gln His Tyr Thr Thr Pro Leu Thr
1 5

<210> 32
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 32
Lys Ser Ser Gln Ser Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 33
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 33
Trp Ala Ser Thr Arg Glu Ser
1 5

<210> 34
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 34
Gln Gln Tyr Tyr Ser Tyr Pro Phe Thr
1 5

<210> 35
<211> 345
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..345
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 35
gaggtccagc tgcaacaatc tggacctgaa ctggtgaagc ctggggcttc agtgaagata 60

tcctgtaagg cttctggata tacgttcact gactactaca tgaactgggt gaagcagagc 120

catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt tcaagggcaa ggccacgttg actgtagaca agtcctccag cacagcctac 240

atggaactcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 36
<211> 345
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..345
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 36
gaggtccagc tgcaacaatc tggacctgaa ctggtgaagc ctgggacttc agtgaagata 60

tcctgtaagg cttctggata tacgttcact gactactaca tgaactgggt gaagcagagc 120

catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt ttaagggcaa ggccacgttg actgtagaca agtcctccag cacagcctac 240

atggaactcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 37
<211> 345
<212> DNA
<213> Mus musculus

<220>
<221> source

<222> 1..345
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 37
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tcctgtaagg cttctggata cacgttcact gactactaca tgaactgggt gaagcagagc 120

catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt tcaagggcaa ggccacgttg actgtagaca cgtcctccag cacagcctac 240

atggagctcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 38
<211> 360
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..360
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 38
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tcctgtgcac cctctggatt cactttcagt gactatggaa tgcactgggt tcgtcaggct 120

ccagagaagg gactggagtg ggttgcatac attagtagtg gcagtagtac catctactat 180

ggagacacag tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgttc 240

ctgcaaatga ccagtctgag gtctgaggac acggccatgt attactgtgc aagaagggga 300

cagctcaggc tacgcctgtt tgcttactgg ggccaaggga ctctggtcac tgtctctgca 360

<210> 39
<211> 357
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..357
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 39
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tactgtgcag cttctggatt cacctttact gattactaca tgagctgggt ccgccagcct 120

ccagggaagg cacctgagtg gttggctttg attagaaaca aagctaatgg ttacacaaca 180

gagtatactg catctgttaa gggtcggttc accatctcca gagataattc ccaaaacatc 240

ctctatcttc aaatgaacac cctgagggct gaggacagtg ccacttatta ctgtgtaaaa 300

gctctgggac gttacttcga tgtctggggc acagggacca cggtcaccgt ctcctca 357

<210> 40
<211> 339
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..339
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 40
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atgagctgca agtccagtca gagtgttttt aatagtggca atcaaaagaa ctctttggcc 120

tggtaccagc agaaaccagg acagtctcct aaacttctgg tatactttgc atccactagg 180

gaatctgggg tccctgatcg cttcataggc agtggatctg ggacagattt cagtcttacc 240

atcagcagtg tgcaggctga ggacctggca gattacttct gtcaggaaca ttataccact 300

cctcccacgt tcggtactgg gaccaagctg gagctgaaa 339

<210> 41
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 41
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atctcttgca gatctagtca gaggcttgta cacagtcatg gaaaaaccta tttacattgg 120

tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tatttctgtt ctcaaactgc acattttccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 42
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 42
gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca gatctagtca gagccttgta cacagtcatg gaaaaaccta tttacattgg 120

tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tatttctgtt ctcaaactgc acattttccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 43
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 43
gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca gatctagtca gagccttcta cacagtcatg gaaacaccta tttacattgg 120

tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

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tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 44
<211> 321
<212> DNA

<213> Mus musculus

<220>
<221> source
<222> 1..321
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 44
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atcacctgca aggccagtca ggatgtgagt actgctgtag cctggtatca acagaaacca 120

ggacaatctc ctaaactact gatttactcg gcatcctacc ggtacactgg agtccctgat 180

cgcttcactg gcagtggatc tgggacggat ttcactttca ccatcagcag tgtgcaggct 240

gaagacctgg cagtttatta ctgtcagcaa cattatacta ctccgctcac gttcggtgct 300

gggaccaagc tggagctgaa a 321

<210> 45
<211> 339
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..339
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 45
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atgagctgca agtccagtca gagcctttta tatagtagca atcaaaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tttactgggc atccactagg 180

gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc 240

atcagcagtg tgaaggctga agacctggca gtttattact gtcagcaata ttatagctat 300

ccattcacgt tcggctcggg gacaaagttg gaaataaaa 339

<210> 46
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..34
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"

/organism="Artificial Sequence"

<400> 46
gggaattcat graatgsasc tgggtywtyc tctt 34

<210> 47
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..35
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<220>
<221> variation
<222> 30..30
<223> /replace="n=i"

<400> 47
cccaagcttc cagggrccar kggataracn grtgg 35

<210> 48
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..32
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 48
gggaattcat gragwcacak wcycaggtct tt 32

<210> 49
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..41
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 49
actagtcgac atgggcwtca agatgragtc acakwyycwg g 41

<210> 50
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..39
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 50
actagtcgac atgaagttgc ctgttaggct gttggtgct 39

<210> 51
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..30
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 51
cccaagctta ctggatggtg ggaagatgga 30

<210> 52
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..36
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 52
actagtcgac atgggatgga gctrtatcat sytctt 36

<210> 53
<211> 33
<212> DNA
<213> artificial seuqence

<220>
<221> source

<222> 1..33
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="artificial seuqence"

<400> 53
gggaattcat grasttskgg ytmarctkgr ttt 33

<210> 54
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..39
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 54
actagtcgac atggactcca ggctcaattt agttttcct 39

<210> 55
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..33
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 55
actagtcgac atgaagwtgt ggbtraactg grt 33

<210> 56
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..39
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<220>
<221> misc_feature
<222> 27..27
<223> /note="n=i"

<400> 56
actagtcgac atggagwcag acacacnsct gytatgggt 39

<210> 57
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<222> 1..37
<223> /mol_type="DNA"
/note="Description of artificial sequence: primer"
/organism="Artificial Sequence"

<400> 57
actagtcgac atggtyctya tvttrctgct gctatgg 37

<210> 58
<211> 30
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..30
<223> /mol_type="protein"
/note="Description of artifiial sequence: ACI-37"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 18..18
<223> phosphorylated serine

<220>
<221> VARIANT
<222> 26..26
<223> phosphorylated serine

<400> 58
Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly Ala Glu Ile Val Tyr
1 5 10 15
Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu
20 25 30

<210> 59
<211> 16
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..16

<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-33"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 14..14
<223> phosphorylated serine

<400> 59
Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu
1 5 10 15

<210> 60
<211> 16
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-39"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 7..7
<223> phosphorylated threonine

<220>
<221> VARIANT
<222> 9..9
<223> phosphorylated serine

<400> 60
Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg
1 5 10 15

<210> 61
<211> 16
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-39"
/organism="Artificial Sequence"

<220>
<221> VARIANT
<222> 7..7
<223> phosphorylated serine

<220>

<221> VARIANT
<222> 10..10
<223> phosphorylated threonine

<400> 61
Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg
1 5 10 15

<210> 62
<211> 16
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-35"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 4..4
<223> phosphorylated serine

<220>
<221> VARIANT
<222> 12..12
<223> phosphorylated serine

<400> 62
Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu
1 5 10 15

<210> 63
<211> 18
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..18
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-36"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 4..4
<223> phosphorylated serine

<220>
<221> VARIANT
<222> 9..9
<223> phosphorylated serine

<400> 63

Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser
1 5 10 15
Ile Asp

<210> 64
<211> 17
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-34"
/organism="artificial sequences"

<220>
<221> VARIANT
<222> 3..3
<223> phosphorylated serine

<220>
<221> VARIANT
<222> 6..6
<223> phosphorylated threonine

<220>
<221> VARIANT
<222> 13..13
<223> phosphorylated threonine

<220>
<221> VARIANT
<222> 15..15
<223> phosphorylated serine

<400> 64
Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu
1 5 10 15
Pro

<210> 65
<211> 12
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..12
<223> /mol_type="protein"
/note="Description of artificial sequence: ACI-42"
/organism="artificial sequences"

<220>
<221> VARIANT

<222> 3..3
<223> phosphorylated serine

<400> 65
His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp
1   5   10

<210> 66
<211> 10
<212> PRT
<213> artificial sequences

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
      /note="Description of artificial sequence: ACI-43"
      /organism="artificial sequences"

<220>
<221> VARIANT
<222> 6..6
<223> phosphorylated serine

<400> 66
Val Ser Gly Asp Thr Ser Pro Arg His Leu
1   5   10

<210> 67
<211> 441
<212> PRT
<213> Homo sapiens

<220>
<221> SOURCE
<222> 1..441
<223> /mol_type="protein"
      /organism="Homo sapiens"

<400> 67
Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly
1   5   10   15
Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Gly Tyr Thr Met His
            20   25   30
Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Glu Ser Pro Leu
        35   40   45
Gln Thr Pro Thr Glu Asp Gly Ser Glu Glu Pro Gly Ser Glu Thr Ser
    50   55   60
Asp Ala Lys Ser Thr Pro Thr Ala Glu Asp Val Thr Ala Pro Leu Val
65   70   75   80
Asp Glu Gly Ala Pro Gly Lys Gln Ala Ala Ala Gln Pro His Thr Glu
                85   90   95
Ile Pro Glu Gly Thr Thr Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro
            100   105   110
Ser Leu Glu Asp Glu Ala Ala Gly His Val Thr Gln Ala Arg Met Val
        115   120   125
Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly

130 135 140
Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro
145 150 155 160
Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro
165 170 175
Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly
180 185 190
Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
195 200 205
Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys
210 215 220
Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys
225 230 235 240
Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val
245 250 255
Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly
260 265 270
Gly Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln
275 280 285
Ser Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly
290 295 300
Ser Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser
305 310 315 320
Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln
325 330 335
Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser
340 345 350
Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn
355 360 365
Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala
370 375 380
Lys Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser
385 390 395 400
Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser
405 410 415
Ile Asp Met Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val
420 425 430
Ser Ala Ser Leu Ala Lys Gln Gly Leu
435 440

<210> 68
<211> 117
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..117
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 68
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Leu Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30
Trp Met His Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile

```
        35                  40                  45
Gly Arg Ile Asp Pro Asn Ser Asp Arg Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60
Lys Arg Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Asp Asp Tyr Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ala
        115
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<210> 69
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 69

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Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
            20                  25                  30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Lys Leu Leu Ile Tyr Lys Leu Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95
Ser His Val Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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<210> 70
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 70

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Gly Tyr Thr Phe Thr Ser Tyr Trp Met His
1               5                   10
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<210> 71
<211> 17
<212> PRT

<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 71
Arg Ile Asp Pro Asn Ser Asp Arg Thr Lys Tyr Asn Glu Lys Phe Lys
1 5 10 15
Arg

<210> 72
<211> 8
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..8
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 72
Asp Asp Tyr Ala Trp Phe Ala Tyr
1 5

<210> 73
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 73
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1 5 10 15

<210> 74
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 74
Lys Leu Ser Asn Arg Phe Ser

1 5

<210> 75
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 75
Phe Gln Gly Ser His Val Pro Pro Thr
1 5

<210> 76
<211> 119
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..119
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 76
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
35 40 45
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Ala Ser
50 55 60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val
65 70 75 80
Phe Leu Lys Ile Thr Cys Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg Leu Leu Arg Pro Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
100 105 110
Thr Ser Val Thr Val Ser Ser
115

<210> 77
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 77
Asn Ile Leu Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30
Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Leu Gln
85 90 95
Tyr Leu Ser Ser Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 78
<211> 12
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..12
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 78
Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Ser
1 5 10

<210> 79
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 79
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Ala Ser Leu Lys Ser
1 5 10 15

<210> 80
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 80
Leu Leu Arg Pro Tyr Ala Leu Asp Tyr
1 5

<210> 81
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 81
Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 82
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 82
Trp Ala Ser Thr Arg Glu Ser
1 5

<210> 83
<211> 8
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..8
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 83
Leu Gln Tyr Leu Ser Ser Leu Thr
1 5

<210> 84
<211> 351
<212> DNA
<213> Mus musculus

<220>

<221> source
<222> 1..351
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 84
caggtccaac tgcagcagcc tggggctgag cttctgaagc ctggggcttc agtgaaactg 60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg 120

cctggacgag gccttgagtg gattggaagg attgatccta atagtgatcg tactaagtac 180

aatgagaagt tcaagcgcaa ggccacactg actgtagaca aatcctccag cacagcctac 240

atgcagctca gcagcctgac atctgaggac tctgcggtct attattgtgc aagggatgat 300

tacgcctggt ttgcttactg gggccaaggg actctggtca ctgtctctgc a 351

<210> 85
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 85
gatgttttga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca gatctagtca gagcattgta catagtaatg gaaacaccta tttagaatgg 120

tacctgcaga aaccaggcca gtctccaaag ctcctgatct acaaactttc caaccgattt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tattactgct ttcaaggttc acatgttcct 300

ccgacgttcg gtggaggcac caagctggaa atcaaa 336

<210> 86
<211> 357
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..357
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 86
caggttactc tgaaagagtc tggccctggg atattgcagt cctcccagac cctcagtctg 60

acttgttctt tctctgggtt ttcactgagc acttctggta tgggtgtgag ctggattcgt 120

cagccttcag gaaagggtct ggagtggctg gcacacattt actgggatga tgacaagcgc 180

tataacgcat ccctgaagag ccggctcaca atctccaagg atacctccag aaaccaggta 240

ttcctcaaga tcacctgtgt ggacactgca gatactgcca catactactg tgctcggtta 300

ctgcgtcctt atgctttgga ctactggggt caaggaacct cagtcaccgt ctcctca 357

<210> 87
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organism="Mus musculus"

<400> 87
aacattttga tgacacagtc gccatcatct ctggctgtgt ctgcaggaga aaaggtcact 60

atgagctgta agtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcacaggc agtggatctg ggacagattt tactcttacc 240

atcagcagtg tacaagctga agacctggca gtttattact gtcttcaata cctctcctcg 300

ctcacgttcg gtgctgggac caagctggag ctgaaa 336

<210> 88
<211> 115
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..115
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 88
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45
Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Val Arg Glu Gly Arg Phe Ala Tyr Trp Gly His Gly Thr Leu Val Thr
100 105 110
Val Ser Ala
115

<210> 89
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 89
Gly Phe Thr Phe Thr Asp Tyr Tyr Met Asn
1 5 10

<210> 90
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 90
Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15
Gly

<210> 91
<211> 6
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..6
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 91
Glu Gly Arg Phe Ala Tyr
1 5

<210> 92

<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SITE
<222> 3..3
<223> Xaa is Met or Val

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 92
Asp Ile Xaa Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15
Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30
Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95
Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 93
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 93
Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 94
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="protein"
/organism="Mus musculus"

<400> 94
Arg Met Ser Asn Leu Ala Ser
1 5

<210> 95
<211> 9
<212> PRT
<213> Mus musculus

<220>
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35 40 45
Ala Arg Ile Tyr Pro Gly Arg Gly Asn Ile Tyr Tyr Asn Glu Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Glu Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
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20 25 30
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35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
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85 90 95
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100 105 110

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20 25 30
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35 40 45
Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr His Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
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85 90 95
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35 40 45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
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catggaaaga gccttgagtg gattggagat attaatccta acaatggtgg tactagctac 180

aaccagaagt tcaagggcaa ggccacattg actgtagaca agtcctccag cacagcctac 240

atggagctcc gcagtctgac atctgaggac tctgcagtct attattgtgt aagagagggg 300

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ttcctgcaga ggccaggcca gtctcctcag ctcctgatat atcggatgtc caaccttgcc 180

tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

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cctggacagg gacttgagtg gattgcaagg atttatcctg gaagaggtaa tatttactac 180

aatgagaagt tcaagggcaa ggccacactg actgcagaaa aatcctccag cactgcctac 240

atgcagctca gcagcctgac atctgaggac tctgctgtct atttctgtgc aagattctgg 300

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ttcctgcaga aaccaggcca gtctccaaag ctcctgatct acaaagtttc caaccgattt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

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caccagaagt tcaagggcaa ggccacattg actgtagaca agtcctccag cacagcctac 240

atggagctcc gcagcctgac atctgaggac tctgcagtct attactgtgt aagagaggga 300

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cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcagcaa tgtgcagtct 240

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Gly

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<223> /mol_type="protein"
/organism="Mus musculus"

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Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile His Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile

65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95
Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

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tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

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20 25 30
Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
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Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

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tcaggagtcc cagacaggtt cagtggcagt gggtcaggaa ctgctttcac actgagaatc 240

agtagagtgg aggctgagga tgtgggtgtt tattactgta tgcaacatct aaaatctccg 300

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(71) Applicants *(for all designated States except US)*: **AC IMMUNE S.A.** [CH/CH]; EPFL-PSE Building B, CH-1015 Lausanne (CH). **K. U. LEUVEN RESEARCH & DEVELOPMENT** [BE/BE]; Waaistraat 6, Box 5105, B-3000 Leuven (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PFEIFER, Andrea** [DE/CH]; Route de Fenil 16A, CH-1806 St-Légier (CH). **MUHS, Andreas** [DE/CH]; Avenue des Cerisiers 39b, CH-1009 Pully (CH). **VAN LEUVEN, Fred** [BE/BE]; Hertogenlaan 18, B-3210 Linden (BE). **PIHLGREN, Maria** [SE/CH]; Chemin du Tessin 6B, CH-1052 Mont-sur-Lausanne (CH). **ADOLFSSON, Oskar** [IS/CH]; Chemin de Larry 16, CH-1038 Bercher (CH).

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(54) Title: PHARMACEUTICAL COMPOSITION

(57) Abstract: The present invention relates to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

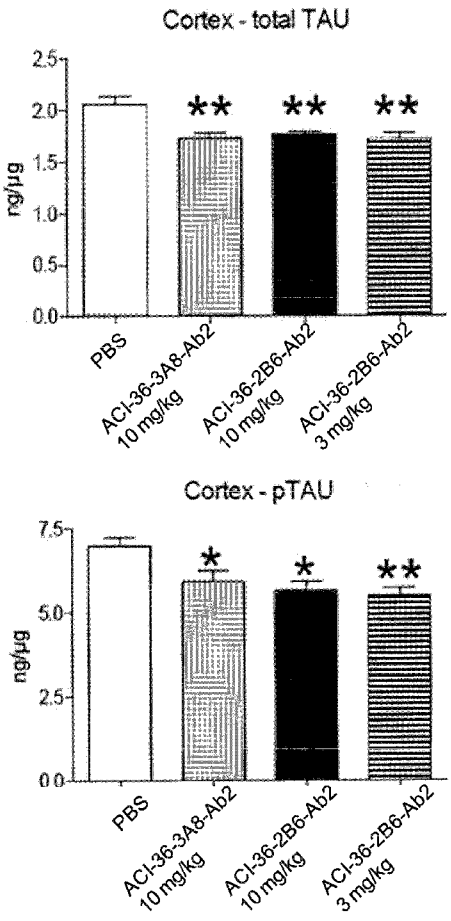


FIGURE 3

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(74) **Agent**: **WERNER BASTIAN**; Vossius & Partner, Siebertstrasse 4, 81675 München (DE).

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PHARMACEUTICAL COMPOSITION

The present invention is related to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

Neurofibrillary tangles and neuropil threads (NTs) are the major neuropathological hallmarks of Alzheimer's Disease (AD). They are composed of the microtubule-associated protein tau that has undergone posttranslational modifications, including phosphorylation, deamidation and isomerization on asparaginyl or aspartyl residues. They originate by the aggregation of hyper-phosphorylated protein tau and its conformers. AD shares this pathology with many neurodegenerative tauopathies, in particularly with specified types of frontotemporal dementia (FTD).

Protein Tau is a freely soluble, "naturally unfolded" protein that binds avidly to microtubuli (MT) to promote their assembly and stability. MTs are of major importance for the cytoskeletal integrity of neurons - and thereby for the proper formation and functioning of neuronal circuits, hence for learning and memory. The binding of tau to MT is controlled by dynamic phosphorylation and de-phosphorylation, as demonstrated mainly *in vitro* and in non-neuronal cells. Due to the large number of possible phosphorylation sites (>80), the exact contribution of each and the identity of the responsible kinases remain largely undefined *in vivo*.

In AD brain, tau pathology develops later than, and therefore probably in response to amyloid pathology, which constitutes the essence of the amyloid cascade hypothesis. This is based on and indicated by studies in AD and Down syndrome patients, and is corroborated by studies in transgenic mice with combined amyloid and tau pathology (Lewis et al., 2001; Oddo et al., 2004; Ribe et al., 2005; Muyllaert et al, 2006; 2008; Terwel et al, 2008).

The exact timing of both pathologies in human AD patients as well as mechanisms that link amyloid to tau pathology remain largely unknown, but are proposed to involve activation of

neuronal signaling pathways that act on or by GSK3 and cdk5 as the major "tau-kinases" (reviewed by Muyllaert et al, 2006, 2008).

The hypothesis that tauopathy is not an innocent side-effect but a major pathological executor in AD is based on sound genetic, pathological and experimental observations that corroborate each other fully:

- in early-onset familial AD cases that are due to mutations in amyloid protein precursor (APP) or presenilin, the obligate pathogenic cause is amyloid accumulation, but invariably the pathology comprises collateral tauopathy, identical to that in the late-onset sporadic AD cases;
- severity of cognitive dysfunction and dementia correlates with tauopathy, not with amyloid pathology, exemplified most recently by several clinical phase-1&2 studies that include PIB-PET imaging for amyloid and identify many “false positives”: cognitively normal individuals with high brain amyloid load;
- in familial FTD, the tauopathy is provoked by mutant tau and causes neurodegeneration directly, without amyloid pathology;
- in experimental mouse models the cognitive defects caused by amyloid pathology are nearly completely alleviated by the absence of protein tau (Roberson et al, 2007).

The combined arguments support the hypothesis that protein tau is a major player in the cognitive demise in AD and related neurodegenerative tauopathies.

A prominent emerging treatment of AD is by passive immunotherapy with specific mAbs, to clear amyloid peptides and their aggregates that are presumed to be neuro-toxic or synapto-toxic.

Immunotherapy targeting tau pathology, as proposed here, is anticipated to counteract the pathological protein tau-conformers that are known or postulated to cause synaptic dysfunction and neurodegeneration. Amyloid pathology caused and intra-neuronal aggregates of hyper-phosphorylated protein tau are proposed to act synergistically in the cognitive and degenerative cascade of pathological events that lead from mild cognitive impairment (MCI) to the severe dementia of AD. The combination of tau-directed medication with amyloid-directed (or any other) medication will therefore constitute the preferred and, substantially more efficacious treatment of AD, as opposed to current mono-therapy.

Other therapeutic approaches that target protein tau are scarce and comprise mainly:

- inhibitors of the kinases that are thought to increase the phosphorylation of tau to pathological levels
- compounds that block the cytoplasmic aggregation of hyper-phosphorylated protein tau.

These approaches suffer various draw-backs of specificity and efficacy, a problem they share with attempts to modify the metabolism of APP and amyloid, all emphasizing the importance of a continuous search for additional treatment options, including immunotherapy against tau.

Practically no efforts have been devoted to define - let alone target - the pathological tau conformers *in vivo*. In the Aβ42 phase II clinical trial, the tangle pathology did not appear to be well considered nor analyzed in much depth (Nicoll et al., 2003; Masliah et al., 2005). On the other hand, experimental immunotherapy targeting amyloid in a preclinical mouse model with combined AD-like pathology demonstrated also an effect on tau pathology although tau aggregates persisted (Oddo et al., 2004).

Some doubts have been cast on the feasibility of approaching intra-cellular protein tau by immunotherapy. These have been countered by the most recent experimental study in a tauopathy mouse model (Asuni et al., 2007). They showed reduction in tangle pathology and functional improvements by vaccination with a protein tau derived phospho-peptide. These data corroborate previous reports of immunotherapy targeting α-synuclein in Parkinson's Disease (PD) and Lewy body disease models (Masliah et al., 2005, 2011) and of superoxide dismutase in an amyotrophic lateral sclerosis (ALS) model (Urushitiani et al., 2007). These diseases are examples whereinintra-cellular proteins lead to synaptic defects and neurodegeneration by as yet not fully understood mechanisms. On the other hand, full-length recombinant protein tau produced in and isolated from bacteria appears not suitable as vaccine, although the adjuvants used, i.e. complete Freunds and pertussis toxin, could have contributed to the negative outcome of that study (Rosenmann et al., 2006).

There is an unmet need for passive and/or active immunotherapies that work to counteract the pathological protein conformers that are known - or presumed - to cause neurodegenerative disorders, such as amyloid pathology in AD caused, for example, by intra-neuronal aggregates of hyper-phosphorylated protein tau that are as typical for AD as amyloid.

This unmet need could be met within the scope of the present invention by providing binding proteins recognizing and binding to major pathological phospho-epitopes of the tau protein. In particular, the present invention provides specific antibodies against linear and

conformational, simple and complex phospho-epitopes on protein tau, particularly on aggregated tau protein that are believed to be responsible for synapto- and neuro-toxicity in tauopathies, including AD.

Accordingly, the present invention relates in one embodiment to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, which binding peptide or protein or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a phospho-epitope on aggregated Tau protein, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity to soluble and insoluble Tau protein, and modulates soluble and insoluble Tau levels, particularly in the brain, particularly with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM, particularly of at least 400 pM, particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM.

In a second embodiment, the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly of between 3 - 5 x 10^4 $M^{-1}s^{-1}$ or greater, particularly of 10^5 $M^{-1}s^{-1}$ or greater; particularly of 2 – 9 x 10^5 $M^{-1}s^{-1}$ or greater; particularly of 10^6 $M^{-1}s^{-1}$ or greater, particularly of 1 – 4 x 10^6 $M^{-1}s^{-1}$ or greater, particularly of 10^7 $M^{-1}s^{-1}$ or greater.

In a third embodiment, the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity with a dissociation

constant of at least 4 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 3 nM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 2 nM and an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 1 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 200 pM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater, particularly a dissociation constant of at least 100 pM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

One embodiment (4) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody binds to an epitope on a mammalian, particularly on the human Tau protein as shown in SEQ ID NO: 67, selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); and Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position 214 (pS214).

One embodiment (5) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 15-20 with a phosphorylated Tyr at position 18 (Y18).

One embodiment (6) relates to the binding peptide or antibody of any o the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 405-412 with a phosphorylated Ser at position 409 (pS409).

One embodiment (7) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 405-411 with a phosphorylated Ser at position 409 (pS409).

One embodiment (8) relates to the binding peptide or antibody of any of the preceding embodiments, wherein said peptide binds to an epitope on a mammalian, particularly on the human Tau protein, but especially the human Tau protein as shown in SEQ ID NO: 67, comprising Tau aa 208-218 with a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position 214 (pS214).

In another embodiment (9), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains, particularly in sequence, a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, or an amino acid sequence at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, or an amino acid sequence at least 60%, particularly at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, 89, 98, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, 115, or an amino acid sequence at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least

97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, 91, 100, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

One embodiment (10) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, or an amino acid sequence at least 85% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, or an amino acid sequence at least 80% identical thereto; and/or an antibody domain which contains a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, or an amino acid sequence at least 85% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, or an amino acid sequence at least 85% identical thereto.

One embodiment (11) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, or an amino acid sequence at least 90% identical thereto; a CDR2 with the

amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, or an amino acid sequence at least 90% identical thereto

One embodiment (12) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain (antibody domain) which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, or an amino acid sequence at least 90% identical thereto.

One embodiment (13) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in

sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

One embodiment (14) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 98% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 98% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

One embodiment (15) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29,

32, 73, 81, 93, 101, or 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, or 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, or 108, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, 89, or 98, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, or 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, 91, or 100.

One embodiment (16) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, or an amino acid sequence at least 76%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, or an amino acid sequence at least 66%, particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66%, particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto.

One embodiment (17) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a

functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 24, or SEQ ID NO: 27, or SEQ ID NO: 28, or an amino acid sequence at least 88%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 25, or an amino acid sequence at least 95%, particularly 98%, particularly 99% and a CDR3 with the amino acid sequence shown in SEQ ID NO: 26, or an amino acid sequence at least 66%, particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66% , particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto.

One embodiment (18) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (15) comprising a first binding domain, wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 27, or an amino acid sequence at least 88% identical thereto.

One embodiment (19) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (15), comprising a first binding domain wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 28, or an amino acid sequence at least 88% identical thereto.

One embodiment (20) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 29, a CDR2 with the amino acid sequence shown in SEQ ID NO: 30, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 31, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 15, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 16, or an amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 17, or an amino acid sequence at least 36%, particularly at least 40%, particularly at least 50%, particularly at least 60%, particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto.

One embodiment (21) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 32, a CDR2 with the amino acid sequence shown in SEQ ID NO: 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 34, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 18, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2

with the amino acid sequence shown in SEQ ID NO: 19, or an amino acid sequence at least 95%, 96%, 97%, 98% or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 20, or an amino acid sequence at least 63%, particularly at least 70%, particularly at least 75%, particularly at least 80%, particularly at least 85%, particularly at least 90%, particularly at least 95%, particularly at least 98%, particularly at least 99% identical thereto.

One embodiment (22) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 73, a CDR2 with the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 75, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 70, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 71, or an amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 72, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

One embodiment (23) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does

not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 81, a CDR2 with the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 80, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

One embodiment (24) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 93, a CDR2 with the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 95, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least

97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

One embodiment (25) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 101, a CDR2 with the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 103, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 98, a CDR2 with the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 100, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

One embodiment (26) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 108, or an amino acid sequence at least 60%, at least

70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

In another embodiment (27), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 85% identical thereto.

In one embodiment (28), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

One embodiment (29) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain (antibody domain) which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

In another embodiment (30), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69, 77, 116/92, 97, 105, or an amino acid sequence particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68, 76, 88, 96, 104, or an amino acid sequence at least 80%, particularly at least 85%, particularly at least 86%, particularly at least 87%, particularly at least 88%, particularly at least 89%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

One embodiment (31) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment

thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence at least 90% and 94%, respectively, identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, or an amino acid sequence at least 91% identical thereto.

One embodiment (32) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto.

One embodiment (33) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 9, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3, or an amino acid sequence at least 90% identical thereto.

One embodiment (34) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does

not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4, or an amino acid sequence at least 89% identical thereto.

One embodiment (35) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11, or an amino acid sequence at least 98% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5, or an amino acid sequence at least 87% identical thereto.

One embodiment (36) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69, or an amino acid sequence at least 98% or 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

One embodiment (37) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment

thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 77, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 76, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

One embodiment (38) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 116, 92, or 118, or an amino acid sequence at least 93%, 94% or 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88, or an amino acid sequence at least 90%, 91%, 92% or 93% identical thereto.

One embodiment (39) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 97, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 96, or an amino acid sequence at least 86%, 87%, 88% or 90% identical thereto.

One embodiment (40) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a

phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 105, or an amino acid sequence at least 98%, or 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 104, or an amino acid sequence at least 88%, 89%, or 90% identical thereto.

In another embodiment (41), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiments (22) – (24), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 21-34 and said second binding domain contains the CDRs as shown in SEQ ID NOs: 12-20.

One embodiment (42) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (31), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 21-23 and SEQ ID NOs: 24-26, respectively, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 12-14.

One embodiment (43) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (32), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 27, 25, 26 and said second binding domain contains the CDRs as shown in SEQ ID NOs: 12-14.

One embodiment (44) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (33), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 28, 25 and 26, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 12-14.

One embodiment (45) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (34), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 29-31, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 15-17.

One embodiment (46) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (35), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 32-34, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 18-20.

One embodiment (47) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (27), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 73-75, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 70-72.

One embodiment (48) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (27), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 81-83, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 78-80.

One embodiment (49) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (27), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 101-103, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 98-100.

One embodiment (50) of the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, according to embodiment (27), wherein said first binding domain contains the CDRs as shown in SEQ ID NOs: 89, 115, and 91, and said second binding domain contains the CDRs as shown in SEQ ID NOs: 106-108.

In still another embodiment (51), the present invention relates to a binding peptide or protein or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, particularly a binding peptide or antibody of any of the preceding embodiments, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes wherein said binding peptide or antibody comprises a

a. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or a

b. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 7 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or a

c. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2; or a

d. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 9 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3; or a

e. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4; or a

f. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5; or a

g. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68; or a

h. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 77 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 76; or a

i. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 116 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a;

j. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 92 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a

k. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 97 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 6; or a

l. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 105 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 104.

In one embodiment (52) of the invention, the binding peptide of any of the preceding embodiments is an antibody, particularly an antibody of the IgG2a, IgG2b or the IgG3 isotype, particularly a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody.

One embodiment (48) of the invention relates to a polynucleotide encoding the binding peptide of any one of the preceding embodiments.

In on embodiment (53), said polynucleotide comprises a nucleic acid molecule selected from the group consisting of

a. a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as depicted in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

b. a nucleic acid molecule comprising a nucleotide sequence that has at least 85% sequence identity to the sequence shown in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

c. a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the sequence shown in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

d. a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the sequence shown in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

e. a nucleic acid molecule comprising a nucleotide sequence that has at least 98% sequence identity to the sequence shown in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

f. a nucleic acid molecule comprising a nucleotide sequence that has at least 99% sequence identity to the sequence shown in SEQ ID NOs: 35-45, SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

g. a nucleic acid molecule comprising a nucleotide sequence the complementary strand of which hybridizes to the nucleic acid molecule of any of a) – f);

h. a nucleic acid molecule comprising a nucleotide sequence that deviates from the nucleotide sequence defined in any of a) – g) by the degeneracy of the genetic code,

wherein said nucleic acid molecule as defined in any of a) – h) recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly on the human Tau protein as shown in SEQ ID NO: 67, selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); and Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position 214 (pS214), Tau aa 393-401, comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396), wherein, in one embodiment, said binding peptide has a high binding affinity with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM, particularly of at least 400 pM, particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM and/or has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater, particularly of between 3 - 5 x 10^4 $M^{-1}s^{-1}$ or greater, particularly of 10^5 $M^{-1}s^{-1}$ or greater; particularly of 6 – 9 x 10^5 $M^{-1}s^{-1}$ or greater; particularly of 10^6 $M^{-1}s^{-1}$ or greater, particularly of 1 – 4 x 10^6 $M^{-1}s^{-1}$ or greater, particularly of 10^7 $M^{-1}s^{-1}$ or greater, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

In various embodiments (54) of the invention, a binding peptide is provided or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, according to any one of the preceding embodiments, or a combination thereof, which is capable of specifically recognizing and binding to a phospho-epitope on a mammalian, particularly on the human Tau protein, particularly a microtubule-associated protein tau, particularly an aggregated microtubule-associated and hyperphosphorylated protein tau such as that present in paired helical filaments (PHF), which are the predominant structures in neurofibrillary tangles, neuropil threads and dystrophic neurites, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

In a specific embodiment (55) of the invention, the human tau protein is the human Tau protein as shown in SEQ ID NO: 67.

The binding peptides and antibodies according to any one of the preceding embodiments can thus be used (56) for reducing the levels of total soluble tau protein, particularly of soluble phosphorylated tau protein, in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of soluble tau protein and/or soluble phosphorylated tau protein.

The binding peptides and antibodies according to any one of the preceding embodiments can also be used (57) for reducing the levels of paired helical filaments containing hyperphosphorylated tau protein (pTau PHF) in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of said pTau paired helical filaments (pTau PHF).

Reduction of the level of total soluble tau protein and/or soluble phosphorylated tau protein and/or pTau paired helical filaments (pTau PHF) in the brain, particularly in the brain cortex and/or hippocampus, of a mammal or a human containing increased levels of said tau protein variants, which contribute to tau-protein-associated diseases, disorders or conditions in said mammal or human, may lead to an improvement and/or alleviation of the symptoms associated with such tau-protein-associated diseases, disorders or conditions (58).

The binding peptides and antibodies according to any one of the preceding embodiments can therefore be used (59) in therapy, particularly in human therapy, for slowing or halting the progression of a tau-protein-associated disease, disorder or condition.

The binding peptides and antibodies according to any one of the preceding embodiments can further be used (60) in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc.

In one embodiment (61), the invention relates to the binding peptides and antibodies according to any one of the preceding embodiments for use in therapy, particularly for use in the treatment of tauopathies, a group of tau-protein-associated diseases and disorders, or for alleviating the symptoms associated with tauopathies.

In one embodiment (62), the invention relates to the binding peptides and antibodies according to any one of the preceding embodiments for retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

In a specific embodiment (63) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-3A8-Ab1, and ACI-36-3A8-Ab2 as given in SEQ ID NOs: 25, 26, 27, and SEQ ID NOs: 21, 22, 23, respectively, and/or at least one or all of the heavy chain CDRs of antibodies ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-3A8-Ab1, and ACI-36-3A8-Ab2 as given in SEQ ID NOs: 12, 13, 14, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (64) of the invention, the antibodies comprising the light chain of antibodies ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-3A8-Ab1, and ACI-36-3A8-Ab2 as given in SEQ ID NO: 8_and SEQ ID NOs: 6, 7, respectively, and/or the heavy chain of antibodies ACI-36-2B6-Ab1, ACI-36-2B6-Ab12, ACI-36-3A8-Ab1, and ACI-36-3A8-Ab2 as given in SEQ ID NO: 1 and SEQ ID NO: 2, respectively, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (65) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-33-6C10-Ab1 and ACI-33-6C10-Ab2 as given in SEQ ID NOs: 29, 30, 31, and/or at least one or all of the heavy chain CDRs of antibodies ACI-33-6C10-Ab1 and ACI-33-6C10-Ab2 as given in SEQ ID NOs: 15, 16, 17, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (66) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 as given in SEQ ID NOs: 32, 33, 34, and/or at least one or all of the heavy chain CDRs of antibodies ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 as given in SEQ ID NOs: 18, 19, 20, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (67) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-35-2A1-Ab1; ACI-35-2A1-Ab2; ACI-35-4A6-Ab1; ACI-35-4A6-Ab2; ACI-35-1D2-Ab1; ACI-35-2G5-Ab1; as given in SEQ ID NOs: 73-75, 81-83, 93-95, 101-103, 106-108 and/or at least one or all of the heavy chain CDRs of antibodies ACI-35-2A1-Ab1; ACI-35-2A1-Ab2; ACI-35-4A6-Ab1; ACI-35-4A6-Ab2; ACI-35-1D2-Ab1; ACI-35-2G5-Ab1; as given in SEQ ID NOs: 70-72, 78-80, 89-91, 98-100, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

In another specific embodiment (68) of the invention, binding peptides and antibodies comprising at least one or all of the light chain CDRs of antibodies ACI-35-2G5-Ab2; ACI-35-2G5-Ab3 as given in SEQ ID NOs: 106-108 and/or at least one or all of the heavy chain CDRs of antibodies ACI-35-2G5-Ab2; ACI-35-2G5-Ab3; as given in SEQ ID NOs: 89, 115 and 91, are used in therapy, particularly in human therapy, for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory, learning, special navigation, etc.

Binding of the peptides or antibodies according to the preceding embodiments to tau tangles and pTau on brains may be determined by applying protein immuno-reactivity testing of selected brain sections and by Western blotting of brain homogenates, respectively, as described in the Examples.

In another embodiment (69), the present invention provides a pharmaceutical composition comprising a binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, according to any one of the preceding embodiments, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

In one embodiment (70), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in therapy, particularly in human therapy for the treatment or alleviation of the symptoms of tau-protein-associated diseases or disorders including neurodegenerative disorders such as tauopathies.

The binding peptides, antibodies and/or pharmaceutical compositions according to any one of the preceding embodiments may thus be used (71) for slowing or halting the progression of a tau-protein-associated disease, disorder or condition, upon administration of said binding peptides, antibodies and/or pharmaceutical compositions to an animal, particularly a mammal, particularly a human, suffering from such a disease or condition.

The binding peptides, antibodies and/or pharmaceutical compositions according to any one of the preceding embodiments may further be used (72) for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc, upon administration of said binding peptides, antibodies and/or pharmaceutical compositions to an animal, particularly a mammal, particularly a human, suffering from such a disease or condition.

In one embodiment (73), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

In one embodiment (74), the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof, is used in the treatment of Alzheimer's Disease.

In one embodiment (75) of the invention, a method is provided for modulating soluble and/or insoluble Tau levels, particularly in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human, comprising administering to said animal, particularly to said mammal or human, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one aspect, modulation relates to reducing the levels of soluble tau protein, particularly of soluble phosphorylated tau protein, in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human containing increased levels of soluble tau protein and/or soluble phosphorylated tau protein.

In one embodiment (76) of the invention, a method is provided for reducing the levels of insoluble tau protein, particularly of paired helical filaments containing hyperphosphorylated tau protein (pTau PHF) in the brain, particularly in the brain cortex and/or hippocampus, of an animal, particularly a mammal or a human, containing increased levels of insoluble tau protein, particularly of pTau paired helical filaments (pTau PHF) comprising administering to said animal, particularly to said mammal or human, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (77), the present invention relates to a method for slowing or halting the progression of a tau-protein-associated disease, disorder or condition in an animal, particularly a mammal or human comprising administering to said animal, particularly said mammal or human, suffering from such a disease or condition, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (78), the present invention relates to a method for improving or alleviating the symptoms associated with tau-protein-associated diseases, disorders or conditions such as, for example, impairment or loss of cognitive functions including reasoning, situational judgement, memory capacity, learning, special navigation, etc., in an animal, particularly a mammal or a human, comprising administering to said animal, particularly to said mammal or human, suffering from such a disease or condition, the binding peptide or a functional part thereof, particularly an antibody, particularly a

monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (79), the present invention relates to a method for retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

In still another embodiment (80) of the invention, a method is provided for the treatment of a tau-protein-associated disease or disorder including a neurodegenerative disease or disorder such as a tauopathy comprising administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In one embodiment (81) of the invention, a method is provided for the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy, which method comprises administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide or a pharmaceutical composition according to any one of the preceding embodiments, or a combination thereof.

In another embodiment (82) of the invention, a method is provided for inducing a passive immune response in an animal, particularly a mammal or a human, suffering from a

neurodegenerative disorder such as tauopathy by administering to said animal or human the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, or a combination thereof.

In still another embodiment (83) of the invention, a method of diagnosing a tau-protein-associated disease, disorder or condition in a patient is provided comprising detecting the immunospecific binding of a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide or a fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding claims, wherein said binding peptide or antibody or fragment thereof binds an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau protein in the sample or specific body part or area.

In still another embodiment (84) of the invention, a method for diagnosing a predisposition to tau-protein-associated disease, disorder or condition in a patient is provided comprising detecting the immunospecific binding of a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, to an epitope of the tau protein in a sample or *in situ,* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, which peptide or fragment thereof binds an epitope of the tau protein;

b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area;
e. comparing the amount of said immunological complex to a normal control value;

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an tau- protein-associated disease or condition.

In one embodiment (85) of the invention, a method is provided for monitoring minimal residual disease in a patient following treatment with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, wherein said method comprises:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments, which peptide or fragment thereof binds to an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

In one embodiment (86), a method is provided for predicting responsiveness of a patient being treated with the binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide, or a pharmaceutical composition, according to any one of the preceding embodiments, comprising

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof according to any one of the preceding embodiments, which peptide or fragment thereof binds to an epitope of the tau protein;
b. allowing said binding peptide, particularly said antibody, particularly said monoclonal antibody or a functional part thereof, to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex before and after onset of the treatment,

wherein a decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

In another embodiment (87), the invention relates to a test kit for detection and diagnosis of tau-protein-associated diseases, disorders or conditions comprising a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments.

In one embodiment (88) said test kit comprises a container holding one or more binding peptides or active fragments thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, according to any one of the preceding embodiments and instructions for using the binding peptides or antibodies for the purpose of binding to tau antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of tau antigen.

In still another embodiment (89), the present invention relates to an epitope selected from the group consisting of Tau aa 15-20 of human tau protein shown in SEQ ID NO: 67 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); and Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position 214 (pS214).

In one embodiment (90), said epitope consists of Tau aa 15-20 with a phosphorylated Tyr at position 18 (Y18).

In one embodiment (91), said epitope consists of Tau aa 405-412 with a phosphorylated Ser at position 409 (pS409).

In one embodiment (92), said epitope consists of Tau aa 405-411 with a phosphorylated Ser at position 409 (pS409).

In another embodiment (93), the invention relates to a cell line producing a binding peptide or an active fragment thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof according to any one of the preceding embodiments.

In one embodiment (94), the invention relates to a cell line, which is hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079.

In one embodiment (95), the invention relates to a cell line, which is hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081.

In one embodiment (96), the invention relates to a cell line, which is hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080.

In one embodiment (97), the invention relates to a cell line, which is hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088.

In one embodiment (98), the invention relates to a cell line, which is hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084.

In one embodiment (99), the invention relates to a cell line, which is hybridoma cell line 2B6G7A12 deposited on March 10, 2010 as DSM ACC3087.

In one embodiment (100), the invention relates to a cell line, which is hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086.

In one embodiment (101), the invention relates to a cell line, which is hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085.

In one embodiment (102), the invention relates to a cell line, which is hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082.

In one embodiment (103), the invention relates to a cell line, which is hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083.

In one embodiment (103a), the invention relates to a cell line, which is hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136.

In one embodiment (103b), the invention relates to a cell line, which is hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137.

In one embodiment (103c), the invention relates to a cell line, which is hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138.

In one embodiment (103d), the invention relates to a cell line, which is hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139.

In one embodiment (103e), the invention relates to a cell line, which is hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140.
In one embodiment (103e), the invention relates to a cell line, which is hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141.

In one embodiment (104), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (105), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (106), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (107), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded

by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (108), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (109), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6G7A12 deposited on August 25, 2010 as DSM ACC3087 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (110), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (111), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (112), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 49; SEQ ID NO: 56 and SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (113), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083 using

a. a pair of primers comprising a 5'-primer of SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

In one embodiment (114), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 149 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, and 140 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 134, and 141-148, for amplification of a second binding domain.

In one embodiment (115), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 169-174 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 124, 127, and 150-158 and a 3'-primer selected from the group consisting of SEQ ID NOs: 130, and 159-168, for amplification of a second binding domain.

In one embodiment (116), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 178, 179 and 180 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 127, 139, 154, 155, and 175 and a 3'-primer selected from the group consisting of SEQ ID NOs: 128, 129, 147, 176, and 177, for amplification of a second binding domain.

In one embodiment (117), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 188-192 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 124, 126, 181, 182 and 183 and a 3'-primer selected from the group consisting of SEQ ID NOs: 144, 145 and 184-187, for amplification of a second binding domain.

In one embodiment (118), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 50 and 201-204 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 137, 151 and 193-197 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 141, 144, 166, 198, 199 and 200, for amplification of a second binding domain.

In one embodiment (119), the invention relates to a monoclonal antibody or a functional part thereof comprising a light chain (VL) and/or a heavy chain (VH) domain, which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 209-214, and 219-221 a 3'-primer of SEQ ID NO: 215, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 216, 217 and 218 and a 3'-primer of SEQ ID NOs: 208, for amplification of a second binding domain.

In one embodiment (120), the antibody according to any one of the preceding embodiments may be a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, a camelid antibody, a diabody, or a modified or engineered antibody.

In one embodiment (121), the binding peptide or functional part thereof may be a fragment comprising a heavy chain and/or a light chain, particularly a heavy chain as show in SEQ ID NOs: 1-5 and/or a light chain as shown in SEQ ID NOs: 6-11, particularly a Fab or a $F(ab')_2$ fragment.

In a specific embodiment (122), the invention relates to a heavy chain as show in SEQ ID NOs: 1-5.

In another specific embodiment (123), the invention relates to a light chain as shown in SEQ ID NOs: 6-11.

In one embodiment (124), the invention provides a method for producing the binding peptides or antibodies of any one of the preceding embodiments, comprising the step of culturing the cell line of any of the preceding embodiments in a suitable cultivation medium and, optionally, purifying the binding peptides or antibody from the cell line or cultivation medium.

Brief Description of Figures and Sequences

FIGURES

Figure 1 shows antibody binding to phospho-Tau in brain sections from biGT (Tau bigenic) mice using TAUPIR.

Figure 2 shows antibody binding to phospho-Tau in brain sections from AD and tauopathy patients using TAUPIR using ACI-36-3A8-Ab1 antibody.

Figure 3 shows the effect of anti-Tau antibody treatment following 1 week in vivo study on pTau epitope pT231 using MSD.

Figure 4 shows a diagram demonstrating how brains were prepared for soluble and sarkosyl insoluble (SinT) Tau protein fractions.

Figure 5 shows pTau epitope Western Blot results after anti-Tau antibody treatment for the 1 month (Figure 5A, 5B, 5C, 5G, 5H, 5I) or 3 month in vivo study (Figure 5D, 5E, 5F)

Figure 6. shows pTau epitope Western Blot results after anti-Tau antibody treatment for the 3 month in vivo study using biGT bigenic mice.

Figure 7 shows IHC after anti-Tau antibody treatment by ACI-36-2B6-Ab1 in 3 month in vivo study.

Figure 8 shows IHC after anti-Tau antibody treatment by ACI-36-3A8-Ab1 in 3 month in vivo study.

Figure 9 shows the Morris Water-Maze results after anti-Tau antibody treatment by ACI-36-2B6-Ab1 in 3 month in vivo study.

Figure 10 shows the Morris Water-Maze results after anti-Tau antibody treatment by ACI-36-3A8-Ab1 in 3 month in vivo study.

SEQUENCES

SEQ ID NO: 1 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-3A8-Ab1 produced by hybridoma cell line 3A8A12G7.

SEQ ID NO: 2 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-2B6-Ab1 produced by hybridoma cell line 2B6A10C11.

SEQ ID NO: 3 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 4 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 5 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 6 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK\text{-}AD}$ and ACI-36-3A8-Ab2$_{VK\text{-}AD}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 7 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-G}$ and ACI-36-3A8-Ab2$_{VK-G}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 8 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-2B6-Ab1 and ACI-36-2B6-Ab2 produced by hybridoma cell line 2B6A10C11 and 2B6G7A12, respectively.

SEQ ID NO: 9 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 10 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 11 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 12 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-36-3A8-Ab1, ACI-36-3A8-Ab2, ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 13 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-36-3A8-Ab1, ACI-36-3A8-Ab2, ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 14 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-36-3A8-Ab1, ACI-36-3A8-Ab2, ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 15 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 16 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 17 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 18 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 19 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 20 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 21 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-AD}$ and ACI-36-3A8-Ab2$_{VK-AD}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 22 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-AD}$ and ACI-36-3A8-Ab2$_{VK-AD}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 23 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-AD}$ and ACI-36-3A8-Ab2$_{VK-AD}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 24 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-G}$ and ACI-36-3A8-Ab2$_{VK-G}$ produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 25 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-G}$, ACI-36-3A8-Ab2$_{VK-G}$, ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 26 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1$_{VK-G}$, ACI-36-3A8-Ab2$_{VK-G}$, ACI-36-2B6-Ab1, ACI-36-2B6-Ab2, ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell

line 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 27 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-36-2B6-Ab1 and ACI-36-2B6-Ab2 produced by hybridoma cell line 2B6A10C11 and 2B6G7A12, respectively.

SEQ ID NO: 28 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 29 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 30 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 31 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 32 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 33 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 34 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 35 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-3A8-Ab1 and ACI-36-3A8-Ab2 produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 36 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-2B6-Ab1 and ACI-36-2B6-Ab2 produced by hybridoma cell line 2B6A10C11 and 2B6G7A12, respectively.

SEQ ID NO: 37 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 38 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 39 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 40 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1 and ACI-36-3A8-Ab2 produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 41 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-3A8-Ab1 and ACI-36-3A8-Ab2 produced by hybridoma cell line 3A8A12G7 and 3A8E12H8, respectively.

SEQ ID NO: 42 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-2B6-Ab1 and ACI-36-2B6-Ab2 produced by hybridoma cell line 2B6A10C11 and 2B6G7A12, respectively.

SEQ ID NO: 43 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-36-6H1-Ab1 and ACI-36-6H1-Ab2 produced by hybridoma cell line 6H1A11C11 and 6H1G6E6, respectively.

SEQ ID NO: 44 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-33-6C10-Ab2 and ACI-33-6C10-Ab1 produced by hybridoma cell line 6C10E5E9C12 and 6C10F9C12A11, respectively.

SEQ ID NO: 45 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-41-7C2-Ab1 and ACI-41-7C2-Ab2 produced by hybridoma cell line 7C2(1)F10C10D3 and 7C2(2)B9F11D5, respectively.

SEQ ID NO: 46 – 57 depicts the nucleotide sequences of VH/VK forward and reverse primers.

SEQ ID NO: 58 depicts the amino acid sequence of Tau 379-408 [pS396, pS404]

SEQ ID NO: 59 depicts the amino acid sequence of Tau 5-20 [pY18]

SEQ ID NO: 60 depicts the amino acid sequence of Tau 206-221 [pT212, pS214]

SEQ ID NO: 61 depicts the amino acid sequence of Tau 196-211 [pS202, pT205]

SEQ ID NO: 62 depicts the amino acid sequence of Tau 393-408 [pS396, pS404]

SEQ ID NO: 63 depicts the amino acid sequence of Tau 401-418 [pS404, pS409]

SEQ ID NO: 64 depicts the amino acid sequence of Tau 200-216 [pS202+ pT205 & pT212+pS214]

SEQ ID NO: 65 depicts the amino acid sequence of Tau 407-418 [pS409]

SEQ ID NO: 66 depicts the amino acid sequence of Tau 399-408 [pS404]

SEQ ID NO: 67 depicts the amino acid sequence of longest isoform of human Tau (441aa), also called Tau40

SEQ ID NO: 68 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 69 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 70 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 71 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 72 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 73 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 74 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 75 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1

SEQ ID NO: 76 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 77 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 78 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 79 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1.

SEQ ID NO: 80 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1

SEQ ID NO: 81 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1

SEQ ID NO: 82 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1

SEQ ID NO: 83 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1

SEQ ID NO: 84 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 85 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab1 produced by hybridoma cell line A4-4A6-18.

SEQ ID NO: 86 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 87 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-1D2-Ab1 produced by hybridoma cell line A6-1D2-12.

SEQ ID NO: 88 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively, produced by hybridoma cell line A4-2A1-18, A4-2A1-40 and A4-4A6-48, respectively.

SEQ ID NO: 89 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, ACI-35-4A6-Ab2, ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 90 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively.

SEQ ID NO: 91 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, ACI-35-4A6-Ab2, ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 92 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2 produced by hybridoma cell line A4-2A1-40

SEQ ID NO: 93 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 94 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 95 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2.

SEQ ID NO: 96 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 97 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 98 depicts the amino acid sequence of the CDR1 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 99 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 100 depicts the amino acid sequence of the CDR3 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 101 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 102 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 103 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-Ab1.

SEQ ID NO: 104 depicts the amino acid sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 105 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 106 depicts the amino acid sequence of the CDR1 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 107 depicts the amino acid sequence of the CDR2 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 108 depicts the amino acid sequence of the CDR3 of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively.

SEQ ID NO: 109 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2A1-Ab1, ACI-35-2A1-Ab2, and ACI-35-4A6-Ab2, respectively, produced by hybridoma cell line A4-2A1-18, A4-2A1-40 and A4-4A6-48, respectively.

SEQ ID NO: 110 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab2 produced by hybridoma cell line A4-2A1-40.

SEQ ID NO: 111 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 112 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB1 produced by hybridoma cell line A6-2G5-08.

SEQ ID NO: 113 depicts the nucleotide sequence of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 114 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3, respectively, produced by hybridoma cell line A6-2G5-30 and A6-2G5-41, respectively.

SEQ ID NO: 115 depicts the amino acid sequence of the CDR2 of the heavy chain variable region (VH) of monoclonal antibody ACI-35-2G5-AB2 and ACI-35-2G5-AB3.

SEQ ID NO: 116 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab1produced by hybridoma cell line A4-2A1-18.

SEQ ID NO: 117 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-2A1-Ab1 produced by hybridoma cell line A4-2A1-18.

SEQ ID NO: 118 depicts the amino acid sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab2 produced by hybridoma cell line A4-4A6-48.

SEQ ID NO: 119 depicts the nucleotide sequence of the light chain variable region (VK) of monoclonal antibody ACI-35-4A6-Ab2 produced by hybridoma cell line A4-4A6-48.

SEQ ID NO: 120 – 221 depicts the nucleotide sequences of VH/VK forward and reverse primers

Definition of Terms

The terms “polypeptide”, “peptide”, and “protein”, as used herein, are interchangeably and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

The term "peptides," or “binding peptide” are used herein interchangeably and refer to chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. The terminal amino acid at one end of the chain (i.e., the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (i.e., the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free alpha-amino group on the amino acid at the amino terminal of the peptide, or to the alpha-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide. A binding peptide may constitutes antibodies such as polyclonal or monoclonal antibodies, human or humanized antibodies, diabodies, camelid antibodies, etc, or functional parts thereof as defined herein.

The terms “fragment thereof” or “fragment” as used herein refer to a functional peptide fragment which has essentially the same (biological) activity as the peptides defined herein (e.g. as shown in SEQ ID NOs 59-66 in Table 1 respectively), i.e. said fragments are still capable of eliciting a highly specific, particularly a conformation specific, immune response in an organism, but particularly within an animal, particularly a mammal or a human, which is highly effective and capable of preventing or alleviating tauopathies, or the symptoms associated with tauopathies. In particular, said fragments still contain the specific pathological phospho-epitope or -epitopes of the tau peptide, as used and defined herein.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminal and increasing in the direction toward the carboxy terminal of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminal of the peptide than the preceding amino acid.

The term "residue" is used herein to refer to an amino acid that is incorporated into a peptide by an amide bond. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that function in a manner similar to the naturally occurring amino acids (i.e., amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

Furthermore, one of skill will recognize that, as mentioned above, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides described herein do not contain materials normally associated with their in situ environment. Typically, the isolated, immunogenic peptides described herein are at least about 80% pure, usually at least about 90%, and preferably at least about 95% as measured by band intensity on a silver stained gel.

Protein purity or homogeneity may be indicated by a number of methods well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

When the immunogenic peptides are relatively short in length (i.e., less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the immunogenic peptides described herein. Techniques for solid phase synthesis are known to those skilled in the art.

Alternatively, the immunogenic peptides described herein are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or polypeptide and, if required, renaturing the peptide. Techniques sufficient to guide one of skill through such procedures are found in the literature.

Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 % to 95 % homogeneity are preferred, and 80 % to 95 % or greater homogeneity is most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the immunogenic peptides may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the antiproliferative peptide and then to cause the peptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

Antigenicity of the purified protein may be confirmed, for example, by demonstrating reaction with immune serum, or with antisera produced against the protein itself.

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

The terms "detecting" or "detected" as used herein mean using known techniques for detection of biologic molecules such as immunochemical or histological methods and refer to qualitatively or quantitatively determining the presence or concentration of the biomolecule under investigation.

By “isolated” is meant a biological molecule free from at least some of the components with which it naturally occurs.

The terms "antibody", "antibodies" or "functional parts thereof" as used herein is an art recognized term and is understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e molecules that contain a binding site that immunospecifically binds an antigen. The immunoglobulin according to the invention can be of any type (IgG, IgM, IgD, IgE, IgA and IgY) or class (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclasses of immunoglobulin molecule.

"Antibodies" are intended within the scope of the present invention to include monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, human and humanized antibodies, camelid antibodies, diabodies, as well as functional parts or active fragments thereof. Examples of active fragments of molecules that bind to known antigens include Fab and $F(ab')_2$ fragments, including the products of a Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above. These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, (1986).

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s).

A humanized antibody may further refer to an antibody having a variable region where one or more of its framework regions have human or primate amino acids. In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain "humanized antibodies" are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technoloy, 9:421 (1991)).

A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured humanlike polyclonal antibodies in large animals such as, for example, rabbits (http://www.rctech.com/bioventures/therapeutic.php).

The term "fully human antibody" or "human" antibody is meant to refer to an antibody derived from transgenic mice carrying human antibody genes or from human cells. To the

human immune system, however, the difference between "fully human", "human", and "humanized" antibodies may be negligible or nonexistent and as such all three may be of equal efficacy and safety.

The term “monoclonal antibody” is also well recognized in the art and refers to an antibody that is mass produced in the laboratory from a single clone and that recognizes only one antigen. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an “immortal” cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces large quantities of the antibody.

The term “antigen” refers to an entity or fragment thereof which can induce an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term includes immunogens and regions responsible for antigenicity or antigenic determinants.

As used herein, the term “soluble” means partially or completely dissolved in an aqueous solution.

Also as used herein, the term "immunogenic" refers to substances which elicit or enhance the production of antibodies, T-cells and other reactive immune cells directed against an immunogenic agent and contribute to an immune response in humans or animals.

An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present invention to moderate or alleviate the disorder to be treated.

The term "hybridoma" is art recognized and is understood by those of ordinary skill in the art to refer to a cell produced by the fusion of an antibody-producing cell and an immortal cell, e.g. a multiple myeloma cell. This hybrid cell is capable of producing a continuous supply of antibody. See the definition of "monoclonal antibody" above and the Examples below for a more detailed description of the method of fusion.

The term "carrier" as used herein means a structure in which antigenic peptide or supramolecular construct can be incorporated into or can be associated with, thereby presenting or exposing antigenic peptides or part of the peptide to the immune system of a human or animal. Any particle that can be suitably used in animal or human therapy such as, for example, a vesicle, a particle or a particulate body may be used as a carrier within the context of the present invention.

The term “carrier” further comprises methods of delivery wherein supramolecular antigenic construct compositions comprising the antigenic peptide may be transported to desired sites by delivery mechanisms. One example of such a delivery system utilizes colloidal metals such as colloidal gold.

Carrier proteins that can be used in the supramolecular antigenic construct compositions of the present invention include, but are not limited to, maltose binding peptide "MBP"; bovine serum albumin "BSA"; keyhole lympet hemocyanin "KLH"; ovalbumin; flagellin; thyroglobulin; serum albumin of any species; gamma globulin of any species; syngeneic cells; syngeneic cells bearing Ia antigens; and polymers of D- and/or L- amino acids.

Further, the term "therapeutically effective amount" or “pharmaceutically effective amount” refers to the amount of binding peptide which, when administered to a human or animal, is sufficient to result in a therapeutic effect in said human or animal. The effective amount is readily determined by one of ordinary skill in the art following routine procedures.

“pTau PHF”, “PHF”, and “paired helical filaments” are used herein synonymously and refer to pairs of approximately 10 nm filaments wound into helices with a periodicity of 160 nm visible on electron microscopy. Width varies between 10 and 22 nm. PHF are the predominant structures in neurofibrillary tangles of Alzheimer's Disease (AD) and neuropil threads. PHF may also be seen in some but not all dystrophic neurites associated with neuritic plaques. The major component of PHF is a hyperphosphorylated form of microtubule-associated protein tau. PHF are composed of disulfide-linked antiparallel hyper-phosphorylated tau proteins. PHF tau may be truncated of its C-terminal 20 amino acid residues. The mechanisms underlying PHF formation are uncertain but hyper-phosphorylation of tau may disengage it from microtubules, increasing the soluble pool of tau.

Within the scope of the present invention, it was demonstrated that the antibody induced response to the antigenic composition according to the invention is largely T-cell independent. A nude mouse model was used in this respect and nude mice were vaccinated and antibody responses measured to evaluate the Aβ-specific antibody response induced by the antigenic composition according to the invention in the immunized nude mice. The nude mice carry the Foxn1nu mutation and as a consequence, have reduced T-cell function due to the lack of a proper thymus.

A “pharmaceutically effective amount” as used herein refers to a dose of the active ingredient in a pharmaceutical composition adequate to cure, or at least partially arrest, the symptoms of the disease, disorder or condition to be treated or any complications associated therewith.

The present invention provides binding peptides recognizing and binding to major pathological phospho-epitopes of the tau protein. In particular, the present invention provides specific antibodies against linear and conformational, simple and complex phospho-epitopes on protein tau that are believed to be responsible for synapto- and neuro-
5 toxicity in tauopathies, including AD.

Accordingly, the present invention relates in one embodiment to a binding peptide or a functional part thereof, particularly to an antibody, particularly a monoclonal antibody or a functional part thereof, which binding peptide or antibody recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a
10 fragment thereof, particularly to a pathological protein tau conformer, but, in one embodiment, does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said binding peptide or antibody has a high binding affinity with a dissociation constant of at least 10 nM, particularly of at least 8 nM, particularly of at least 5 nM, particularly of at least 2 nM, particularly of at least 1 nM, particularly of at least 500 pM,
15 particularly of at least 400 pM particularly of at least 300 pM, particularly of at least 200 pM, particularly of at least 100 pM, particularly of at least 50 pM.

"Soluble Tau" protein as used herein refers to proteins consisting of both completely solubilized Tau protein/peptide monomers or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins monomers,
20 and of Tau protein oligomers. "Soluble Tau" excludes particularly neurofibrillary tangles (NFT).

"Insoluble Tau" as used herein refers to multiple aggregated monomers of Tau peptides or proteins, or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins forming oligomeric or polymeric structures
25 which are insoluble both *in vitro* in aqueous medium and *in vivo* in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of Tau or of modified or truncated Tau peptides/proteins or of derivatives thereof, which are insoluble in the mammalian or human body more particularly in the brain, respectively. "Insoluble Tau" particularly includes neurofibrillary tangles (NFT).

30 ""Monomeric Tau" or "Tau monomer" as used herein refers to completely solubilized Tau proteins without aggeregated complexes in aqueous medium.

"Aggregated Tau", "oligomeric Tau" and "Tau oligomer" refer to multiple aggregated monomers of Tau peptides or proteins , or of Tau-like peptides/proteins, or of modified or truncated Tau peptides/proteins or of other derivates of Tau peptides/proteins forming

oligomeric or polymeric structures which are insoluble or soluble both in vitro in aequeous medium and in vivo in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of Tau or of modified or truncated Tau peptides/proteins or of derivatives thereof, which are insoluble or soluble in the mammalian or human body more particularly in the brain, respectively."

In one embodiment, the present invention provides a pharmaceutical composition comprising a binding peptide or a functional part thereof, particularly an antibody, particularly a monoclonal antibody or a functional part thereof, or a polynucleotide comprising a nucleic acid sequence encoding said binding peptide or antibody, according to any one of the embodiments described and claimed herein, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.

The binding peptides according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, can be prepared in a physiologically acceptable formulation and may comprise a pharmaceutically acceptable carrier, diluent and/or excipient using known techniques. For example, the binding peptides according to the invention and as described herein including any functionally equivalent binding peptides or functional parts thereof, in particular, the monoclonal antibodies of the invention including any functionally equivalent antibodies or functional parts thereof, are combined with a pharmaceutically acceptable carrier, diluent and/or excipient to form a therapeutic composition. Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions, various types of wetting agents, sterile solutions, etc.

Formulation of the pharmaceutical composition according to the invention can be accomplished according to standard methodology know to those of ordinary skill in the art.

The compositions of the present invention may be administered to a subject in the form of a solid, liquid or aerosol at a suitable, pharmaceutically effective dose. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumor site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection intramuscularly, subcutaneously,

intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

The compositions may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal, interdermal, intraperitoneal, or parenteral (for example, intravenous, subcutaneous, or intramuscular) routes.

In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor. The method includes administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

A sustained release matrix, as used herein, is a matrix made of materials, usually polymers which are degradable by enzymatic or acid/base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix desirably is chosen by biocompatible materials such as liposomes, polylactides (polylactide acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

It is well known to those of ordinary skill in the pertinent art that the dosage of the composition will depend on various factors such as, for example, the condition of being treated, the particular composition used, and other clinical factors such as weight, size, sex and general health condition of the patient, body surface area, the particular compound or composition to be administered, other drugs being administered concurrently, and the route of administration.

The composition according to the invention may be administered in combination with other compositions comprising an biologically active substance or compound such as, for example, a known compound used in the medication of tauopathies and/or of amyloidoses, a group of diseases and disorders associated with amyloid or amyloid-like protein such as the amyloid β protein involved in Alzheimer's Disease.

The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the therapeutic vaccine according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

Generally, the other biologically active compound may include neutron-transmission enhancers, psychotherapeutic drugs, acetylcholine esterase inhibitors, calcium-channel blockers, biogenic amines, benzodiazepine tranquillizers, acetylcholine synthesis, storage or release enhancers, acetylcholine postsynaptic receptor agonists, monoamine oxidase-A or –B inhibitors, N-methyl-D-aspartate glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, antioxidants, and serotonergic receptor antagonists.

In particular, the biologically active agent or compound may comprise at least one compound selected from the group consisting of compounds against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3- amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), secretase activators, [beta]- and 7-secretase inhibitors, tau proteins, neurotransmitter, /3-sheet breakers, antiinflammatory molecules, "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine and other drugs and nutritive supplements such as, for example, vitamin B 12, cysteine, a precursor of acetylcholine, lecithin, choline, Ginkgo biloba, acyetyl-L-carnitine, idebenone, propentofylline, or a xanthine derivative, together with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient and instructions for the treatment of diseases.

In a further embodiment, the composition according to the invention may comprise niacin or memantine together with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

In still another embodiment of the invention compositions are provided that comprise “atypical antipsychotics” such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine for the treatment of positive and negative psychotic symptoms including hallucinations, delusions, thought disorders (manifested by marked incoherence, derailment, tangentiality), and bizarre or disorganized behavior, as well as anhedonia, flattened affect, apathy, and social withdrawal, together with the binding peptide according

to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

Other compounds that can be suitably used in compositions in addition to the binding peptide according to the invention, are those disclosed, for example, in WO 2004/058258 (see especially pages 16 and 17) including therapeutic drug targets (page 36-39), alkanesulfonic acids and alkanolsulfuric acid (pages 39-51), cholinesterase inhibitors (pages 51-56), NMDA receptor antagonists (pages 56-58), estrogens (pages 58-59), non-steroidal anti-inflammatory drugs (pages 60-61), antioxidants (pages 61-62), peroxisome proliferators-activated receptors (PPAR) agonists (pages 63-67), cholesterol–lowering agents (pages 68-75); amyloid inhibitors (pages 75-77), amyloid formation inhibitors (pages 77-78), metal chelators (pages 78-79), anti-psychotics and anti-depressants (pages 80-82), nutritional supplements (pages 83-89) and compounds increasing the availability of biologically active substances in the brain (see pages 89-93) and prodrugs (pages 93 and 94), which document is incorporated herein by reference, but especially the compounds mentioned on the pages indicated above.

Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose. Generally, the regime of administration should be in the range of between 0.1 µg and 10 mg of the antibody according to the invention , particularly in a range 1.0 µg to 1.0 mg, and more particularly in a range of between 1.0 µg and 100 µg, with all individual numbers falling within these ranges also being part of the invention. If the administration occurs through continuous infusion a more proper dosage may be in the range of between 0.01 µg and 10 mg units per kilogram of body weight per hour with all individual numbers falling within these ranges also being part of the invention.

Administration will generally be parenterally, e.g. intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Non-aqueous solvents include, without being limited to, propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous solvents may be chosen from the group consisting of water, alcohol/aqueous solutions, emulsions or suspensions including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and others. Preservatives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, etc.

The pharmaceutical composition may further comprise proteinaceous carriers such as, for example, serum albumin or immunoglobulin, particularly of human origin. Further biologically active agents may be present in the pharmaceutical composition of the invention dependent on its the intended use.

When the binding target is located in the brain, certain embodiments of the invention provide for the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to traverse the blood-brain barrier. Certain neurodegenerative diseases are associated with an increase in permeability of the blood-brain barrier, such that the binding peptide according to the invention including antibodies, particularly monoclonal antibodies or active fragment thereof can be readily introduced to the brain. When the blood-brain barrier remains intact, several art-known approaches exist for transporting molecules across it, including, but not limited to, physical methods, lipid-based methods, and receptor and channel-based methods.

Physical methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof across the blood-brain barrier include, but are not limited to, circumventing the blood-brain barrier entirely, or by creating openings in the blood-brain barrier. Circumvention methods include, but are not limited to, direct injection into the brain (see, e.g., Papanastassiou et al., Gene Therapy 9: 398-406 (2002)) and implanting a delivery device in the brain (see, e.g., Gill et al., Nature Med. 9: 589-595 (2003); and Gliadel Wafers(TM), Guildford Pharmaceutical). Methods of creating openings in the barrier include, but are not limited to, ultrasound (see, e.g., U.S. Patent Publication No. 2002/0038086), osmotic pressure (e.g., by administration of hypertonic mannitol (Neuwelt, E. A., Implication of the Blood-Brain Barrier and its Manipulation, Vols 1 & 2, Plenum Press, N. Y. (1989)), permeabilization by, e.g., bradykinin or permeabilizer A-7 (see, e.g., U.S. Patent Nos. 5,112,596, 5,268,164, 5,506,206, and 5,686,416), and transfection of neurons that straddle the blood-brain barrier with vectors containing genes encoding the binding peptide or antigen-binding fragment (see, e.g., U.S. Patent Publication No. 2003/0083299).

Lipid-based methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or an active fragment thereof across the blood-brain barrier include, but are not limited to, encapsulating the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof in liposomes that are coupled to active fragments thereof that bind to receptors on the vascular endothelium of the blood-brain barrier (see, e.g., U.S. Patent Application Publication No. 20020025313), and coating the binding peptide according to the

invention including antibodies, particularly monoclonal antibodies, or active fragment thereof in low-density lipoprotein particles (see, e.g., U.S. Patent Application Publication No. 20040204354) or apolipoprotein E (see, e.g., U.S. Patent Application Publication No. 20040131692).

Receptor and channel-based methods of transporting the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or active fragment thereof across the blood-brain barrier include, but are not limited to, using glucocorticoid blockers to increase permeability of the blood-brain barrier (see, e.g., U.S. Patent Application Publication Nos. 2002/0065259, 2003/0162695, and 2005/0124533); activating potassium channels (see, e.g., U.S. Patent Application Publication No. 2005/0089473), inhibiting ABC drug transporters (see, e.g., U.S. Patent Application Publication No. 2003/0073713); coating antibodies with a transferrin and modulating activity of the one or more transferrin receptors (see, e.g., U.S. Patent Application Publication No. 2003/0129186), and cationizing the antibodies (see, e.g., U.S. Patent No. 5,004,697).

Single or repeated administrations of the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or an active fragment thereof, or of a pharmaceutical composition according to the invention may be provided to a subject over an extended period of time. The duration of administration may be between 1 week and up to 12 month or more. During this time the binding peptide, antibody or pharmaceutical composition may be administerd once a week, once every two weeks, three weeks, four weeks, etc, or at a higher or lower frequency depending on the needs of the subject to be treated.

In a further embodiment the present invention provides methods and kits for the detection and diagnosis of tau-protein-associated diseases, disorders or conditions, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further of diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick

disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy. The pathological abnormalities may be caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy.

Further, the present invention provides methods and kits for diagnosing a predisposition to tau-protein-associated diseases, disorders or conditions, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein. These methods include known immunological methods commonly used for detecting or quantifying substances in biological samples or in an in situ condition.

Diagnosis of a tau-protein-associated disease or condition or of a predisposition to an tau-protein-associated disease or condition in a subject in need thereof, particularly a mammal, more particularly a human, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies, may be achieved by detecting the immunospecific binding of a binding peptide of the invention, particularly of an antibody, particularly of a monoclonal antibody or an active fragment thereof, to an epitope of the tau protein in a sample or in situ, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with an antibody which binds an epitope of the tau protein, allowing the antibody to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of the immunologic complex to a normal control value, wherein an increase in the amount of the immunologic complex compared to a normal control value indicates that the subject is suffering from or is at risk of developing an tau protein-associated disease or condition.

Monitoring minimal residual disease in a subject, particularly a mammal, more particularly a human, following treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a

composition according to the invention may be achieved by detecting the immunospecific binding of a binding peptide of the invention, particularly of an antibody, particularly a monoclonal antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ*, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, which binds an epitope of the tau protein, allowing the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of said immunologic complex to a normal control value, wherein an increase in the amount of said immunologic complex compared to a normal control value indicates that the subject may still suffer from a minimal residual disease.

Predicting responsiveness of a subject, particularly a mammal, more particularly a human, to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention may be achieved by detecting the immunospecific binding of a binding peptide, particularly of a monoclonal antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ*, which includes bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, which binds an epitope of the tau protein, allowing the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, to bind to the tau protein to form an immunologic complex, detecting the formation of the immunologic complex and correlating the presence or absence of the immunologic complex with the presence or absence of tau protein in the sample or specific body part or area, optionally comparing the amount of said immunologic complex before and after onset of the treatment, wherein an decrease in the amount of said immunologic complex indicates that said patient has a high potential of being responsive to the treatment.

Biological samples that may be used in the diagnosis of a tau protein-associated disease or condition, for diagnosing a predisposition to a tau protein-associated disease or condition, including neurodegenerative diseases or disorders such as tauopathies comprising a

heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein are, for example, fluids such as serum, plasma, saliva, gastric secretions, mucus, cerebrospinal fluid, lymphatic fluid and the like or tissue or cell samples obtained from an organism such as neural, brain, cardiac or vascular tissue. For determining the presence or absence of the tau protein in a sample, any immunoassay known to those of ordinary skill in the art may be used such as, for example, assays which utilize indirect detection methods using secondary reagents for detection, ELISA's and immunoprecipitation and agglutination assays. A detailed description of these assays is, for example, given in Harlow and Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory, New York 1988 555-612, WO96/13590 to Maertens and Stuyver, Zrein et al. (1998) and WO96/29605.

For *in situ* diagnosis, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, of the invention or any active and functional part thereof may be administered to the organism to be diagnosed by methods known in the art such as, for example, intravenous, intranasal, intraperitoneal, intracerebral, intraarterial injection such that a specific binding between an antibody according to the invention with an eptitopic region on the amyloid protein may occur. The binding peptide/antigen complex may conveniently be detected through a label attached to the binding peptide according to the invention including antibodies, particularly monoclonal antibodies, or a functional fragment thereof or any other art- known method of detection.

The immunoassays used in diagnostic applications or in applications for diagnosing a predisposition to a tau protein-associated disease or condition, including neurodegenerative diseases or disorders such as tauopathies comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-existence of tau and amyloid pathologies, or for monitoring minimal residual disease in a patient or for predicting responsiveness of a patient to a treatment with a binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, or a composition according to the invention and as described herein typically rely on labelled antigens, binding peptides, or secondary reagents for detection. These proteins or reagents can be labelled with compounds generally known to those of ordinary skill in the art including enzymes, radioisotopes, and fluorescent,

luminescent and chromogenic substances including, but not limited to colored particles, such as colloidal gold and latex beads. Of these, radioactive labelling can be used for almost all types of assays and with most variations. Enzyme-conjugated labels are particularly useful when radioactivity must be avoided or when quick results are needed. Fluorochromes, although requiring expensive equipment for their use, provide a very sensitive method of detection. Binding peptides useful in these assays are those disclosed claimed herein including antibodies, particularly monoclonal antibodies, polyclonal antibodies, and affinity purified polyclonal antibodies.

Alternatively, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be labelled indirectly by reaction with labelled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be conjugated with a second substance and detected with a labelled third substance having an affinity for the second substance conjugated to the antibody. For example, the binding peptide according to the invention including antibodies, particularly monoclonal antibodies and active fragments thereof, may be conjugated to biotin and the binding peptide/biotin conjugate detected using labelled avidin or streptavidin. Similarly, the binding peptide may be conjugated to a hapten and the binding peptide/hapten conjugate detected using labelled anti-hapten binding peptide.

Those of ordinary skill in the art will know of these and other suitable labels which may be employed in accordance with the present invention. The binding of these labels to binding peptides or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy, J. H., et al., 1976 (Clin. Chim. Acta 70:1-31), and Schurs, A. H. W. M., et al. 1977 (Clin. Chim Acta 57:1-40). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, and others, all of which are incorporated by reference herein.

Current immunoassays utilize a double antibody method for detecting the presence of an analyte, wherein, the antibody is labeled indirectly by reactivity with a second antibody that has been labeled with a detectable label. The second antibody is preferably one that binds to antibodies of the animal from which the monoclonal antibody is derived. In other words, if the monoclonal antibody is a mouse antibody, then the labeled, second antibody is an anti-mouse antibody. For the antibody to be used in the assay described herein, this label is preferably an antibody-coated bead, particularly a magnetic bead. For the antibody to be

employed in the immunoassay described herein, the label is preferably a detectable molecule such as a radioactive, fluorescent or an electrochemiluminescent substance.

An alternative double antibody system, often referred to as fast format systems because they are adapted to rapid determinations of the presence of an analyte, may also be employed within the scope of the present invention. The system requires high affinity between the antibody and the analyte. According to one embodiment of the present invention, the presence of the amyloid protein is determined using a pair of antibodies, each specific for amyloid protein. One of said pairs of antibodies is referred to herein as a "detector antibody" and the other of said pair of antibodies is referred to herein as a "capture antibody". The monoclonal antibody of the present invention can be used as either a capture antibody or a detector antibody. The monoclonal antibody of the present invention can also be used as both capture and detector antibody, together in a single assay. One embodiment of the present invention thus uses the double antibody sandwich method for detecting amyloid protein in a sample of biological fluid. In this method, the analyte (amyloid protein) is sandwiched between the detector antibody and the capture antibody, the capture antibody being irreversibly immobilized onto a solid support. The detector antibody would contain a detectable label, in order to identify the presence of the antibody-analyte sandwich and thus the presence of the analyte.

Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes of polystyrene, magnetic, plastic or glass beads and slides which are well known in the field of radioimmunoassay and enzyme immunoassay. Methods for coupling antibodies to solid phases are also well known to those of ordinary skill in the art. More recently, a number of porous material such as nylon, nitrocellulose, cellulose acetate, glass fibers and other porous polymers have been employed as solid supports.

The present invention also relates to a diagnostic kit for detecting tau protein in a biological sample comprising a composition as defined above. Moreover, the present invention relates to the latter diagnostic kit which, in addition to a composition as defined above, also comprises a detection reagent as defined above. The term "diagnostic kit" refers in general to any diagnostic kit known in the art. More specifically, the latter term refers to a diagnostic kit as described in Zrein et al. (1998).

It is still another object of the present invention to provide novel immunoprobes and test kits for detection and diagnosis of tau protein-associated diseases and conditions, comprising binding peptides according to the present invention. For immunoprobes, the binding peptides are directly or indirectly attached to a suitable reporter molecule, e.g., an enzyme

or a radionuclide. The test kit includes a container holding one or more binding peptides according to the present invention and instructions for using the binding peptides for the purpose of binding to tau antigen to form an immunologic complex and detecting the formation of the immunologic complex such that presence or absence of the immunologic complex correlates with presence or absence of tau protein.

EXAMPLES

EXAMPLE 1: Generation and screening of hybridomas and antibodies

The objective of this study was to generate and screen anti-Tau mAbs (monoclonal antibodies). Hybridomas were generated by fusion of tau vaccine immunized mouse spleen with a myeloma cell line. The hybridomas were assessed for reactivity against both phosphorylated and non-phosphorylated full-length Tau protein, as well as the phosphorylated and non-phosphorylated Tau antigenic peptides used in the vaccine preparation. Hybridoma screening was also performed for reactivity of hybridomas supernatant for tau tangles using immunochemistry on Tau transgenic mouse brain slices.

1.1 Methods

1.1.1 Fusion

A wild type C57BL/6 mouse vaccinated with ACI-33 (Tau5-20 [pY18]) was used for hybridoma production. The mouse was boosted with ACI-33 vaccine on day 0 then again on day 4 and the fusion was performed on day 7. $173x10^6$ (ACI-33), splenocytes from the immunized mouse were fused with SP2-O-Ag14 myeloma cells at a ratio of 5 splenocytes / 1 myeloma cell.

A wild type C57BL/6 mouse vaccinated with ACI-35 (Tau393-408 [pS396, pS404]) was used for hybridoma production. The mouse was boosted with ACI-35 vaccine on day 0 then again on day 4 and the fusion was performed on day 6 $x10^7$ (ACI-35), splenocytes from the immunized mouse were fused with 2 x 10^7 SP2-O-Ag14 myeloma cells at a ratio of 3 splenocytes / 1 myeloma cell.

A wild type C57BL/6 mouse vaccinated with ACI-36 (Tau401-418 [pS404/S409]) was used for hybridoma production. The mouse was boosted with ACI-36 vaccine on day 0 then again on day 4 and the fusion was performed on day 7. $84x10^6$ splenocytes from the immunized

mouse were fused with SP2-O-Ag14 myeloma cells at a ratio of 5 splenocytes / 1 myeloma cell.

A wild type C57BL/6 mouse vaccinated with ACI-41 (mix of Tau206-221 [pT212/pS214] and Tau196-211 [pS202/pT205]) was used for hybridoma production. The mouse was boosted with ACI-41 vaccine on day 0 then again on day 4 and the fusion was performed on day 8. 162x106 splenocytes from the immunized mouse were fused with SP2-O-Ag14 myeloma cells at a ratio of 5 splenocytes / 1 myeloma cell.

The four fusions resulted in 8x96 well plates and the clones were name according to the plate (1-8) then the row (A-G) and finally the column (1-12).

1.1.2 Screening method to select clones

The 8x96 well plates were first screened twice for IgG expression. Positive expressing clones were then transferred in 24 well plates and cell supernatants (=clones) of growing cells were tested in a Tau ELISA screen and a immunohistochemistry TAUPIR screen. Positive supernatants in ELISA and/or TAUPIR were transferred to T25 flasks and clones were screened again for IgG expression in aTau ELISA screen and TAUPIR screen.

1.1.3 IgG screen

Elisa plates were coated with 50 ul/well of anti-mouse IgG antibody (CER Groupe, Marloie, Belgium) in coating buffer for 16 hrs at 4°C. After washing plates with PBS/Tween 100 ul/well of a blocking solution was applied for 1 hr at RT. 50 ul of undiluted hybridoma supernatant were incubated for 1 hr at RT. After a washing step, a mix of the HorseRadish Peroxydase (HRP)-conjugated anti-mouse IgG1, IgG2a, IgG2b and IgG3 (Ab Serotec, Raleigh, NC, USA) was applied on the plates for 1 hr at RT. After a final washing, detection was performed with TMB (3-3',5,5'-tetramethylbenzidine), the phosphatase substrate for HRP, and plates were read at 405 nm using an ELISA plate reader. Results are expressed as O.D. (Optical Density).

1.1.4 Hybridomas Tau ELISA screen

Hybridomas ELISA screen was performed on pTau peptide (ACI-33, T1.5: Tau5-20 [pY18]; ACI-35, T3.5: Tau393-408[pS396/pS404]; ACI-36, T4.5: Tau401-418 [pS404/S409]; ACI-41, T8.5: Tau206-221 [pT212/pS214] and T9.5: Tau196-211 [pS202/pT205] PolyPeptide Laboratories, Hillerød, Denmark), corresponding Tau peptide (ACI-33, T1.6: Tau5-20; ACI-36, T4.6: Tau401-4; ACI-41, T8.6: Tau206-221 and T9.6: Tau196-211, PolyPeptide Laboratories, Hillerød, Denmark), phosphorylated full-length (441aa) Tau protein (pTau protein, Vandebroek et al., 2005) and full-length (441aa) Tau protein (Tau protein,

SignalChem, Richmond, Canada). Finally Bovine Serum Albumin (BSA) was used as negative control.

Plates were coated with 10 µg/ml of corresponding Tau peptide and 1 µg/ml of corresponding Tau protein overnight at 4°C. After washing each well with PBS-0.05% Tween 20 and blocking with 1% BSA in PBS-0.05% Tween 20, undiluted hybridoma supernatant or medium negative control were added to the plates and incubated at 37°C for 2 hours. After washing plates were incubated with an alkaline phosphatase (AP)-conjugated anti-mouse IgG total antibody (Jackson Laboratories, Baltimore, PA, USA) for 2 hours at 37°C. After washing plates were incubated with pNPP (para-nitro-phenyl-phosphate), the phosphatase substrate for AP, and read at 405 nm using an ELISA plate reader. Results are expressed as O.D. (Optical Density).

1.1.5 Hybridomas IHC screen: Binding of anti-Tau antibodies to tangles in brain sections from transgenic mice (TAUPIR)

TAUPIR experiments were done according to protocol from EXAMPLE 3.1.2.

1.1.6 T25 flasks IgG screen

Elisa plates were coated with 5ug/ml of anti-mouse IgG F(ab')2 fragment specific antibody (Jackson Laboratories, Baltimore, PA, USA) in carbonate-bicarbonate coating buffer pH 9.6 (Sigma, Buchs, Switzerland) overnight at 4°C. After washing plates, undiluted hybridoma supernatant, positive control IgG1 antibody (6E10 at 1ug/ml: Covance, Emeryville, CA, USA) or negative control (culture medium alone) were incubated for 1 hr at RT. After a washing step, the secondary AP-conjugated goat anti-mouse IgG (subclasses 1+2a+2b+3) Fcγ fragment specific antibody (Jackson Laboratories, Baltimore, PA, USA) was incubated on the plates for 2 hrs at 37°C. After a final washing, detection was performed with pNPP (para-nitro-phenyl-phosphate), the phosphatase substrate for AP, and plates were read at 405 nm using an ELISA plate reader. Results are expressed as O.D. (Optical Density).

1.2 Results

1.2.1 ACI-33 hybridomas

The cell supernatants from the 8x96 well plates resulting from the fusion were screened for production of IgG. In the 768 wells (8x96 wells) tested 277 wells were positive for IgG expression and were transferred to 24 wells plates. In the 24 well plates 79 clones were growing and supernatant from those cells were analysed. Positive clones were further

transferred in T25 flasks and supernatants screened for IgG production, ELISA and TAUPIR (Table 2).

The clone 6C10 was the only one positive in the 3 screens and was selected for subcloning.

1.2.2 ACI-36 hybridomas

The cell supernatants from the 8x96 well plates resulting from the fusion were screened for production of IgG. In the 768 wells (8x96 wells) tested 333 wells were positive for IgG expression and were transferred to 24 wells plates. In the 24 well plates 75 clones were growing and supernatant from those cells were analysed. Positive clones were further transferred in T25 flasks and supernatants screened for IgG production, ELISA and TAUPIR (Table 3).

In order to select clones for the next steps a ranking of all supernatants positives for IgG/ELISA/TAUPIR screens was performed based on the ELISA and TAUPIR results. Ranking the ELISA and TAUPIR results was performed as explained in the methods section. TAUPIR staining was almost identical for the five first clones and this corresponded to the ELISA results. 4C12 was discarded as it was found in the same plate as 4C1 which increased the likelihood of the 2 clones being the same (recognizing the same epitope). The best 4 clones selected were 3A8, 2B6, 4C1 and 6H1. The other 6 clones (4C12, 2G1, 2F9, 7D6, 3B9, 4E12) were kept as back-up.

A ranking of the 10 clones that showed positivity in ELISA screen and TAUPIR screen was performed to select the best ones (Table 4). Highlighted in grey are the best 5 clones.

1.2.3 ACI-41 hybridomas

The cell supernatants from the 8x96 well plates resulting from the fusion were screened for production of IgG. In the 768 wells (8x96 wells) tested 215 wells were positive for IgG expression and were transferred to 24 wells plates. In the 24 well plates 81 clones were growing and supernatant from those cells were analysed. Positive clones were further transferred in T25 flasks and supernatants screened for IgG production, ELISA and TAUPIR (table 5).

The clones 5D10 and 7C2 were the only ones positive in the 3 screens and were selected for subcloning. The clone 5D10 binds only the peptide T8.5, while the clone 7C2 binds to the two peptides of the ACI-41 vaccine (T8.5 and T9.5) (see Figure 10 in PCT application PCT/EP2010/054418).

The subclone 5D10A4 originating from 5D10 was specific for pTau peptide.

1.3. Conclusion

The antibodies generated have shown high specificity to pTau peptides with only marginal binding to non-phosphorylated peptides.

From the 4 fusions (ACI-33, ACI-36, ACI-35 and ACI-41), a total of 16 clones were deposited at DSMZ (table 1) and selected for further subcloning.

The positive motherclones mentioned above were further cultivated in 96 well plates, then 24 well plates and finally T25 flasks. At each stage, the supernatants of the hybridoma clones were screened by ELISA, Taupir and Western Blot.

EXAMPLE 2: Cloning of Antibody Light Chain and Heavy Chain Variable Regions

Antibody heavy and light variable region genes from the hybridoma cells are cloned and the DNA sequences and location of the complementarity determining regions (CDRs) determined as well as the antibodies binding features.

Total RNA was prepared from 3 x 10^6 hybridoma cells (1 vial) using the Qiagen RNeasy mini kit (Cat No: 74104). RNA was eluted in 50mL water and checked on a 1.2% agarose gel.

V_H and V_K cDNAs were prepared using reverse transcriptase with IgG and kappa constant region primers. The first strand cDNAs were amplified by PCR using a large set of signal sequence primers. The amplified DNAs were gel-purified and cloned into the vector pGem® T Easy (Promega). The V_H and V_K clones obtained were screened for inserts of the expected size. The DNA sequence of selected clones was determined in both directions by automated DNA sequencing. The locations of the complementarity determining regions (CDRs) in the sequences were determined with reference to other antibody sequences (Kabat EA *et al.*, 1991).

EXAMPLE 3: Binding Studies I

The objective was to measure the phospho-Tau (pTau) binding of the antibodies generated from subcloned hybridomas derived from mice immunized with the tau liposomal vaccines. To test this, an enzyme-linked immunosorbant assay (ELISA) was used to measure the binding of the purified antibodies to both phosphorylated and non-phosphorylated full-length Tau protein, as well as the phosphorylated and non-phosphorylated Tau antigenic peptides used for the liposomal vaccine preparation.

The screening was completed by two other methods. Immunohistochemistry (IHC) on brain sections from a Tau transgenic animal (TAUPIR) using an anti-tau antibody as the primary antibody was done. Additionally, a western blot (WB) on brain protein homogenates from Tau transgenic mice was performed, using an anti-tau antibody as the blotting antibody.

3.1 Methods

3.1.1 Phospho-Tau binding assay

The anti-phospho Tau antibodies (mouse IgG3 isotype) were generated from liposomal tau vaccinated mice. The liposomal vaccines are phosphorylated preparations of a phospho-Tau (pTau) peptide. The hybridoma sub-clones producing the anti-tau antibodies were selected by limiting dilution from the mother-clones. Isotyping was done to indicate the presence of a single isotype clone. The antibodies was produced in roller-bottles, purified by affinity chromatography, subjected to sterile 0.22 µm filtration, and quantified. To test the binding of the antibody to Tau and pTau, an ELISA assay was used. Briefly, Nunc MaxiSorp 96-well plates (Nunc, Roskilde, Denmark) were coated with 1 µg/mL of full-length (441 aa) Tau protein (SignalChem, Richmond, Canada) or phosphorylated full-length (441 aa) Tau protein (Vandebroek et al., 2005). Additionally, plates were coated with 10 µg/mL of the Tau-derived peptide. To test for cross-reactivity to Tau and pTau sequences that were not used in the vaccine preparation, plates were coated with 10 µg/mL of the following peptides: Tau5-20 (phosphorylated or not on Y18), Tau393-408 (phosphorylated or not on S396 and S404), Tau401-418 (phosphorylated or not on S404 and S409), Tau206-221 (phosphorylated or not on T212 and S214), and Tau196-211 (phosphorylated or not on S202 and T205). Coating was done overnight in phosphate-buffered saline (PBS) at 4°C. Plates were washed thoroughly with 0.05% Tween20/PBS and then blocked with 1% bovine serum albumin (BSA) in 0.05% Tween20/PBS for 1 hr at 37°C. The antibody being tested was then added in an 8 or 16 two-fold dilution series between 20 and 0 µg/mL, and allowed to incubate for 2 hr at 37°C. Plates were then washed as described previously, and AP-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Suffolk, England) was added at 1/6000 dilution in 0.05% Tween20/PBS for 2 hr at 37°C. After washing, plates were incubated with *p*-nitrophenyl phosphate disodium hexahydrate (pNPP; Sigma-Aldrich, Buchs, Switzerland) phosphatase substrate solution, and read at 405 nm following 2 or 16 hr incubation using an ELISA plate reader. Results are expressed as optical density (O.D.).

3.1.2 Binding of anti-Tau antibody to Tau tangles in brain sections from a Tau transgenic animal (TAUPIR)

Brain slices used were from old (>18 months old) double transgenic biGT (GSK-3β transgenic mice crossed with TPLH mice, containing the longest isoform (441aa) of human Tau with the P301L mutation) transgenic mice. Additionally, sections from Tau knock-out mice (TKO; 6 months old) were also used. Brain sections were washed for 5 min in PBS then incubated for 15 min at RT in 1.5% H_2O_2 in PBS:MeOH (1:1) to block endogenous peroxidase activity. After washing the sections 3 times in PBST (PBS/0.1% TritonX100) they were incubated for 30 min at RT in PBST+10% FCS (fetal calf serum) blocking solution. The incubation with the anti-Tau antibody being tested was done overnight at 4°C at indicated dilutions in PBST/10% FCS. Sections were next washed 3 times in PBST before incubation with an HRP-conjugated goat anti-mouse (purchased from Dako, Glostrup, Denmark) secondary antibody in PBST/10% FCS for 1 hour at RT. Prior to detection, sections were washed 3 times with PBST and incubated in 50 mM Tris/HCl pH7.6 for 5 min. Detection was done by incubating the sections for 3 min in Diaminobenzidine (DAB: 1 tablet in 10 ml of 50 mM Tris.HCl + 3 ul H_2O_2 30%; MP Biomedicals, Solon, OH, USA). The reaction was stopped by washing the sections 3 times in PBST. Sections were then transferred onto silanized glass-plates and air-dried on warm-plate at 50°C for 2 hours. Counterstaining was done using incubation with Mayers hematoxylin (Fluka Chemie, Buchs, Switzerland) for 1 min, followed by a washing step for 4 min in running tap-water. Sections were dehydrated by passing in 50%, 70%, 90% and twice in 100% ethanol bath then in Xylol 2 times for 1 min. Finally sections were mounted with DePeX (BDH Chemicals Ltd., Poole, England) under glass cover-slips.

Additionally, hybridoma supernatants at 1/10 dilution (all ACI-35-derived antibodies shown in Table 1) were used to blot membranes containing SDS-PAGE separated brain homogenate proteins from Tau transgenic mice, wild-type mice, or Tau knock-out mice.

3.1.3. Binding of anti-Tau antibody to Tau tangles in brain sections from AD and tauopathy patients (TAUPIR)

The assay for the immunoreaction of the anti-pTau antibody ACI-36-3A8-Ab1 to pTau in human brain was done by TAUPIR. Brain paraffin sections were de-paraffinized by passing in Xylol 2 times for 5 min and 2 times for 1 min in 100% EtOH, followed by 1 min wash in 90%, 70%, and 50% EtOH and distilled water, followed by 2 times 5 min washes in PBS. For antigen retrieval, sections were treated by heating for 10 min in 0.01 M citric acid solution in water (pH 6.0) and cooled down for 20 min. Sections were incubated for 15 min at RT in 1.5% H_2O_2 in PBS:MeOH (1:1) to block endogenous peroxidase activity. After washing the sections 3 times in PBST (PBS/ 0.05%Tween-20), they were incubated for 30

min at RT in PBST + 10% fetal calf serum (FCS) as blocking solution. The incubation with the primary anti-pTau antibody ACI-36-3A8-Ab1 (410 ng/mL in blocking buffer) was done overnight at 4°C. Sections were then washed 3 times in PBST before incubation with HRP-conjugated goat anti-mouse secondary antibody (Dako, Glostrup, Denmark) diluted 1/500 in PBST/10% FCS, for 1 hour at RT. Prior to detection, sections were washed 3 times with PBS and incubated in 50 mM Tris/HCl pH 7.6 for 5 min. Detection was done by incubating the sections for 3 min in diaminobenzidine (DAB: 1 tablet in 10 mL of 50 mM Tris-HCl + 3 µL H_2O_2 30%; MP Biomedicals, Solon, OH, USA). The reaction was stopped by washing the sections 3 times in PBS. Counterstaining was done by incubating with Mayer's hematoxylin (Fluka Chemie, Buchs, Switzerland) for 1 min, followed by washing for 4 min in running tap-water.Sections were dehydrated by passing through 50%, 70%, 90% and twice in 100% ethanol baths, followed by Xylol for 2 times 1 min.

Finally, sections were mounted with DePeX (BDH Chemicals Ltd., Poole, England) under glass cover-slips. Stained sections were examined by white light microscopy and digital images taken with a 3CCD camera (Leica, Wetzlar, Germany). Images were captured and analyzed using dedicated software (IM500, Leica). Images are shown at 20x1.6 magnification.

3.1.4. Western Blot (WB)

Binding of the test antibody to pTau in the brain extract from transgenic animal was done by WB. Brain homogenization from wild-type FVB, TPLH, biGT and TKO mice was done in the following buffer: 25 mM Tris/HCl pH7.6, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 30 mM NaF, 0.2 mM Na_3VO_4, 1 nM Okadaic acid, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM Na4P2O7, 1 tablet complete protease inhibitor cocktail (CPIC) per 12 ml total. To obtain total brain homogenate the brain was homogenized on ice in 1 vol / weight hemisphere (ml / g) with a motor-driven potter-like glass tube / teflon pestle at 700 rpm. Total brain homogenates were diluted by half in sample buffer (125 mM Tris/HCl pH6.8, 4% (w/v) sodium dodecyl sulfate (SDS), 20% glycerol, 0.01% bromophenol blue and 5% beta-mercapto-ethanol), then heated rapidly to 95°C. Samples were kept 5 min, diluted ¼ in sample buffer, heated again to 95°C and then cooled down and spun at 14.000 rpm for 5 min to clear debris that were not solubilized. Supernatants were collected and loaded onto a SDS-PAGE gel. The transfer to nitrocellulose membrane (Hybond-ECL) was done in transfer buffer (25 mM Tris pH 8.6, 190 mM Glycine, 20% methanol). Membranes were transferred to the blocking solution (0.1% Tween in TBS (50 mM Tris.HCl, pH7.6, 150 mM NaCl, and 5% dry-milk powder) prior to overnight incubation at 4°C with the test antibody

diluted in the blocking solution. Incubation with secondary antibody HRP-conjugated goat anti-mouse (Dako, Glostrup, Denmark) diluted 1/10'000 in blocking solution was performed at RT for 1 hour. Detection was done using the ECl Western Blotting Detection Reagents from GE Healthcare.

3.2 Results

3.2.1 ELISA assays and TAUPIR using brain sections from tangle positive Tau transgenic mice

The binding of antibodies were measured against the phosphorylated Tau peptide used as the immunogen, and against the phosphorylated full-length human Tau protein. This is the longest isoform of human Tau protein consisting of 441 amino acids. The corresponding non-phosphorylated peptide and full-length human Tau protein were also included. As indicated in the table 6 antibodies demonstrated high binding to the phosphorylated Tau peptide, with only limited or no binding to the phosphorylated full-length human Tau protein. No binding was observed to the corresponding non-phosphorylated Tau peptide or to the non-phosphorylated full-length human Tau protein. This demonstrates high binding of anti-tau antibodies to phosphorylated human Tau peptides.

To test for non-specific binding to other phosphorylated and non-phosphorylated Tau sequences, the antibody was tested for binding to five phospho and non-phospho Tau peptides one of which was used as the antigen peptide sequence. No cross-reactivity to phospho or non-phospho Tau peptides, other than the peptide used in the vaccine was observed, even at high concentrations of peptide.

The binding of anti-tau antibodies to pTau in brains of Tau transgenic mice was evaluated by TAUPIR staining (Figure 1) and by WB (Figure 1). Antibodies demonstrated binding to Tau tangles and neuropil threads present in cortex and hippocampus in brains of Tau transgenic (biGT) mice. The antibody dilutions used for the TAUPIR ranged from 0.05 to 0.0033 ug/mL. Anti-tau antibodies were also used as a primary antibody in a WB using total brain homogenates from wild-type FVB, TPLH, biGT and TKO mice, and separated by SDS-PAGE. Two commercial anti-pTau antibodies were used as controls, MC1 and Tau5. All anti-tau antibodies bound to pTau present in brains of Tau transgenic mice. Blotting

On membranes containing SDS-PAGE separated protein homogenates from Tau transgenic mice, wild-type mice, and Tau knock-out mice, all ACI-35 antibodies (disclosed in Table 1) bound to protein bands having identical 46 kDa migration pattern as Tau and pTau (data not shown).

3.2.2 TAUPIR study in brain sections from AD and tauopathy patients

The ability of antibody ACI-36-3A8-Ab1 to bind to Tau-aggregates, lodged in human brain sections from subjects with diagnosed tauopathies, including AD, FAD, AGD, FTDP-17, CBD, and PSP, was examined by TAUPIR immunohistochemistry (Figure 2). The anti-pTau antibody ACI-36-3A8-Ab1 bound to pTau containing neurofibrillary tangles (NFTs), neuropil threads in human brain sections, and other forms of pTau accumulations present in neurons and in glial cell-types. More specifically, ACI-36-3A8-Ab1 prominently stained NFTs, neuropil threads, and dystrophic neurites surrounding amyloid plaques in AD brains, which was readily apparent in the subjects diagnosed with AD and FAD. In brain sections from AGD, ACI-36-3A8-Ab1 stained both NFTs and neuropil threads, with multiple argyrophilic grains/granules clearly visible (Figure2,). Staining of brain sections from PSP with ACI-36-3A8-Ab1 showed NFTs, neuropil threads, and dystrophic neurites. Additionally, Pick body-like inclusions and tufted pTau positive astrocytes were clearly noted, being an abundant feature in PSP, where pTau staining extends throughout the cell, including in distal processes. In FTDP-17, the staining pattern also illustrated the known heterogeneity of the disease, with not only NFTs but also achromatic "ballooned" neurons detected. The ACI-36-3A8-Ab1 antibody also stained swollen achromatic neurons that were faintly Tau-positive, the main characteristic of CBD. Another prominent pathological feature of CBD, i.e. oligodendroglial inclusions, called coiled bodies, were also well detected by the ACI-36-3A8-Ab1 antibody. No staining was detected in an AT8-negative control subject whereas weak staining was identified in an AT8-positive control subject.

Using TAUPIR on human brain sections from subjects previously diagnosed with different forms of tauopathy, the anti-pTau antibody ACI-36-3A8-Ab1 demonstrated good binding to various known pTau-rich pathological features present in the brains of these subjects.

EXAMPLE 4: Binding Studies II

The objective of the study was to determine the binding affinity between anti-tau antibodies and the phospho-tau peptide using Surface Plasmon Resonance (SPR). Phospho-tau peptide correspond to the peptide sequence used in the vaccine preparation to generate the anti-tau antibody. To study this interaction, phosphopeptides were immobilized to the surface of a sensor chip and the binding monitored in real-time using SPR upon passing antibody over the chip.

4.1 Methods

4.1.1 SPR binding assay

All SPR experiments were carried out on a Biacore X instrument (GE Healthcare). Reagents for immobilization (EDC, NHS and Ethanolamine), sensor chip CM5 (carboxymethyl dextran) as well as running buffer HBS-EP were purchased from GE Healthcare. Phospho-tau peptide were solubilized in PBS / sodium acetate buffer (10 mM, pH 5.0) in a 1:1 (v/v) ratio to give a final peptide concentration of 250 µg/ml. This peptide solution was then coupled via to flow cell (fc) 2 of a CM5 sensor chip that was preactivated using EDC/NHS. After coupling, Ethanolamine was passed over the surface and giving a final immobilization level of 218 RUs. Five concentrations of the anti-tau antibodies were assayed by serial dilutions using running buffer. Injections were performed starting from the lowest concentration and were passed over both fc 1 and 2 at a flow rate of 30 µL/min for 180 s. Flow cell 1 was underivatized and responses were subtracted from fc 2 to correct for instrument noise and bulk refractive changes. After injection was finished, the surfaces were washed immediately with running buffer for 300 s. To remove remaining bound antibody from the chip, surface regeneration was performed by injecting a pulse (typically 3 µl) of 8 mM NaOH in water containing 1M NaCl. Kinetic analysis was performed using algorithms for numerical integration and global analysis using BIAevaluation 3.0. The sensograms obtained for injections of antibody at different concentrations were overlaid and the baselines adjusted to zero. For curve fitting, all data were fit simultaneously to a 1:1 homogeneous (Langmuir) model.

Alternatively, immobilized biotinylated T3 peptide (T3.30) was immobilized to a Streptavidin Biacore SA chip (GE Healthcare) using a Biacore X instrument. Antibodies were diluted in HBS-EP running buffer (GE Healthcare) and injected at 50ul/min for 120s followed by 100s dissociation. Surface regeneration was performed using a pulse (1-3 ul) of 16 mM NaOH. Fitting was performed using BIAevaluation and assuming a 1:1 Langmuir binding interaction.

Peptides used

T1.5	H-K(Ac)K(Ac)-RQEFEVMEDHAGTY[PO3H2]GL-K(Ac)K(Ac)-NH2	lot AW11309D
T4.5	H-K(Ac)K(Ac)-GDTS[PO3H2]PRHLS[PO3H2]NVSSTGSID-K(Ac)K(Ac)-NH2	lot CF09168
T3.30	Biotin-LC linker-GVYKS[PO3H2]PVVSGDTS[PO3H2]PRHL-NH2	lot MI89P9-P12-2

4.2 Results

The binding of the anti-tau antibodies to the phosphorylated Tau peptide was monitored in real-time using SPR. Analyses of the association and dissociation phases of antibody binding could be used to determine the association rate constant (k_a), dissociation rate constant (k_d) as well as dissociation constant K_D. Antibody ACI-33-6C10-Ab1 binds specifically to peptide T1.5 over the non-derivatized carboxymethyl dextran surface in the range 3.7 → 367 nM of antibody. Kinetic analyses of the sensograms revealed a fast association rate constant of 9.46 x 10^5 $M^{-1}s^{-1}$ and a dissociation rate constant of 3.27 x 10^{-3} s^{-1} (Table7). The dissociation constant K_D was determined therefore to be 3.46 nM showing that the antibody recognizes the phosphopeptide T1.5 with very high affinity. All tested antibodies displayed a high affinity to their respective phosphopeptides used for immunization and hybridoma generation, but they displayed little affinity to non-phosphopeptides.

EXAMPLE 5: Epitope mapping of anti pTau antibodies

5.1 Methods

Epitope mapping of anti-phospho Tau mouse monoclonal antibodies was performed by ELISA using different phospho and non-phospho peptide libraries. The amino acid sequences of peptide libraries used are shown in Table 8. Each library consisted of short biotinylated peptides spanning phospho and non-phospho sequences present in the peptide vaccine. Peptide libraries were purchased from ANAWA Trading SA. Epitope mapping was done according to the manufacturer's (Mimotopes) instructions. Briefly, streptavidin coated plates (NUNC) were blocked with 0.1% BSA in phosphate-buffered saline (PBS) overnight at 4°C. After washing with PBS-0.05% Tween 20, plates were coated for 1 hr at RT with the different peptides from each library, diluted in 0.1% BSA, 0.1% sodium azide in PBS to a final concentration of 10 µM. After washing, plates were incubated for 1 hr at RT with the antibody to be tested diluted to 40 ng/ml in 2% BSA, and 0.1% sodium azide in PBS. Plates were washed again and incubated with AP-conjugated anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Suffolk, England) at 1/6000 dilution for 1 hr at RT. After a final wash, plates were incubated with *p*-nitrophenyl phosphate disodium hexahydrate (pNPP; Sigma-Aldrich, Buchs, Switzerland) phosphatase substrate solution, and read at 405 nm following 2 hr incubation using an ELISA plate reader. Binding was considered positive if the optical density (O.D.) was at least 2-times over background O.D.

5.2 Results

As a result of the epitope mapping experiments, epitopes could be identified including the required phosphorylated amino acid residue (see table 9) to which the antibodies disclosed herein specifically bind.

- Tau aa 15-20, with requirement for pY18 (6C10F9C12A11; 6C10E5E9C12)
- Tau aa 405-412, with requirement for pS409 (6H1A11C11; 6H1G6E6)
- Tau aa 405-411, with requirement for pS409 (2B6A10C11; 2B6G7A12; 3A8A12G7; 3A8E12H8)
- Tau aa 208-218, with requirement for pT212 and pS214 (7C2(1)F10C10D3)
- Tau aa 393-401, with requirement for pS396 (A4-2A1-18; A4-2A1-40)
- Tau aa 396-401, with requirement for pS396 (A4-4A6-18)
- Tau aa 394-400, with requirement for pS396 (A6-1D2-12)
- Tau aa 402-406, with requirement for pS404 (A6-2G5-08)
- Tau aa 393-400, with requirement for p396 (A6-2G5-30; A6-2G5-41)

EXAMPLE 6: 1-week passive Immunization of Tau Transgenic Mice

6.1. Methods

For all in vivo studies, Tau transgenic mice were used and administred the treatment antibodies as shown in the Table below.

Transgenic mice and antibodies used for *in vivo* studies

Study no.	Tau transgenic model	Age of mice at study start (months)	Study duration (weeks)	Antibodies administered	Doses (mg/kg)	Number of i.p. administrations	Readout
1	TMHT (h*Tau*$^{V337M/R406W}$)	6.3	1	ACI-36-2B6-Ab1	0*, 3 or 10	2	MSD, IHC, WB
				ACI-36-3A8-Ab1	0 or 3		
2	TMHT (h*Tau*$^{V337M/R406W}$)	4.2	4	ACI-36-2B6-Ab1 or ACI-36-3A8-Ab1	0, 1 or 3	4	MSD, IHC, WB, MWM
3	TMHT (h*Tau*$^{V337M/R406W}$)	3.0	12	ACI-36-2B6-Ab1 or ACI-36-3A8-Ab1	0, 1 or 3	13	MSD, IHC, WB, MWM

4	biGT (hTau^{P301L} x hGSK3β)	4.5	12	ACI-36-2B6-Ab1 or ACI-36-3A8-Ab1	0, 1 or 3	13	WB

*vehicle control for all of the studies; intraperitoneally (i.p.)

6.1.1. Mice and treatments

Female and male 6.3 months old (±3 days) Tg mice over-expressing the full-length human TAU isoform TAU441, bearing the missense mutations V337M and R406W under the control of murine Thy-1 promoter (TMHT mice), were used for Study no.1 (see Table above). Mice were euthanized 1 day following the last administration to determine TAU pathology in the brain.

6.1.2 Animal Identification and Housing

In the course of tail tipping for genotyping, animals were numbered consecutively by classical earmarking. All animals were re-genotyped prior to the start of the study. Mice were kept according to the JSW Standard Operating Procedures based on international standards. Animals were housed in individual ventilated cages on standardized rodent bedding supplied by Rettenmaier®. The temperature was maintained at approximately 24 °C and the relative humidity was maintained between 40 to 70 %. Animals were housed under a constant light-cycle (12 hours light/dark). Dried, pelleted standard rodent chow (Altromin®) and normal tap water were available to the animals ad libitum. Each individual animal was checked regularly for any clinical signs that were noted in the individual animal datasheet.

6.1.3 In vivo bleedings

Seven days before the first immunization, in vivo bleedings were performed by mandibular sampling from the facial vein/artery. The blood samples are a mixture of venous and arterial blood. To get plasma, blood was collected in heparin tubes and centrifuged (1000 x g, 10 minutes, room temperature). Plasma was frozen in two aliquots until used.

6.1.4. Immunohistochemical (IHC) quantitation

All cryo-frozen brain hemispheres were analyzed. 15 cryo-sections per level (altogether 5 levels), each 10µm thick (Leica CM 3050S) were sagittally cut. Brain levels were chosen according to the morphology atlas "The Mouse Brain" from Paxinos and Franklin (2nd edition). The cut of the five levels started with a random slice then sampling continued uniformly and systematically, always retaining 15 slices per level in series and discarding 150 µm in between the levels. For determination of TAU pathology in the hippocampus and

the amygdala 5 slices (1 from each level) per brain region and animal were stained using AT180 (# MN1040, Thermo Scientific) and HT7 (# MN1000, Thermo Scientific) antibodies. The primary antibodies were visualized by Cy-3-coupled secondary antibody (Jackson Laboratories) and subsequently immunoreactive area were evaluated using Image Pro Plus (v6.2) software.

Immunoreactive objects were measured above a size restriction (30 μm^2 in the amygdala, 7 μm^2 in the hippocampus) and above a dynamic intensity threshold. Total area and intensity of objects and the individual threshold were automatically filed. If used, a dynamic threshold was defined as "mean intensity within AOI plus factor times the standard deviation of pixel intensities within the AOI". In any case, values had to exceed a minimal set threshold. Exact threshold levels are given in the table below.

Thresholds	Minimum	Dynamice factor
AT180 Amygdala	25	2
AT180 Hippocampus	28	-
HT7 Amygdala	35	2
HT7 Hippocampus	25	0.5

The region size was measured by manual delineation of the hippocampus and amygdala. HT7 and AT180 IR area data were normalized to the regions size.

All IHC related data with n > 4 followed a Gaussian distribution according to Kolmogorov Smirnov normality test and are represented as mean + SEM. For the vehicle group consisting of four animals only, thus too few for normality testing, Gaussian distribution was assumed. Group differences were calculated by means of a parametric one-way ANOVA followed by Newman Keuls post hoc testing, calculated with GraphPadPrism software. The alpha-error level was set to 0.05.

Brain TAU pathology was determined in hippocampus and amygdala by immunohistochemical (IHC) quantitation using AT180 (anti-pTAU) and HT7 (anti-TAU) antibodies. Furthermore, the treatment effects on soluble pTAU and TAU in cortex and hippocampus was measured in the soluble homogenate fraction using MesoScale Discovery (MSD) duplex technology, probing for pTAU and total TAU.

None of the antibodies used for either the IHC or the MSD assays have an epitope that overlaps with the two treatment antibodies used in this study.

6.1.5. Generation of fraction for the quantification of soluble Tau protein level in the soluble brain fractions of Tg mice

Mice treated according to method 6.1.1. were euthanized 1 day following the second administration to determine Tau pathology in the brain. Briefly, soluble cortex samples from one brain hemisphere were homogenized in 100 to 200 µL of cold extraction buffer (25 mM Tris-HCl pH=7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate, 30 mM NaF, 2 mM Na_3VO_4, protease and phosphatase inhibitor cocktail). The homogenates were centrifuged (74,200 x g for 15 min at 4°C) and the supernatants were used for the analysis of soluble Tau. The concentration of total protein in the soluble fractions of cortex samples was determined by a BCA protein quantitation assay (Thermo Fisher Scientific, Rockford, IL, USA).

6.1.6. Analysis of pTau presence by Western Blot

To probe for immunoreactivity in the brains of mice administered ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2, two antibodies reported to bind pTau PHF epitopes (Greenberg et al., 1992; Reig et al., 1995; Hoffmann et al., 1997) were used in Western-blot (WB) assays. Soluble fractions from cortex were diluted by adding an equal volume of sample buffer A (125 mM Tris-HCl pH 6.8, 4% [w/v] sodium dodecyl sulfate [SDS], 20% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol), and the samples were heated to 95°C for 10 min. 30 µg of sample was loaded onto a 4-12% Bis-Tris gel (Invitrogen, Basel, Switzerland) and run in MOPS SDS buffer (Invitrogen). Proteins were transferred to a 0.45 µm PVDF membrane in transfer buffer (25 mM Tris pH 8.6, 190 mM glycine, 20% methanol). To verify protein transfer, the membranes were stained with Ponceau S for 5 min, washed, and blocked for 1 hour in blocking buffer (5% BSA in TBS [50 mM Tris-HCl, pH 7.6, 150 mM NaCl]). Membranes were blotted over-night at 4°C with the primary antibodies in blocking buffer and 0.1% Tween. The two pTau PHF-specific primary antibodies used for the WBs were: anti-pS396 (PHF-13 epitope; AbCam, Cambridge, UK; used at 3 µg/mL), specific to phosphorylated Ser396 (pS396) of human or murine pTau (Hoffmann et al., 1997), and AD2 (PHF-1 epitope; BioRad, Reinach, Switzerland; used at 0.4 µg/mL), specific for human and murine pS396 and phosphorylated Ser404 (pS404; Reig et al., 1995). For total Tau WBs, Tau5 (0.5 µg/mL), an antibody that binds both human and murine Tau (BD Biosciences, Allschwil, Switzerland), was used. Following incubation with the primary antibody, membranes were washed with 0.1% Tween in TBS, and incubated with the secondary antibodies: goat anti-mouse-IRDye800 or goat anti-rabbit-IRDye680 (both from Li-Cor Biosciences, NE, USA), both diluted 1:15000 in BB and 0.1% Tween. Membranes were then incubated 1 hour at room-temperature protected from light, washed for 15 min 3-times with 0.1% Tween in TBS, and for 5 min 2-times with TBS, and bands quantified using Li-Cor Odyssey near-infrared imaging system (Li-Cor). Bands were normalized to β-actin

expression (AbCam; used at 0.4 µg/mL). To verify the identification of the human transgenic versus the mouse endogenous Tau bands, blots were probed with an antibody specific for human total Tau (Tau13, AbCam; not shown). Additionally, membranes were probed with an anti-mouse primary antibody, to verify that the treatment antibodies, ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2, were not present in the denatured test samples in sufficient quantity to interfere with the binding of anti-pS396 or AD2. No intact or denatured treatment antibodies were detected (results not shown) in the samples used for this study.

6.1.7. Statistical analysis

Data were analyzed using non-parametric Kruskal-Wallis rank sum statistics, and if significant at the $P<0.05$ level, a Dunn's post-hoc test was used comparing all groups (GraphPad Prism, GraphPad Software, CA, USA). Results are presented as individual data points showing mean ± SEM. Differences with $P<0.05$ were considered as statistically significant.

6.2 Results

6.2.1. Brain TAU pathology by immunohistochemical (IHC) quantitation

Two i.p. injections of ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 did not show any gross adverse effects during the study period. Staining for pT231 and pS235 using AT180 by IHC, showed increased immunoreactive area (IR) in the amygdala following ACI-36-3A8-Ab2 treatment (). Mice treated with 3 mg/kg ACI-36-2B6-Ab2 had significantly less AT180 IR area in the hippocampus ().

ACI-36-3A8-AB2 treatment increased AT180 IR pTAU compared to the PBS group in the amygdala. In the hippocampus ACI-36-2B6-AB2 treatment decreased pTAU. AT180 specifically labels pTAU. The frequency of AT180 IR cells was decreased in ACI-36-2B6-AB2 treated mice. This effect was stronger in the low dose (3 mg/kg) group (ACI-36-2B6-AB2 LD). The somal staining pattern does not differ among groups.

At the higher 10 mg/kg dose, a non-significant trend for less AT180 IR was seen in the hippocampus for both ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2, when compared to vehicle control treated mice. Qualitatively, ACI-36-2B6-Ab2 treated animals showed a lower number of hippocampal neurons with highly intense AT180 labeling.

6.2.2. Reduction of total Tau level in brain fraction following passive immunization

The effect of the treatments on pTAU and TAU in the brain fraction containing soluble proteins was measured using an MSD duplex assay. Levels of total soluble TAU in the

cortex was significantly reduced in mice treated with ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 (p<0.01; Figure 3 upper panel). The levels of soluble pTAU was also significantly reduced (p<0.05; Figure 3 lower panel), with the 3 mg/kg dose of ACI-36-2B6-Ab2 demonstrating the greatest decrease (p<0.01). The ratio of pTAU to total TAU remained unchanged. The levels of soluble TAU and pTAU did not change in samples from hippocampus (not shown here).

6.2.3. Effects of ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 Administration on the Presence of Phospho-Tau Epitopes present in Paired Helical Filaments (PHFs)

Structurally, neurofibrillary tangles (NFTs) consist of paired helical filaments (PHFs) composed of the microtubule-associated protein Tau, found primarily in a hyper-phosphorylated state (Alonso et al., 1997). The objective of this study was to use antibodies that recognize pTau PHF to probe for and quantify these pTau PHF epitopes in the brains of Tau transgenic mice, following the administration of ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2.

To measure the effects of two ACI-36-2B6-Ab2 or ACI-36-3A8-Ab2 administrations on the quantity of well documented Tau PHF phospho epitopes, brain cortex soluble fractions from treated Tau Tg mice were probed with AD2 (PHF-1 epitope, pS396/pS404) and anti-pS396 antibody (PHF-13 epitope, pS396) using WBs. The immunoreactivity was quantified using an infrared imaging system. The effects of ACI-36-3A8-AB2 and ACI-36-2B6-AB2 treatment on AD2 PHF immunoreactivity in the cortex of Tau Tg mice were determined using AD2 which probes for pS396 and pS404, two previously documented PHF phospho residues of Tau (Greenberg et al., 1992; Reig et al., 1995).

Bands, indicating human and mouse pTau phosphorylated on S396 and S404 using the AD2 (PHF-1) antibody, were quantified using a Li-Cor infrared imaging system. Values for individual mice as well as the mean ± SEM are determined.

A non-significant trend was observed for a reduction in AD-2-positive pTau immunoreactivity was observed for the transgenic human pTau band. However, a significant reduction in the quantity of mouse AD2-positive pTau was observed in mice treated with 3 mg/kg of ACI-36-2B6-Ab2, and a non-significant trend when treated with either 10 mg/kg of ACI-36-2B6-Ab2 or ACI-36-3A8-Ab2 .

When a different antibody that specifically recognizes pTau pS396 was used for staining (Hoffmann et al., 1997), an even greater effect was observed. Mice treated with 3 mg/kg of ACI-36-2B6-Ab2 had significantly less pS396-positive human transgenic and mouse endogenous pTau, with a trend towards reduction when treated with 10 mg/kg ACI-36-2B6-Ab2 or ACI-36-3A8-Ab2. To assess the effects on total human and mouse Tau, which

includes both non-phosphorylated and all pTau, blots were probed with the Tau5 antibody. Compared to vehicle control, total Tau was not modulated by ACI-36-2B6-Ab2 or ACI-36-3A8-Ab2 administered at 10 mg/kg, however a trend for reduced total Tau was observed for mice administered ACI-36-2B6-Ab2 at 3 mg/kg.

6.2.4 Summary

Two peripheral administrations of Tau Tg mice with the anti-pTAU antibody ACI-36-3A8-Ab2 significantly reduced soluble TAU and soluble pTAU in the brain cortex. Two peripheral administrations of Tau Tg mice with the anti-pTAU antibody ACI-36-2B6-Ab2 significantly reduced soluble TAU and soluble pTAU in the brain cortex. Additionally, ACI-36-2B6-Ab2 significanly reduced pTAU immunoreactivity in the hippocampus. These results demonstrate the ability of passive anti-pTAU immunization, using ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 antibodies, in reducing tauopathy.

Two peripheral administrations of ACI-36-2B6-Ab2 at 3 mg/kg to Tau Tg mice reduced the presence of pTau PHF epitopes in the cortex as measured by Western-blotting. At a higher dose of 10 mg/kg, both ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 showed a trend towards reduced pTau PHF epitope immunoreactivity. These results show that ACI-36-2B6-Ab2 and ACI-36-3A8-Ab2 antibodies may be suitably used in passive immunotherapy against tauopathies such as Alzheimer's Disease.

EXAMPLE 7: 1-month treatment of human Tau over-expressing mice

7.1 Methods

7.1.1 Mice and treatments

Tau transgenic mice were used and administred the treatment antibodies as shown in the Table in Method 6.1. (study no.2.)

7.1.2. Behavioral testing – Morris water-maze (MWM) task

Following the last administration, a water-maze (MWM) task was performed to test for spatial memory performance on mice treated according to 6.1.1. The MWM testing was performed with all enclosed animals in week 4 after start. The MWM consists of a white circular pool with a diameter of 100 cm, filled with tap water at a temperature of 21±2 °C. The pool is virtually divided into four sectors. A transparent platform (8 cm diameter) is placed about 0.5 cm beneath the water surface. During all test sessions, the platform is located in the southwest quadrant of the pool. Each mouse had to perform three trials on

each of four consecutive days. A single trial lasted for a maximum of one minute. During this time, the mouse had the chance to find the hidden, diaphanous target. After each trial mice were allowed to rest on the platform for 10-15 sec to orientate in the surrounding. At least one hour after the last trial on day 4, mice had to fulfill a so-called probe trial (PT). During the PT, the platform was removed from the pool and the number of crossings over the former target position was recorded by the experimenter together with the abidance in this quadrant. For the quantification of escape latency (the time [seconds] the mouse needed to find the hidden platform and therefore to escape from the water), of pathway (the length of the trajectory [meter] to reach the target), of target zone crossings and of the abidance in the target quadrant in the PT, a computerized tracking system (Biobserve Software) was used. All animals had to perform a visual test after the PT on the last day to exclude influence of insufficient seeing abilities on behavioral results.

7.1.3. Brain Tau pathology determination by immunohistochemical (IHC) quantitation

Mice were euthanized 1 day following the MWM (1 week following last administration) to determine Tau pathology in the brain. Brain Tau pathology was determined in hippocampus and amygdala by immunohistochemical (IHC) quantitation using AT180 (anti-pTau, pT231/pS235) and HT7 (human-specific anti-Tau) antibodies. Furthermore, the treatment effects on soluble pTau and soluble Tau in cortex and hippocampus was measured in the homogenate fraction using MesoScale Discovery (MSD) duplex technology, probing for pTau (pT231) and total Tau. None of the antibodies used for either the IHC or the MSD assays have an epitope that overlaps with the treatment antibody used in this study.

7.1.4. Sample preparation for the analysis of soluble Tau in cortex and hippocampus

Mice were euthanized for tissue collection, one week following the last treatment administration. Cortex and hippocampus were homogenized in 100 to 200 µL of cold extraction buffer 1 (25 mM Tris HCl pH=7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM β-glycerophosphate, 30 mM NaF, 2 mM Na_3VO_4, protease and phosphatase inhibitor cocktails). The homogenates were centrifuged (74,200 g for 15 min at 4°C) and the supernatants were used for the analysis of soluble Tau in cortex and hippocampus (Figure 4-1). The pellets were resuspended in 100-200 µL extraction buffer 2 (10 mM Tris HCl pH=7.4, 800 mM NaCl, 300 mM sucrose, 1mM EGTA, protease and phosphatase inhibitor cocktails) and transferred to a 1.5 mL tube. The solutions were centrifuged (4,000 g for 20 min at 4°C) and the supernatants transferred to ultracentrifugation tubes. Sarkosyl (a 30% aqueous solution) was then added to a final concentration of 1% and incubated for 1.5 hours at room temperature. After centrifugation (74,200 g for 30 min at 4°C) the

supernatants were discarded and the pellets were re-suspended in 100 µL buffer 3 (50 mM Tris-HCl, pH=7.4). The re-suspended pellets were used as sarkosyl-insoluble (SinT) Tau in cortex and hippocampus. The concentration of total protein in the soluble and SinT fractions samples was determined by a BCA protein quantitation assay (Thermo Fisher Scientific, Rockford, IL, USA).

7.1.5. Western Blots for pTau PHF and Tau

To evaluate the effect of ACI-36-2B6-Ab1 administration on the presence of pTau PHF in brain cortex and hippocampus, two antibodies reported to bind pTau PHF epitopes (Greenberg et al., 1992; Reig et al., 1995; Hoffmann et al., 1997) were used in Western-blot (WB) assays. Soluble and SinT fractions from cortex and hippocampus were diluted by adding an equal volume of sample buffer A (125 mM Tris-HCl pH 6.8, 4% [w/v] sodium dodecyl sulfate [SDS], 20% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol), and the samples were heated to 95°C for 10 min. 30 µg of sample was loaded onto a 4-12% Bis-Tris gel (Invitrogen, Basel, Switzerland) and run in MOPS SDS buffer (Invitrogen). Proteins were transferred to a 0.45 µm PVDF membrane in transfer buffer (25 mM Tris pH 8.6, 190 mM glycine, 20% methanol). To verify protein transfer, membranes were stained with Ponceau S for 5 min. Membranes were then washed, and blocked for 1 hour in blocking buffer (5% BSA in TBS [50 mM Tris-HCl, pH 7.6, 150 mM NaCl]). Membranes were blotted over-night at 4°C with the primary antibodies in blocking buffer and 0.1% Tween.

The two pTau PHF-specific primary antibodies used for the WBs were: anti-pS396 (PHF-13 epitope; AbCam, Cambridge, UK; used at 3 µg/mL), specific to phosphorylated Ser396 (pS396) of human or murine pTau (Hoffmann et al., 1997), and AD2 (PHF-1 epitope; BioRad, Reinach, Switzerland; used at 0.4 µg/mL), specific for human and murine pS396 and phosphorylated Ser404 (pS404; Reig et al., 1995). For detection of target effects, ACI-36-2B6-Ab1 was used for blotting at 1.6 µg/mL. For total Tau WBs, Tau5, an antibody that binds both human and murine Tau (BD Biosciences, Allschwil, Switzerland), was used at 0.5 µg/mL. All membranes were additionally blotted for β-actin (AbCam; used at 0.4 µg/mL) to normalize for protein loading.

Following incubation with the primary antibody, membranes were washed with 0.1% Tween in TBS, and incubated with the secondary antibodies: goat anti-mouse-IRDye800 or goat anti-rabbit-IRDye680 (both from Li-Cor Biosciences, NE, USA), both diluted 1:15,000 in BB and 0.1% Tween. Membranes were then incubated 1 hour at room-temperature protected from light, washed for 15 min 3-times with 0.1% Tween in TBS, and for 5 min 2-times with

TBS, and bands quantified using Li-Cor Odyssey near-infrared imaging system (Li-Cor). Bands of interest were normalized to β-actin expression. To verify the identification of the human transgenic versus the mouse endogenous Tau bands, blots were probed with an antibody specific for human total Tau and does not cross-react with murine Tau (Tau13, AbCam; not shown). Additionally, membranes were probed with an anti-mouse primary antibody, to verify that the treatment-antibody, was not present in the denatured test samples in sufficient quantity to interfere with the binding of the primary blotting antibodies. No intact or denatured treatment antibodies were detected (results not shown) in the samples used for this study. Values are expressed as arbitrary β-actin-corrected immunoreactivity (IR).

7.1.6. Statistical analysis

Data were analyzed using a one-way ANOVA, followed by Dunnett's multiple comparison post-hoc test (GraphPad Prism, GraphPad Software, CA, USA) comparing each treatment to Tg control-treated mice. Results are presented as individual data points showing mean ± SEM. Differences with $p<0.05$ were considered as statistically significant. Single values that were identified as significant ($p<0.05$) outliers by Grubb's extreme studentized deviate test, were excluded

7.2 Results

7.2.1. Behavioral testing – Morris water-maze (MWM) task following passive immunization

Four i.p. injections of ACI-36-2B6-Ab1 administered weekly at 3 mg/kg or 1 mg/kg over a four week period did not show any gross adverse effects.

During the last week of treatment, spatial navigation learning and memory of animals were evaluated. Animals had to fulfill 4 days of training with 3 trials per day followed by one probe trial and visual test. Escape latency (the time [seconds] the mouse needed to find the hidden platform and therefore to escape from the water), the pathway (the length of the trajectory [meter] to reach the target), the swim speed (calculated quotient of pathway and escape latency), the number of target crossings and the abidance in the target quadrant were evaluated.

Tg (group A) as well as nTg (F) control animals treated with vehicle showed the expected learning curves when evaluating escape latency and length of the swimming path to reach the platform over the four testing days. Tg control (A) animals had a significant learning impairment as shown by flatter learning curves in escape latencies and swimming paths compared to nTg control animals (F). Escape latencies and swimming path were

significantly (Two Way ANOVA) longer on training days 3 and 4 ($p<0.001$; Bonferroni´s post test). Treatment with ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1, low or high dose (groups B and C and D and E, respectively) did not lead to a significant improvement of spatial learning abilities compared to Tg control animals (group A) and showed similar learning curves. When adjusting the day 1 performance of each group to 100% and all further days as percentage of day 1, an improvement can be seen for the ACI-36-3A8-Ab1 treated mice (both dosages). The effect reached statistical significance for swimming path length on day 3 ($p<0.01$ group D and $p<0.05$ group E) and day 4 ($p<0.05$ group D).

For the ACI-36-2B6-Ab1 treated mice (both dosages) a slight improvement can be seen in swimming path length, although without statistical significance.

No differences between treatment groups were detected in terms of swimming speed on all four training days.

The results from the MWM test demonstrated trends toward improved spatial learning for mice treated with ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1.

7.2.2. Brain TAU pathology by immunohistochemical (IHC) quantification

The AT180 antibody stains the endogenous and human pTAU (doubly phosphorylated at Thr231 and Ser235).

AT180 IR in the amygdala and hippocampus after ACI-36-2B6-AB1 and ACI-36-3A8-AB1 immunization was determined. The AT180 IR area percentage in the amygdala and hippocampus was measured.

The amount of intrasomal pTAU in nTg controls was significantly lower compared to Tg groups ($p<0.001$). In the amygdala, a tendency to increase somal pTAU was observed for the 3 mg/kg ACI-36-2B6-Ab1 treatment. In contrast, both dosages of ACI-36-2B6-Ab1 tended to lower pTAU compared to vehicle treated animals in the hippocampus. Mean staining intensities were comparable in all transgenic groups.

ACI-36-3A8-Ab1 treatment did not alter somal pTAU in the hippocampus and amygdala and neuronal pTAU levels in the amygdala and hippocampus did not differ significantly among treated transgenic groups. Mean and sum of staining intensities were comparable in all transgenic groups.

Since the HT7 antibody is specific for human TAU, only little signal was measured in nTg controls, that derives from autofluorescence of lipofuscine dots above seven pixel in size. ACI-36-2B6-Ab1 treatment did not alter somal HT7 positive IR area in the hippocampus () compared to the vehicle control (PBS). In the amygdala, mice receiving the lower dose of

ACI-36-2B6-Ab1 tended to have higher levels of total human TAU (T-test: p = 0.0954) in terms of IR area (). This increase was also qualitatively visible as increase of the area of staining and the staining intensity in individual neuronal somata. No statistically significant treatment induced differences were observed in the hippocampal neurons.

ACI-36-3A8-Ab1 treatment did not significantly alter somal HT7 positive IR area in the amygdala () and the hippocampus () compared to the vehicle control (PBS). Mean and sum of staining intensities were comparable in all transgenic groups (data not shown).

Brain Tau pathology did not show a change in total Tau or pTau levels in the brain soluble fraction, however immunostaining of brain sections demonstrated a reduction in hippocampus pTau in mice treated with ACI-36-2B6-Ab1.

7.2.3. Effects of anti-Tau antibody Administration on Phospho-Tau Epitopes present in Paired Helical Filaments (PHFs)

Alzheimer's Disease (AD) is characterized neuropathologically by neurofibrillary tangles (NFTs; Braak, Braak, & Bohl, 1993). Structurally, NFTs consist of paired helical filaments (PHFs) composed of the microtubule-associated protein Tau, found primarily in a hyper-phosphorylated state (Alonso et al., 1997). The objective of this study was to reduce these pTau PHF epitopes in the brains of Tau transgenic mice by four administrations of the anti-pTau antibody ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1.

To measure the effects of four ACI-36-2B6-Ab1 administrations on the quantity of well documented Tau PHF phospho epitopes, brain cortex and hippocampus soluble and SinT fractions from treated Tau Tg mice were probed with AD2 (PHF-1 epitope, pS396/pS404) and anti-pS396 antibody (PHF-13 epitope, pS396) using WBs. As markers of Tau PHFs, the presence of pS396/pS404 have been previously documented (Greenberg et al., 1992; Reig et al., 1995), and more specifically the pS396 site (Hoffmann et al., 1997). In the Tau Tg mice, Tau is expressed as endogenous murine Tau and as the human Tau transgene, with a molecular-weight difference that can be clearly identified on WBs when a blotting antibody binds Tau from both species and Tau from the two different transcripts is expressed in sufficient amounts. Therefore when possible, the endogenous mouse and human transgenic Tau bands were identified for each blotting antibody and quantified separately. To verify the migration patterns for these Tau bands in our WB assays, an anti-Tau antibody that binds to total Tau, but is human specific (Tau13), and therefore only shows the human transgene in Tau Tg brains was used for Tau Tg control samples to verify migration patterns for human and mouse Tau in the Tau transgenic mice. Additionally, all quantified bands were normalized to β-actin.

The presence of PHF epitopes, probed for in the soluble fraction from brain cortex, was reduced in ACI-36-2B6-Ab1 and in ACI-36-3A8-Ab1 treated mice. This was significant for both the mouse and human bands, using the pS396 (PHF-13) antibody for the WBs (Figure 5A and B).

When the AD2 (PHF-1, pS396/pS404) antibody was used for WBs of extracts from ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1 treated mice, a significant reduction was observed (Figure 5C).

A note should be made that even though the two PHF-specific antibodies that were used for these WBs have similar epitopes and good specificity to their phosphorylated target(s), the pS396 (PHF-13) antibody appears to have a better signal-to-noise ratio and was the better overall antibody for these WBs.

The direct target effect of the treatment-antibody was probed for in the cortex using ACI-36-2B6-Ab1 and the ACI-36-2B6-Ab1, respectively, as the blotting antibody. This anti-pTau antibody binds to the same phospho-Tau epitope as the treatment antibody used in this study. Blots had previously been probed with a secondary anti-mouse IgG antibody only.

Bands were quantified using an infrared imaging system. Values for individual mice as well as the mean ± SEM are determined.

No signal above background was detected, verifying the lack of blocking effects or interference by the treatment antibody in these samples (data not shown).

In ACI-36-2B6-Ab1 treated mice, a trend towards a reduced signal down to the level of control-treated nTg mice was observed, indicative of a direct target effect (Figure 5G).In ACI-36-3A8-Ab1 treated mice no significant effects of treatment were observed (Figure 5G)..

The significant effect of of ACI-36-2B6-Ab1 treatment on total Tau in the soluble cortex of Tau Tg mice was observed, using a Tau5 antibody for blotting which binds both the endogenous mouse Tau and the transgenic human Tau (Figure 5H and 5I). A significant reduction in total Tau was observed for both endogenous mouse Tau and the transgenic human Tau in the soluble fraction of brain cortex.

Bands, indicating the mouse A) and human B) total Tau (Tau5), were quantified using an infrared imaging system. Values for individual mice as well as the mean ± SEM are determined.

The presence of PHF epitopes in the detergent insoluble Tau fraction was done by preparing sarkosyl-insoluble (SinT) brain fractions.

Bands were quantified using an infrared imaging system. Values for individual mice as well as the mean ± SEM are determined.

Much less Tau was present in this fraction when compared to the soluble Tau fraction, both in the cortex and in hippocampus. This may be due to the age of the Tau Tg mice used in this study, which at 4 months may not have accumulated a significant amount of insoluble and aggregated Tau in the hippocampus and cortex. Therefore, when probing for PHF epitopes in the SinT fraction, only the AD2 (PHF-1, pS396/pS404) antibody provided a signal that was sufficient for reliable quantization of bands.

Mice treated with 1 mg/kg of ACI-36-2B6-Ab1 and with 1 mg/kg of ACI-36-3A8-Ab1, respectively, had a significant reduction in the PHF-1 epitope in bands representing the endogenous mouse Tau (Figure 5C). Signals observed for the transgenic human band were not intense enough to be quantitated reliably.

The hippocampus was also probed, using the same antibodies and fractions as that for the cortex. Lower signals for all blotting antibodies were detected in fractions from hippocampus compared to that of cortex.

The effects of ACI-36-2B6-AB1 and ACI-36-3A8-AB1 treatment on pS396 (PHF-13) immunoreactivity in the soluble hippocampus of Tau Tg mice was determined. Bands, indicating the mouse A) and human B) pTau pS396 (PHF-13) epitopes, were quantified using an infrared imaging system. Values for individual mice as well as the mean ± SEM are determined.

ACI-36-2B6-Ab1 treatment did not significantly alter the presence of the pS396 (PHF-13) epitope in the mouse Tau soluble hippocampus fraction, with a small trend for a reduction in the human transgene band.

ACI-36-3A8-Ab1 treatment showed trends towards a reduction in the presence of the pS396 (PHF-13) epitope in the mouse Tau soluble hippocampus fraction and the human transgene band.

Similarly to what was observed for pS396 (PHF-13) WBs, a trend for a reduced signal was detected in both extracts of ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1 treated mice for total Tau in the human Tau soluble hippocampus fraction.

Akin to the cortex SinT samples, the SinT fraction from hippocampus had very low levels of pTau. Mice treated with ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1, respectively, had no change in the PHF-1 (pS396/pS404) epitope in bands representing the endogenous mouse Tau.

Signals observed for the transgenic human band were not intense enough for reliable quantization.

7.2.4 Summary

The study indicates that passive immunization using four administrations of a phosphosite-specific anti-pTau antibody ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1 antibodies improves spatial learning and reduces brain pTau pathology.

Four peripheral administrations of the anti-pTau antibody ACI-36-2B6-Ab1 at 1 and 3 mg/kg to Tau Tg mice reduced the presence of pTau PHF epitopes in the cortex as measured by Western-blotting. A trend for reduction was observed in the hippocampus. Similarly, a reduction in total Tau was also observed. A significant reduction in pTau PHF-1 immunoreactivity was observed in the insoluble cortex fraction, and a trend was also observed which indicated direct target effects of the antibody treatment. These results provide further support for anti-pTau antibodies ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1 in passive immunotherapy against tauopathies such as Alzheimer's Disease.

EXAMPLE 8: 3-month treatment of human Tau over-expressing mice

8.1 Methods

8.1.1 Mice and treatments

Tau transgenic mice were used and administred the treatment antibodies as shown in the Table in Method 6.1. (study no. 3) and mice were assigned to 4 different treatment groups as described in the table below.

Group	Mouse strain	Geno type	Age at start	Sex	n	Treatment		
A	TMHT	Tg	3 months (±2 weeks)	mixed	15+1	PBS (control)	i.p. 10 µl/ g b.w.	weekly
B	TMHT	Tg	3 months (±2 weeks)	mixed	15+1	ACI-36-2B6-Ab1 (1 mg/kg)	i.p. 10 µl/ g b.w.	weekly
C	TMHT	Tg	3 months (±2 weeks)	mixed	15+1	ACI-36-2B6-Ab1 (3 mg/kg)	i.p. 10 µl/g b.w.	weekly
F	TMHT	nTg	3 months (±2 weeks)	mixed	15+1	PBS (control)	i.p. 10 µl/g b.w.	weekly

In total 45 Tg mice plus 3 reserves allocated to treatment groups A to C and 15 nTg mice plus 1 reserve (group F) were treated on day 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and

84 by i.p. injection of either PBS (vehicle control) or anti-pTAU antibody, ACI-36-2B6-Ab1 or ACI-36-3A8-Ab1. Animals were randomly enclosed in 5 different starting groups (scales) comprising animals of all treatment groups. The number of animals in a scale was limited to ensure same age and uniform handling. Following the 12th administration, a water-maze (MWM) task was performed to test for spatial memory performance. Following the MWM, mice were administered the test article one additional time (13th injection) before being euthanized 24 hours later do determine Tau pathology. Brain Tau pathology was determined in hippocampus and amygdala by immunohistochemical (IHC) quantitation using the AT180 (anti-pTau, pT231/pS235) antibody. Furthermore, the treatment effects on soluble and sarkosyl-insoluble Tau and pTau in cortex and hippocampus were measured using MesoScale Discovery (MSD) technology, probing for pTau (pT231 and pS396) and total Tau.

8.1.2. Behavioral testing – Morris water-maze (MWM) task

This experiment was performed according to the protocol described in Example 7.1.2. In week 12, spatial navigation was tested in the Morris Water Maze (MWM) to evaluate learning and memory.

8.1.3. Molecular Biology

Total TAU and Tau phosphorylated at Thr231 and at pS396 was quantified in brain homogenates of Tg animals by using an immunosorbent assay from MesoScale Discovery (MSD)

8.1.4. Brain Tau pathology determination by immunohistochemical (IHC) quantitation

This experiment was performed according to the protocol described in Example 7.1.3. TAU pathology was determined by AT180 immunoreactivity in the hippocampus and amygdala of 8 animals per group.

8.1.5. Effects of Three Month anti-Tau antibody Administration on Phospho-Tau Epitopes present in Paired Helical Filaments (PHFs)

This experiment was performed according to the protocols described in 7.1.4., 7.1.5. and 7.1.6. To measure the effects of four ACI-36-2B6-Ab1 and ACI-36-3A8-Ab1 administrations on the quantity of well documented Tau PHF phospho epitopes, brain cortex and hippocampus soluble and SinT fractions from treated Tau Tg mice were probed with AD2 (PHF-1 epitope, pS396/pS404), anti-pS396 antibody (PHF-13 epitope, pS396) and AT180 (pT231/pS235) using WBs.

8.1.6. Effect of 3 month anti-Tau antibody administration on Phospho-Tau epitopes using biGT Tau bigenic mice

Study no. 4 was done using bigenic Tau mice as shown in Method 6.1. Brain cortex samples were prepared as shown in Figure 4-2, using the total homogenate (TH) or the soluble fraction (S1) for western blotting. Membranes were probed using the following blotting antibodies for pTau or total Tau:

- HT-7 (26ng/ml), specific to total human Tau
- PHF-13 (pS396) at 1/7500 dilution
- AT180 (pT231) at 2.47ug/ml
- AT8 (pS202) at 3ug/ml
- pS404 at 1:5000 dilution
- pS400 at 1:5000 dilution

All quantifications were normalized to β-actin.

8.2 Results for ACI-36-2B6-Ab1 antibody

Thirteen i.p. injections of ACI-36-2B6-Ab1 administered weekly at 1 or 3 mg/kg over a twelve week study period did not show any gross adverse effects.

8.2.1 Behavioral results – Morris water maze

The results from the MWM test demonstrated strong trends toward improved spatial learning for mice treated with ACI-36-2B6-Ab1 (Figure 9).

During the last week of treatment, spatial navigation learning and memory of animals were evaluated. Animals had to fulfill 4 days of training with 3 trials per day followed by one probe trial and visual test. Escape latency (the time [seconds] the mouse needed to find the hidden platform and therefore to escape from the water), the pathway (the length of the trajectory [meter] to reach the target), the swim speed (calculated quotient of pathway and escape latency), the number of target crossings and the abidance in the target quadrant were evaluated. Vehicle treated Tg (group A) and nTg (group F) control animals showed expected learning curves in terms of escape latency and length of the swimming path to reach the platform over the four testing days. Tg control (A) animals showed a significant impairment of learning abilities reflected in flatter learning curves of escape latencies and swimming paths compared to nTg control animals (group F). Escape latencies and swimming paths were significantly (Two Way ANOVA) different on training days 3 ($p<0.01$, latency; $p<0.001$, length; Bonferroni´s post test) and 4 ($p<0.01$; Bonferroni´s post test). Treatment with ACI-36-2B6-Ab1, low or high dose (B and C) did not significantly improve spatial learning abilities compared to Tg control animals (A). When adjusting the performance of each group to 100% on training day 1 and all further days as percentage of day 1, a slight improvement can be seen for the ACI-36-2B6-Ab1 treated mice (low and high dosage) in swimming path length, although without statistical significance. No differences between treatment groups were detected when calculating the swimming speed on all four training days. In the probe trial (PT), the abidance in the target quadrant (south west quadrant) as well as target zone crossings were recorded. nTg controls (group F) spent more time in the target quadrant and crossed the target zone more often relative to Tg controls (group A) but without statistical significance.

Treatment with neither the low nor the high dose led to an improvement of spatial learning abilities in comparison to Tg control mice as evaluated in the PT.

8.2.2 Molecular biology

8.2.2.1 TAU in soluble fraction of cortex homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the soluble fraction of cortex homogenates of n=16 animals from group A (Tg vehicle group; PBS), B (Tg, ACI-36-2B6-Ab1 1 mg/kg), and C (Tg, ACI-36-2B6-Ab1 3 mg/kg). A treatment with ACI-36-2B6-Ab1 did not significantly affect total TAU and pTAU in the in soluble cortex homogenates. However, a slight increase (without significance) of mean total TAU, p231TAU, and p396TAU was observed upon ACI-36-2B6-Ab1 treatment. TAU phosphorylation evaluated as the ratios of pTAU to total TAU was not affected.

8.2.2.2 TAU in sarcosyl insoluble fraction of cortex homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the sarcosyl insoluble fractions of cortex homogenates of n=16 animals from group A (Tg vehicle group; PBS), B (Tg, ACI-36-2B6-Ab1 1 mg/kg), and C (Tg, ACI-36-2B6-Ab1 3 mg/kg). A treatment with ACI-36-2B6-Ab1 did not significantly affect total TAU and pTAU in the in sarcosyl insoluble cortex homogenates. A slight decrease (without significance) of mean total TAU and p231TAU was observed upon ACI-36-2B6-Ab1 treatment. TAU phosphorylation evaluated as the ratios of pTAU to total TAU was not affected.

8.2.2.3 TAU in soluble fraction of hippocampus homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the soluble fraction of hippocampus homogenates of n=16 animals from group A (Tg vehicle group; PBS), B (Tg, ACI-36-2B6-Ab1, 1 mg/kg), and C (Tg, ACI-36-2B6-Ab1, 3 mg/kg). treatment with ACI-36-2B6-Ab1 did not significantly affect total TAU and pTAU in the in soluble hippocampus homogenates. A slight decrease (without significance) of TAU phosphorylation evaluated as the ratios of pTAU to total TAU was observed upon ACI-36-2B6-Ab1 treatment.

8.2.2.4 TAU in sarcosyl insoluble fraction of hippocampus homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the sarcosyl insoluble fractions of hippocampus homogenates of n=16 animals from group A (Tg vehicle group; PBS), B (Tg, ACI-36-2B6-Ab1, 1 mg/kg), and C (Tg, ACI-36-2B6-Ab1, 3 mg/kg). A treatment with ACI-36-2B6-Ab1 did not significantly affect total TAU and pTAU in the in sarcosyl insoluble hippocampus homogenates. Treatment with 3 mg/kg increased the mean total TAU, p231TAU as well as p396TAU, although without reaching significance, whereas a slight reduction of the mean total TAU, p231TAU as

well as p396TAU upon 1 mg/kg treatment was observed. TAU phosphorylation evaluated as the ratios of pTAU to total TAU was slightly increased upon 3 mg/kg ACI-36-2B6-Ab1 treatment.

8.2.2.5. Western Blots for soluble cortex

Treatment with ACI-36-2B6-Ab1 dose-dependently reduced the presence of both the pS396/pS404 (Figure 5D) and pT181 (Figure 5E and 5F) pTau epitopes in the soluble fraction of brain cortex, with a significant effect at the 3 mg/kg dose.

8.2.2.6 TAU in biGT Tau bigenic mice

biGT mice treated with ACI-36-2B6-Ab1 for 3 months had significantly reduced total Tau in brain cortex soluble fraction (Figure 6A and 6B). A significant reduction was observed for pTau epitopes pT231/AT180 (Figure 6C and 6D), pS202/AT8 (Figure 6E), and pS396 (Figure 6F and 6G). A significant reduction was also observed in both total homogenate (TH) for pTau epitope pS400 (Figures 6H and 6I) and pS404 (Figures 6L and 6M). Furthermore, a significant reduction was also observed in soluble fraction for pTau epitopes pS400 (Figure 6J and 6K) and a trend for reduction for pTau epitope pS404 (Figure 6N and 6O).

8.2.3 Histology

8.2.3.1 Morphometry – determination of region areas

Measured region areas of the hippocampus and the amygdala not differ significantly throughout all investigated brains which excludes negative effects on tissue during dissection and IHC or staining (e.g. unequivocal shrinkage, different sectioning) and to a certain degree treatment induced atrophy. Individual sections may deviate from the individual and group mean because of e.g. folding of tissue or loss of parts of the section during execution of the labeling protocol. Therefore, the total immunoreactive area [in µm2] of any labeling was normalized to the section's individual region area [in mm2] by calculating the percentage of the labeled area within the region area [labeled area / (region area * 10.000)].

8.2.3.2 Results of AT180 IH

The AT180 antibody detects the endogenous and human pTAU (doubly phosphorylated at Thr231 and Ser235). The amount of intrasomal pTAU in nTg controls was significantly lower compared to Tg groups ($p<0.01$ as well as $p<0.001$). In the amygdala, the higher dose of ACI-36-2B6-Ab1 (3 mg/kg – group C) significantly decreased the somal pTAU compared to vehicle treated animals (Figure 7, left). The lower dose (1 mg/kg – group C)

showed the same tendency but did not reach significance. The same effect was detectable in the hippocampus where ACI-36-2B6-Ab1 reduced pTAU dose-dependently, significant for the higher dose and tendentiously for the lower (Figure 7, right). This decrease was also qualitatively visible as decrease of the area of staining and the staining intensity in individual neuronal somata. Results of the sum staining intensities normalized to AOI size of the measured AT180 IR in the neuronal somata were comparable with measured AT180 IR area percentage, including a significant dose-dependence of the greater effect of the higher dose in the amygdala. In the hippocampus the post hoc comparisons did not reach significance level.

8.2.4 Summary

Brain Tau pathology as measured by MSD did not show a significant change, however immunostaining of brain sections demonstrated a dose-dependent with up to 60% reduction in AT180 (pT231/pS235) immunostaining in neuronal somata.

The study shows that passive immunization using thirteen administrations of a phosphosite-specific anti-pTau antibody ACI-36-2B6-Ab1 can improve spatial learning and significantly reduces brain pTau pathology.

8.3 Results ACI-36-3A8-Ab1 antibody

Thirteen i.p. injections of ACI-36-3A8-Ab1 administered weekly at 1 or 3 mg/kg over a twelve week study period did not show any gross adverse effects.

8.3.1 Behavioral results – Morris water maze

The results from the MWM test demonstrated a significant effect of improved spatial learning for mice treated with ACI-36-3A8-Ab1 at 3 mg/kg (Figure 10).

Vehicle treated Tg (group A) and nTg (group F) control animals showed expected learning curves in terms of escape latency and length of the swimming path to reach the platform over the four testing days. Tg control (A) animals showed a significant impairment of learning abilities reflected in flatter learning curves of escape latencies and swimming paths compared to nTg control animals (group F). Escape latencies and swimming paths were significantly (Two Way ANOVA) different on training days 3 ($p<0.01$, latency; $p<0.001$, length; Bonferroni´s post test) and 4 ($p<0.01$; Bonferroni´s post test). Treatment with ACI-36-3A8-Ab1, low or high dose (D and E) did not significantly improve spatial learning abilities compared to Tg control animals (A) when absolute values are analyzed. When adjusting the performance of each group to 100%

on training day 1 and all further days as percentage of day 1, an improvement of learning and memory abilities can be upon ACI-36-3A8-Ab1 treatment (low and high dosage). Animals treated with the low dose of ACI-36-3A8-Ab1 (group D) performed only slightly better in the MWM compared to Tg controls (group A). The effect of a weekly treatment with 3 mg/kg ACI-36-3A8-Ab1 (group E) was much more pronounced and almost restored the performance of nTg animals. Compared to the Tg controls (A) the effect of 3 mg/kg ACI-36-3A8-Ab1 was statistically significant for swimming path length on day 3 and day 4 ($p<0.05$). No differences between treatment groups were detected when calculating the swimming speed on all four training days.

In the probe trial (PT), the abidance in the target quadrant (south west quadrant) as well as target zone crossings were recorded. nTg controls (group F) spent more time in the target quadrant and crossed the target zone more often relative to Tg controls (group A) but without statistical significance. Treatment with neither the low nor the high dose led to a statistically significant improvement in comparison to Tg control mice as evaluated in the PT. However, ACI-36-3A8-Ab1 treated animals had – although statistically insignificantly - more target zone crossings compared to Tg control, that is in accordance with the outcome of the swim length over the 4 training days.

8.3.2 Molecular biology

8.3.2.1 TAU in soluble fraction of cortex homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the soluble fraction of cortex homogenates of n=16 animals from group A (Tg vehicle group; PBS) and D (Tg, ACI-36-3A8-Ab1, 1 mg/kg) and of n=15 animals from group E (Tg, ACI-36-3A8-Ab1, 3 mg/kg). A treatment with ACI-36-3A8-Ab1 did not significantly affect total TAU and pTAU in the in soluble cortex homogenates. However, a slight increase (without significance) of mean total TAU, p231TAU, and p396TAU was observed upon ACI-36-3A8-Ab1 treatment. TAU phosphorylation at 231 evaluated as the ratio of p231TAU to total TAU was slightly decreased after treatment with 3 mg/kg ACI-36-3A8-AB1.

8.3.2.2 TAU in sarcosyl insoluble fraction of cortex homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the sarcosyl insoluble fractions of cortex homogenates of n=16 animals from group A (Tg vehicle group; PBS) and D (Tg, ACI-36-3A8-Ab1 , 1 mg/kg) and of n=15 animals from group E (Tg, ACI-36-3A8-Ab1, 3 mg/kg). A treatment with ACI-36-3A8-AB1 did not significantly affect total TAU and pTAU in the in sarcosyl insoluble cortex homogenates.

A slight decrease (without significance) of mean total TAU, p231TAU, and p396TAU was observed upon 1 mg/ ACI-36-3A8-Ab1 treatment. Further the p231TAU to total TAU ratios of 1 mg/kg ACI-36-3A8-Ab1 treated animals showed slightly lower variability compared to vehicle treated animals (but with lacking significance in F-Test: p=0.184) without changing the mean p231TAU to total TAU ratios of the two groups. For 1 mg/kg and 3 mg/kg ACI-36-3A8-Ab1 treated groups a slight increase of p396TAU phosphorylation was observed.

8.3.2.3 TAU in soluble fraction of hippocampus homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the soluble fraction of hippocampus homogenates of n=16 animals from group A (Tg vehicle group; PBS) and D (Tg, ACI-36-3A8-Ab1, 1 mg/kg) and of n=15 animals from group E (Tg, , ACI-36-3A8-Ab1, 3 mg/kg). TAU and pTAU levels in the soluble hippocampus fractions of IRN6301 (group D) were outliers and were excluded. A treatment with ACI-36-3A8-Ab1 did not significantly affect total TAU and pTAU in the in soluble hippocampus homogenates. A slight decrease (without significance) of TAU phosphorylation at 231 evaluated as the ratios of p231TAU to total TAU was observed upon ACI-36-3A8-Ab1 treatment.

8.3.2.4 TAU in sarcosyl insoluble fraction of hippocampus homogenates

Total TAU, p231TAU, p396TAU, and the ratios of pTAU to total TAU were evaluated in the sarcosyl insoluble fractions of hippocampus homogenates of n=16 animals from group A (Tg vehicle group; PBS), B (Tg ACI-36-3A8-Ab1, 1 mg/kg), and C (Tg, ACI-36-3A8-Ab1, 3 mg/kg). A treatment with ACI-36-3A8-Ab1 did not significantly affect total TAU and pTAU in the in sarcosyl insoluble hippocampus homogenates. A slight increase (without significance) of mean total TAU, p231TAU as well as p396TAU was observed upon ACI-36-3A8-Ab1 treatment. TAU phosphorylation evaluated as the ratio of p231TAU to total TAU was not affected and treatment with 1mg/kg ACI-36-3A8-Ab1 slightly reduced the ratio of p396TAU to total TAU.

8.3.2.5. Western Blots for soluble cortex

A significant reduction of the pS396/pS404 pTau epitope in the soluble brain cortex in mice treated with 1 or 3 mg/kg of ACI-36-3A8-Ab1 (Figure 5D). The presence of the human/transgenic pT181 pTau epitope was reduced in the soluble cortex fraction, with a significant effect in mice treated with 1 mg/kg and trend in mice treated with 3 mg/kg

(Figure 5E). A trend for a reduction was observed in the amount of endogenous pT181 pTau (Figure 5F).

8.3.2.6 TAU in biGT Tau bigenic mice

biGT mice treated with ACI-36-3A8-Ab1 for 3 months had significantly reduced total Tau in brain cortex soluble fraction (Figure 6A and 6B). A significant reduction was observed for pTau epitopes pT231/AT180 (Figure 6C and 6D), pS202/AT8 (Figure 6E), and pS396 (Figure 6F and 6G). A significant reduction was also observed in both total homogenate (TH) for pTau epitope pS400 (Figures 6H and 6I) and pS404 (Figures 6L and 6M). Furthermore, a significant reduction was also observed in soluble fraction for pTau epitopes pS400 (Figure 6J and 6K) and a trend for reduction for pTau epitope pS404 (Figure 6N and 6O).

8.3.3 Histology

8.3.3.1 Morphometry – determination of region areas

See Example 8.2.3.1

8.3.3.2 Results of AT180 IH

The AT180 antibody detects the endogenous and human pTAU (doubly phosphorylated at Thr231 and Ser235). The amount of intrasomal pTAU in nTg controls was significantly lower compared to Tg groups ($p<0.001$). In the amygdala, both doses of ACI-36-3A8-Ab1 [1 mg/kg (group D) and 3 mg/kg (group E)] significantly decreased the somal pTAU compared to vehicle treated animals (Figure 8, left). A similar effect was detectable in the hippocampus where the lower dosage ACI-36-3A8-Ab1 reduced pTAU, however in this case the higher dose was less effective and just led to a tendentious decrease (Figure 8, right). This decrease was also qualitatively visible as decrease of the area of staining and the staining intensity in individual neuronal somata. Results of the normalized sum of intensities of the measured AT180 IR in the neuronal somata were comparable in the amygdala with measured AT180 IR area percentage but reached significance for the higher dose only. In the hippocampus the result was totally comparable to IR area percentage.

8.3.4 Summary

Brain Tau pathology as measured by MSD did not show a significant change, however immunostaining of brain sections demonstrated a dose-dependent with up to 40% reduction in AT180 (pT231/pS235) immunostaining in neuronal somata.

The study shows that passive immunization using thirteen administrations of a phosphosite-specific anti-pTau antibody ACI-36-3A8-Ab1 improves spatial learning and significantly reduces brain pTau pathology.

Deposits:

The following hybridoma cell lines were deposited in the name of AC Immune SA, PSE-EPFL Building B, 1015 Lausanne, Switzerland and Katholieke Universiteit Leuven, Minderbroedersstraat 8a - Box 5105, B-3000 Leuven with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Inhoffenstrasse 7 B, 38124 Braunschweig, under the provisions of the Budapest Treaty:

Hybridoma name	Deposit number	Date of deposit
6C10F9C12A11	DSM ACC3079	August 25, 2010
6C10E5E9C12	DSM ACC3081	August 25, 2010
6H1A11C11	DSM ACC3080	August 25, 2010
6H1G6E6	DSM ACC3088	August 25, 2010
2B6A10C11	DSM ACC3084	August 25, 2010
2B6G7A12	DSM ACC3087	August 25, 2010
3A8A12G7	DSM ACC3086	August 25, 2010
3A8E12H8	DSM ACC3085	August 25, 2010
7C2(1)F10C10D3	DSM ACC3082	August 25, 2010
7C2(2)B9F11D5	DSM ACC3083	August 25, 2010
A4-4A6-48	DSM ACC3136	August 30, 2011
A6-2G5-30	DSM ACC3137	August 30, 2011
A6-2G5-41	DSM ACC3138	August 30, 2011
A4-2A1-18	DSM ACC3139	August 30, 2011
A4-2A1-40	DSM ACC3140	August 30, 2011
A6-1D2-12	DSM ACC3141	September 6th, 2011

Table 1. Tau sequence, vaccine and antibody description

Description	Vaccine	Sequence*, length (n), sequence ID number	Hybridomas	Antibodies
T1: Tau 5-20 [pY18]	ACI-33	RQEFEVMEDHAGTY(p)GL (n = 16) (SEQ ID NO: 59)	6C10F9C12A11	ACI-33-6C10-Ab1
			6C10E5E9C12	ACI-33-6C10-Ab2
T8: Tau 206-221 [pT212, pS214] T9: Tau 196-211 [pS202, pT205]	ACI-41	PGSRSRT(p)PS(p)LPTPPTR (n = 16) (SEQ ID NO: 60) GYSSPGS(p)PGT(p)PGSRSR (n = 16) (SEQ ID NO: 61)	7C2(1)F10C10D3	ACI-41-7C2-Ab1
			7C2(2)B9F11D5	ACI-41-7C2-Ab1
T4: Tau 401-418 [pS404, pS409]	ACI-36	GDTS(p)PRHLS(p)NVSSTGSID (n = 18) (SEQ ID NO: 63)	6H1A11C11	ACI-36-6H1-Ab1
			6H1G6E6	ACI-36-6H1-Ab2
			2B6A10C11	ACI-36-2B6-Ab1
			2B6G7A12	ACI-36-2B6-Ab2
			3A8A12G7	ACI-36-3A8-Ab1
			3A8E12H8	ACI-36-3A8-Ab2
T3: Tau 393-408 [pS396, pS404]	ACI-35	VYKS(p)PVVSGDTS(p)PRHL (n = 16) (SEQ ID NO: 62)	A4-4A6-48	ACI-35-4A6-Ab2
			A6-2G5-30	ACI-35-2G5-Ab2
			A6-2G5-41	ACI-35-2G5-Ab3
			A4-2A1-18	ACI-35-2A1-Ab1
			A4-2A1-40	ACI-35-2A1-Ab2
			A6-1D2-12	ACI-35-1D2-Ab1
T5: Control sequence: Tau 379-408 [pS396, pS404]	ACI-37	RENAKAKTDHGAEIVYKS(p)PVVSGDTS(p)PRHL (n = 30) (SEQ ID NO: 58)		
T8: Tau 206-221 [pT212, pS214]	ACI-39	PGSRSRT(p)PS(p)LPTPPTR (n = 16) (SEQ ID NO: 60)		
T9 : Tau 196-211 [pS202, pT205]	ACI-40	GYSSPGS(p)PGT(p)PGSRSR (n = 16) (SEQ ID NO: 61)		
T2: Tau 200-216 [pS202+ pT205 & pT212+pS214]	ACI-34	PGS(p)PGT(p)PGSRSRT(p)PS(p)LP (n = 17) (SEQ ID NO: 64)		
T10: Tau 407-418 [pS409]	ACI-42	HLS(p)NVSSTGSID (n = 12) (SEQ ID NO: 65)		
T11: Tau 399-408 [pS404]	ACI-43	VSGDTS(p)PRHL (n = 10) (SEQ ID NO: 66)		

*Based on the longest isoform of human Tau (Tau441). p indicates phosphorylated residue.

Table 2. Results of ACI-33 hybridoma screen

24 well plate screen		*T25 Flasks screen*		
Positive in ELISA	**Positive in TAUPIR**	**Positive in IgG screen**	**Positive in ELISA**	**Positive in TAUPIR**
1A7		1A7		
	1A11			
	1C11	1C11		
2C9		2C9		
3C3		3C3	3C3	
3C5		3C5		
3E8		3E8		
3G10	3G10	3G10	3G10	
6C10	6C10	6C10	6C10	6C10
6F3		6F3		
6F8		6F8		

Table 3. Results of ACI-36 hybridoma screen

24 well plate screen		*T25 Flasks screen*		
Positive in ELISA	**Positive in TAUPIR**	**Positive in IgG screen**	**Positive in ELISA**	**Positive in TAUPIR**
2B6	2B6	2B6	2B6	2B6
2F9	2F9	2F9	2F9	2F9
2G1		2G1	2G1	2G1
3A8	3A8	3A8	3A8	3A8
3B9		3B9	3B9	3B9
3F11	3F11	3F11		3F11
	4A3			4A3
4C1		4C1	4C1	4C1
4C12		4C12	4C12	4C12
4E12		4E12	4E12	4E12
5E10		5E10	5E10	
5F5		5F5	5F5	
7D6	7D6	7D6	7D6	7D6
6H1		6H1	6H1	6H1

Table 4. Ranking for positive clones in ELISA and TAUPIR of ACI-36

ranking for ELISA	ranking for TAUPIR
3A8	6H1
2B6	4C1
4C1	3A8
6H1	4C12
4C12	2B6
2G1	2F9
2F9	3B9
7D6	2G1
3B9	7D6
4E12	4E12

Table 5. Results of ACI-41 hybridoma screen

24 well plate screen		*T25 Flasks screen*		
Positive in ELISA	**Positive in TAUPIR**	**Positive in IgG screen**	**Positive in ELISA**	**Positive in TAUPIR**
	3D11	3D11		3D11
4H6		4H6		4H6
5D10	5D10	5D10	5D10	5D10
5E6	5E6			
5F10		5F10		
6B7		6B7	6B7	
7C2	7C2	7C2	7C2	7C2
	8G8			8G8
	8H8	8H8		8H8

Table 6. Screening of hybridomas for binding to target

Hybridomas	Antibodies	ELISA				TAUPIR	Western Blot
		Tau p-peptide	*Tau peptide*	*Full-length pTau*	*Full-length Tau*		
6C10F9C12A11	ACI-33-6C10-Ab1	+	-	+/-	-	+	-
6C10E5E9C12	ACI-33-6C10-Ab2	+	-	+/-	-	+	-
6H1A11C11	ACI-36-6H1-Ab1	+	-	+	-	+	+
6H1G6E6	ACI-36-6H1-Ab2	+	-	+	-	+	+
2B6A10C11	ACI-36-2B6-Ab1	+	-	+	-	+	+
2B6G7A12	ACI-36-2B6-Ab2	+	-	+	-	+	+
3A8A12G7	ACI-36-3A8-Ab1	+	-	+	-	+	+
3A8E12H8	ACI-36-3A8-Ab2	+	-	+	-/+	+	+
7C2(1)F10C10D3	ACI-41-7C2-Ab1	+	-	+	-	+	-
7C2(2)B9F11D5	ACI-41-7C2-Ab2	+	-	+	-	+	-
A4-2A1-18	ACI-35-2A1-Ab1	+	-	+	-		
A4-2A1-40	ACI-35-2A1-Ab2	+	-	+	-		
A4-4A6-18	ACI-35-4A6-Ab1	+	-	-	+		
A4-4A6-48	ACI-35-4A6-Ab2						
A6-1D2-12	ACI-35-1D2-Ab1	+	-	+	-		
A6-2G5-08	ACI-35-2G5-Ab1	+	-	-	-		
A6-2G5-30	ACI-35-2G5-Ab2	+	-	+	-		
A6-2G5-41	ACI-35-2G5-Ab3	+	-	+	-		

Table 7. Binding affinity of anti-tau antibodies

Hybridomas	Antibodies	Association rate constant (k_d) (1/Ms)	Dissociation rate constant (k_a) (1/s)	Dissociation constant (K_D) (nM)
6C10F9C12A 11	ACI-33-6C10-Ab1	9.46×10^5	3.27×10^{-3}	3.46
6H1A11C11	ACI-36-6H1-Ab1	3.53×10^4	6.80×10^{-5}	1.93
6H1G6E6	ACI-36-6H1-Ab2	9.99×10^4	9.58×10^{-5}	0.96
2B6A10C11	ACI-36-2B6-Ab1	6.90×10^5	1.63×10^{-4}	0.24
2B6G7A12	ACI-36-2B6-Ab2	9.11×10^5	1.11×10^{-4}	0.12
3A8A12G7	ACI-36-3A8-Ab1	1.01×10^6	1.09×10^{-4}	0.11
3A8E12H8	ACI-36-3A8-Ab2	8.43×10^5	1.43×10^{-4}	0.17
A4-4A6-18	ACI-35-4A6-Ab1	2.00×10^5	3.10×10^{-3}	16
A6-1D2-12	ACI-35-1D2-Ab1	1.60×10^3	9.30×10^{-6}	≤6
A6-2G5-08	ACI-35-2G5-Ab1	4.80×10^5	5.30×10^{-3}	10

Table 8. Peptide libraries used for epitope mapping

Peptide library for T1

	Tau(441) amino acid number	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Amino acid	**R**	**Q**	**E**	**F**	**E**	**V**	**M**	**E**	**D**	**H**	**A**	**G**	**T**	**Y (P)**	**G**	**L**
Phospho peptides	**Peptide no**																
	T1.18											A	G	T	Y(P)	G	L
	T1.17										H	A	G	T	Y(P)	G	L
	T1.16									D	H	A	G	T	Y(P)	G	L
	T1.15								E	D	H	A	G	T	Y(P)	G	L
	T1.14							M	E	D	H	A	G	T	Y(P)	G	L
	T1.13						V	M	E	D	H	A	G	T	Y(P)	G	L
	T1.12					E	V	M	E	D	H	A	G	T	Y(P)	G	L
	T1.11				F	E	V	M	E	D	H	A	G	T	Y(P)	G	L
	T1.10			E	F	E	V	M	E	D	H	A	G	T	Y(P)	G	L
	T1.9		Q	E	F	E	V	M	E	D	H	A	G	T	Y(P)	G	L
	T1.7	R	Q	E	F	E	V	M	E	D	H	A	G	T	Y(P)	G	L

	Amino acid	**R**	**Q**	**E**	**F**	**E**	**V**	**M**	**E**	**D**	**H**	**A**	**G**	**T**	**Y**	**G**	**L**
Non-phospho peptides	**Peptide no**																
	T1.28											A	G	T	Y	G	L
	T1.27										H	A	G	T	Y	G	L
	T1.26									D	H	A	G	T	Y	G	L
	T1.25								E	D	H	A	G	T	Y	G	L
	T1.24							M	E	D	H	A	G	T	Y	G	L
	T1.23						V	M	E	D	H	A	G	T	Y	G	L
	T1.22					E	V	M	E	D	H	A	G	T	Y	G	L
	T1.21				F	E	V	M	E	D	H	A	G	T	Y	G	L
	T1.20			E	F	E	V	M	E	D	H	A	G	T	Y	G	L
	T1.19		Q	E	F	E	V	M	E	D	H	A	G	T	Y	G	L
	T1.8	R	Q	E	F	E	V	M	E	D	H	A	G	T	Y	G	L

Table 8. Continued

Peptide library for T4

	Tau(441) amino acid number	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418
	Amino acid	**G**	**D**	**T**	**S(p)**	**P**	**R**	**H**	**L**	**S(p)**	**N**	**V**	**S**	**S**	**T**	**G**	**S**	**I**	**D**
Phospho peptides	**Peptide no.**																		
	T3.17	G	D	T	S(p)	P	R	H	L										
	T4.11		D	T	S(p)	P	R	H	L	S(p)									
	T4.12			T	S(p)	P	R	H	L	S(p)	N								
	T4.13				S(p)	P	R	H	L	S(p)	N	V							
	T4.14					P	R	H	L	S(p)	N	V	S						
	T4.15						R	H	L	S(p)	N	V	S	S					
	T4.16							H	L	S(p)	N	V	S	S	T				
	T4.17								L	S(p)	N	V	S	S	T	G			
	T4.18									S(p)	N	V	S	S	T	G	S		
	T4.19										N	V	S	S	T	G	S	I	
	T4.20											V	S	S	T	G	S	I	D

	Amino acid	G	D	T	S	P	R	H	L	S	N	V	S	S	T	G	S	I	D
Non-phospho peptides	**Peptide no.**																		
	T3.26	G	D	T	S	P	R	H	L										
	T4.21		D	T	S	P	R	H	L	S									
	T4.22			T	S	P	R	H	L	S	N								
	T4.23				S	P	R	H	L	S	N	V							
	T4.24					P	R	H	L	S	N	V	S						
	T4.25						R	H	L	S	N	V	S	S					
	T4.26							H	L	S	N	V	S	S	T				
	T4.27								L	S	N	V	S	S	T	G			
	T4.28									S	N	V	S	S	T	G	S		
	T4.19										N	V	S	S	T	G	S	I	
	T4.20											V	S	S	T	G	S	I	D

Table 8. Continued

Peptide library for T8

	Tau(441) amino acid number	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221
	Amino acid	**P**	**G**	**S**	**R**	**S**	**R**	**T(p)**	**P**	**S(p)**	**L**	**P**	**T**	**P**	**P**	**T**	**R**
Phospho peptides	**Peptide no**																
	T8.7	P	G	S	R	S	R	T(p)	P								
	T8.8		G	S	R	S	R	T(p)	P	S(p)							
	T8.9			S	R	S	R	T(p)	P	S(p)	L						
	T8.10				R	S	R	T(p)	P	S(p)	L	P					
	T8.11					S	R	T(p)	P	S(p)	L	P	T				
	T8.12						R	T(p)	P	S(p)	L	P	T	P			
	T8.13							T(p)	P	S(p)	L	P	T	P	P		
	T8.14								P	S(p)	L	P	T	P	P	T	
	T8.15									S(p)	L	P	T	P	P	T	R

	Amino acid	P	G	S	R	S	R	T	P	S	L	P	T	P	P	T	R
Non-phospho peptides	**Peptide no**																
	T8.16	P	G	S	R	S	R	T	P								
	T8.17		G	S	R	S	R	T	P	S							
	T8.18			S	R	S	R	T	P	S	L						
	T8.19				R	S	R	T	P	S	L	P					
	T8.20					S	R	T	P	S	L	P	T				
	T8.21						R	T	P	S	L	P	T	P			
	T8.22							T	P	S	L	P	T	P	P		
	T8.23								P	S	L	P	T	P	P	T	
	T8.24									S	L	P	T	P	P	T	R

Table 8. Continued

Peptide library for T9

	Tau(441) amino acid number	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211
	Amino acid	G	Y	S	S	P	G	S(p)	P	G	T(p)	P	G	S	R	S	R
Phospho peptides	Peptide no																
	T9.7	G	Y	S	S	P	G	S(p)	P								
	T9.8		Y	S	S	P	G	S(p)	P	G							
	T9.9			S	S	P	G	S(p)	P	G	T(p)						
	T9.10				S	P	G	S(p)	P	G	T(p)	P					
	T9.11					P	G	S(p)	P	G	T(p)	P	G				
	T9.12						G	S(p)	P	G	T(p)	P	G	S			
	T9.13							S(p)	P	G	T(p)	P	G	S	R		
	T9.14								P	G	T(p)	P	G	S	R	S	
	T9.15									G	T(p)	P	G	S	R	S	R
	Amino acid	G	Y	S	S	P	G	S	P	G	T	P	G	S	R	S	R
Non-phospho peptides	Peptide no																
	T9.16	G	Y	S	S	P	G	S	P								
	T9.17		Y	S	S	P	G	S	P	G							
	T9.18			S	S	P	G	S	P	G	T						
	T9.19				S	P	G	S	P	G	T	P					
	T9.20					P	G	S	P	G	T	P	G				
	T9.21						G	S	P	G	T	P	G	S			
	T9.22							S	P	G	T	P	G	S	R		
	T9.23								P	G	T	P	G	S	R	S	
	T9.24									G	T	P	G	S	R	S	R

Table 8. Continued

Peptide library for T3

	Tau(441) amino acid number	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408
	Amino acid	V	Y	K	S(p)	P	V	V	S	G	D	T	S(p)	P	R	H	L
Phospho peptides	Peptide no.																
	T3.9	V	Y	K	S(p)	P	V	V	S								
	T3.10		Y	K	S(p)	P	V	V	S	G							
	T3.11			K	S(p)	P	V	V	S	G	D						
	T3.12				S(p)	P	V	V	S	G	D	T					
	T3.13					P	V	V	S	G	D	T	S(p)				
	T3.14						V	V	S	G	D	T	S(p)	P			
	T3.15							V	S	G	D	T	S(p)	P	R		
	T3.16								S	G	D	T	S(p)	P	R	H	
	T3.17									G	D	T	S(p)	P	R	H	L

	Amino acid	V	Y	K	S	P	V	V	S	G	D	T	S	P	R	H	L
Non-phospho peptides	Peptide no.																
	T3.18	V	Y	K	S	P	V	V	S								
	T3.19		Y	K	S	P	V	V	S	G							
	T3.20			K	S	P	V	V	S	G	D						
	T3.21				S	P	V	V	S	G	D	T					
	T3.22					P	V	V	S	G	D	T	S				
	T3.23						V	V	S	G	D	T	S	P			
	T3.24							V	S	G	D	T	S	P	R		
	T3.25								S	G	D	T	S	P	R	H	
	T3.26									G	D	T	S	P	R	H	L

Table 9. Tau amino acids and phospho-residues required for antibody binding.

Vaccine	Hybridoma	Epitope*
ACI-33	6C10F9C12A11	Tau aa 15-20, with requirement for pY18
ACI-33	6C10E5E9C12	Tau aa 15-20, with requirement for pY18
ACI-36	6H1A11C11	Tau aa 405-412, with requirement for pS409
ACI-36	6H1G6E6	Tau aa 405-412, with requirement for pS409
ACI-36	2B6A10C11	Tau aa 405-411, with requirement for pS409
ACI-36	2B6G7A12	Tau aa 405-411, with requirement for pS409
ACI-36	3A8A12G7	Tau aa 405-411, with requirement for pS409
ACI-36	3A8E12H8	Tau aa 405-411, with requirement for pS409
ACI-41	7C2(1)F10C10D3	Tau aa 208-218, with requirement for pT212 and pS214
ACI-35	A4-2A1-18	Tau aa 393-401, with requirement for pS396
ACI-35	A4-2A1-40	Tau aa 393-401, with requirement for pS396
ACI-35	A4-4A6-18	Tau aa 396-401, with requirement for pS396
ACI-35	A6-1D2-12	Tau aa 394-400, with requirement for pS396
ACI-35	A6-2G5-08	Tau aa 402-406, with requirement for pS404
ACI-35	A6-2G5-30	Tau aa 393-400, with requirement for pS396
ACI-35	A6-2G5-41	Tau aa 393-400, with requirement for pS396

*Based on the longest isoform of human Tau (Tau441)

Table 10. Amino Acid Sequence of the heavy chain and light chain variable regions (VH and VK) and the CDRs

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-36	3A8A12G7*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49/ and SEQ ID NO: 51)	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1)	DIVMTQSPSSLAMSVG QKVTMSCKSSQSVFNS GNQKNSLAWYQQKPG QSPKLLVYFASTRESGV PDRFIGSGSGTDFSLTI SSVQAEDLADYFCQEH YTTPPTFGTGTKLELK (SEQ ID NO: 6)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	KSSQSVF NSGNQK NSLA (SEQ ID NO: 21)	FASTRE S (SEQ ID NO: 22)	QEHYTT PPT (SEQ ID NO: 23)
ACI-36	3A8A12G7*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_G (SEQ ID NO: 50 and SEQ ID NO: 51)	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1	DVVMTQTPLSLPVSLG DQASISCRSSQRLVHS HGKTYLHWYLQKPGQS PKLLIYKVSNRFSGVPD RFSGSGSGTDFTLKISR VEAEDLGVYFCSQTAH FPYTFGGGTKLEIK (SEQ ID NO: 7)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQRLV HSHGKT YLH (SEQ ID NO: 24)	KVSNRF S (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)
ACI-36	3A8E12H8*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49 and SEQ ID NO: 51)	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1)	DIVMTQSPSSLAMSVG QKVTMSCKSSQSVFNS GNQKNSLAWYQQKPG QSPKLLVYFASTRESGV PDRFIGSGSGTDFSLTI SSVQAEDLADYFCQEH YTTPPTFGTGTKLELK (SEQ ID NO: 6)	GYTFTD YYMN (SEQ ID NO: 12	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	KSSQSVF NSGNQK NSLA (SEQ ID NO: 21)	FASTRE S (SEQ ID NO: 22)	QEHYTT PPT (SEQ ID NO: 23)
ACI-36	3A8E12H8*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_G (SEQ ID NO: 50 and SEQ ID NO: 51)	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1)	DVVMTQTPLSLPVSLG DQASISCRSSQRLVHS HGKTYLHWYLQKPGQS PKLLIYKVSNRFSGVPD RFSGSGSGTDFTLKISR VEAEDLGVYFCSQTAH FPYTFGGGTKLEIK (SEQ ID NO: 7)	GYTFTD YYMN (SEQ ID NO: 12	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQRLV HSHGKT YLH (SEQ ID NO: 24)	KVSNRF S (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-36	2B6A10C11	SEQ ID NO: 46/SEQ ID NO: 52 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	EVQLQQSGPELVKPGT SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 2)	DVVMTQTPLSLPVSLG DQASISCRSSQSLVHSH GKTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ ID NO: 8)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQSLV HSHGKT YLH (SEQ ID NO: 27)	KVSNRF S (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)
ACI-36	2B6G7A12	SEQ ID NO: 46/SEQ ID NO: 52 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	EVQLQQSGPELVKPGT SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 2)	DVVMTQTPLSLPVSLG DQASISCRSSQSLVHSH GKTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ ID NO: 8)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQSLV HSHGKT YLH (SEQ ID NO: 27)	KVSNRF S (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)
ACI-36	6H1A11C11	SEQ ID NO: 46 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDTSSSTAYME LRSLTSEDSAVYYCASY YAVGYWGQGTTLTVSS (SEQ ID NO: 3)	DVVMTQTPLSLPVSLG DQASISCRSSQSLLHSH GNTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ ID NO: 9)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQSLL HSHGNT YLH (SEQ ID NO: 28)	KVSNRF S (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26))
ACI-36	6H1G6E6	SEQ ID NO: 46 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDTSSSTAYME LRSLTSEDSAVYYCASY YAVGYWGQGTTLTVSS (SEQ ID NO: 3)	DVVMTQTPLSLPVSLG DQASISCRSSQSLLHSH GNTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ ID NO: 9)	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQSLL HSHGNT YLH (SEQ ID NO: 28)	KVSNRF (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-33	6C10E5E9C12	SEQ ID NO: 53/SEQ ID NO:54 and SEQ ID NO: 47	SEQ ID NO: 48/SEQ ID NO:49 and SEQ ID NO: 51	EVQLVESGGGLVKPGG SLKLSCAPSGFTFSDYG MHWVRQAPEKGLEWV AYISSGSSTIYYGDTVK GRFTISRDNAKNTLFLQ MTSLRSEDTAMYYCAR RGQLRLRLFAYWGQGT LVTVSA (SEQ ID NO: 4)	DIVMTQSHKFMSTSVG DRVSITCKASQDVSTAV AWYQQKPGQSPKLLIY SASYRYTGVPDRFTGS GSGTDFTFTISSVQAED LAVYYCQQHYTTPLTFG AGTKLELK (SEQ ID NO: 10)	GFTFSD YGMH (SEQ ID NO: 15)	YISSGSS TIYYGDT VKG (SEQ ID NO: 16)	RGQLR LRLFAY (SEQ ID NO: 17)	KASQDV STAVA (SEQ ID NO: 29)	SASYRY T (SEQ ID NO: 30)	QQHYTT PLT (SEQ ID NO: 31)
ACI-33	6C10F9C12 A11	SEQ ID NO: 53/SEQ ID NO:54 and SEQ ID NO: 47	SEQ ID NO: 54 and SEQ ID NO: 51	EVQLVESGGGLVKPGG SLKLSCAPSGFTFSDYG MHWVRQAPEKGLEWV AYISSGSSTIYYGDTVK GRFTISRDNAKNTLFLQ MTSLRSEDTAMYYCAR RGQLRLRLFAYWGQGT LVTVSA (SEQ ID NO: 4)	DIVMTQSHKFMSTSVG DRVSITCKASQDVSTAV AWYQQKPGQSPKLLIY SASYRYTGVPDRFTGS GSGTDFTFTISSVQAED LAVYYCQQHYTTPLTFG AGTKLELK (SEQ ID NO: 10)	GFTFSD YGMH (SEQ ID NO: 15)	YISSGSS TIYYGDT VKG (SEQ ID NO: 16)	RGQLR LRLFAY (SEQ ID NO: 17)	KASQDV STAVA (SEQ ID NO: 29)	SASYRY T (SEQ ID NO: 30)	QQHYTT PLT (SEQ ID NO: 31)
ACI-41	7C2(1)F10C 10D3	SEQ ID NO: 53/ SEQ ID NO:55 and SEQ ID NO: 47	SEQ ID NO: 49/SEQ ID NO:56/SEQ ID NO: 57 and SEQ ID NO: 51	EVKLMESGGGLVHPGA SLRLYCAASGFTFTDYY MSWVRQPPGKAPEWL ALIRNKANGYTTEYTAS VKGRFTISRDNSQNILY LQMNTLRAEDSATYYC VKALGRYFDVWGTGTT VTVSS(SEQ ID NO: 5)	DIVMSQSPSSLAVSVGE KVTMSCKSSQSLLYSS NQKNYLAWYQQKPGQ SPKLLIYWASTRESGVP DRFTGSGSGTDFTLTIS SVKAEDLAVYYCQQYY SYPFTFGSGTKLEIK (SEQ ID NO: 11)	GFTFTD YYMS (SEQ ID NO: 18)	LIRNKAN GYTTEY TASVKG (SEQ ID NO: 19)	ALGRY FDV (SEQ ID NO: 20)	KSSQSLL YSSNQK NYLA (SEQ ID NO: 32)	WASTRE S (SEQ ID NO: 33)	QQYYSY PFT (SEQ ID NO: 34)
ACI-41	7C2(2)B9F1 1D5	SEQ ID NO: 53/ SEQ ID NO:55 and SEQ ID NO: 47	SEQ ID NO: 57 and SEQ ID NO: 51	EVKLMESGGGLVHPGA SLRLYCAASGFTFTDYY MSWVRQPPGKAPEWL ALIRNKANGYTTEYTAS VKGRFTISRDNSQNILY LQMNTLRAEDSATYYC VKALGRYFDVWGTGTT VTVSS (SEQ ID NO: 5)	DIVMSQSPSSLAVSVGE KVTMSCKSSQSLLYSS NQKNYLAWYQQKPGQ SPKLLIYWASTRESGVP DRFTGSGSGTDFTLTIS SVKAEDLAVYYCQQYY SYPFTFGSGTKLEIK (SEQ ID NO: 11)	GFTFTD YYMS (SEQ ID NO: 18)	LIRNKAN GYTTEY TASVKG (SEQ ID NO: 19)	ALGRY FDV (SEQ ID NO: 20)	KSSQSLL YSSNQK NYLA(SE Q ID NO: 32)	WASTRE S (SEQ ID NO: 33)	QQYYSY PFT (SEQ ID NO: 34)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-35	A4-4A6-18			QVQLQQPGAELLKPGA SVKLSCKASGYTFTSY WMHWVKQRPGRGLE WIGRIDPNSDRTKYNEK FKRKATLTVDKSSSTAY MQLSSLTSEDSAVYYC ARDDYAWFAYWGQGT LVTVSA (SEQ ID NO: 68)	DVLMTQTPLSLPVSLGD QASISCRSSQSIVHSNG NTYLEWYLQKPGQSPK LLIYKLSNRFSGVPDRF SGSGSGTDFTLKISRVE AEDLGVYYCFQGSHVP PTFGGGTKLEIK (SEQ ID NO: 69)	GYTFTS YWMH (SEQ ID NO: 70)	RIDPNS DRTKYN EKFKR (SEQ ID NO: 71)	DDYAW FAY (SEQ ID NO: 72)	RSSQSIV HSNGNT YLE (SEQ ID NO: 73)	KLSNRF S (SEQ ID NO: 74)	FQGSHV PPT (SEQ ID NO: 75)
ACI-35	A6-1D2-12			QVTLKESGPGILQSSQT LSLTCSFSGFSLSTSGM GVSWIRQPSGKGLEWL AHIYWDDDKRYNASLK SRLTISKDTSRNQVFLKI TCVDTADTATYYCARLL RPYALDYWGQGTSVTV SS (SEQ ID NO: 76)	NILMTQSPSSLAVSAGE KVTMSCKSSQSVLYSS NQKNYLAWYQQKPGQ SPKLLIYWASTRESGVP DRFTGSGSGTDFTLTIS SVQAEDLAVYYCLQYLS SLTFGAGTKLELK (SEQ ID NO: 77)	GFSLST SGMGVS (SEQ ID NO: 78)	HIYWDD DKRYNA SLKS (SEQ ID NO: 79)	LLRPYA LDY (SEQ ID NO: 80)	KSSQSVL YSSNQK NYLA (SEQ ID NO: 81)	WASTRE S (SEQ ID NO: 82)	LQYLSSL T (SEQ ID NO: 83)
ACI-35	A4-2A1-18			EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGE SVSISCRSSKSLLHSNG NTYLYWFLQRPGQSPQ LLIHRMSNLASGVPDRF SGSGSGTAFTLRISRVE AEDVGVYYCMQHLKSP YTFGGGTKLEIK (SEQ ID NO: 116)	GYTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYN QKFKG (SEQ ID NO: 90)	EGRFA Y (SEQ ID NO: 91)	RSSKSLL HSNGNT YLY (SEQ ID NO: 93)	RMSNLA S (SEQ ID NO: 94)	MQHLKS PYT (SEQ ID NO: 95)
ACI-35	A4-2A1-40			EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	DIX*MTQAAPSVPVTPG ESVSISCRSSKSLLHSN GNTYLYWFLQRPGQSP QLLIYRMSNLASGVPDR FSGSGSGTAFTLRISRV EAEDVGVYYCMQHLKS PYTFGGGTKLEIK (SEQ ID NO: 92) *X = M or V	GYTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYN QKFKG (SEQ ID NO: 90)	EGRFA Y (SEQ ID NO: 91)	RSSKSLL HSNGNT YLY (SEQ ID NO: 93)	RMSNLA S (SEQ ID NO: 94)	MQHLKS PYT (SEQ ID NO: 95)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI-35	A4-4A6-48			EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGE SVSISCRSSKSLLHSNG NTYLYWFLQRPGQSPQ LLIYRMSNLASGVPDRF SGSGSGTAFTLRISRVE AEDVGVYYCMQHLKSP YTFGGGTKLEIK (SEQ ID NO: 118)	GYTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYN QKFKG (SEQ ID NO: 90)	EGRFA Y (SEQ ID NO: 91)	RSSKSLL HSNGNT YLY (SEQ ID NO: 93)	RMSNLA S (SEQ ID NO: 94)	MQHLKS PYT (SEQ ID NO: 95)
ACI-35	A6-2G5-08			QVQLKQSGAELVRPGA SVKLSCKASGYTFTDYY INWVKQRPGQGLEWIA RIYPGRGNIYYNEKFKG KATLTAEKSSSTAYMQL SSLTSEDSAVYFCARF WDVTYWGQGTLVTVSA (SEQ ID NO: 96)	DVLMTQTPLSLPVSLGD QASISCRSSQSIVHSNG NTYLEWFLQKPGQSPK LLIYKVSNRFSGVPDRF SGSGSGTDFTLKISRVE AEDLGVYYCFQGSHVP YTFGGGTKLEIK (SEQ ID NO: 97)	GYTFTD YYIN (SEQ ID NO: 98)	RIYPGR GNIYYN EKFKG (SEQ ID NO: 99)	FWDVT Y (SEQ ID NO: 100)	RSSQSIV HSNGNT YLE (SEQ ID NO: 101)	KVSNRF S (SEQ ID NO: 102)	FQGSHV PYT (SEQ ID NO: 103)
ACI-35	A6-2G5-30			EVQLQQSGPELVKPGA SVKISCKASGFTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYHQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DIVMTQSQKFMSTSVG DRVSVTCKASQNVGTN VAWYQQKPGQSPKALI YSASYRYSGVPDRFTG SGSGTDFTLTISNVQSE DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)	GFTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYH QKFKG (SEQ ID NO: 115)	EGRFA Y (SEQ ID NO: 91)	KASQNV GTNVA (SEQ ID NO: 106)	SASYRY S (SEQ ID NO: 107)	QQYNSY PYT (SEQ ID NO: 108)
ACI-35	A6-2G5-41			EVQLQQSGPELVKPGA SVKISCKASGFTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYHQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DIVMTQSQKFMSTSVG DRVSVTCKASQNVGTN VAWYQQKPGQSPKALI YSASYRYSGVPDRFTG SGSGTDFTLTISNVQSE DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)	GFTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYH QKFKG (SEQ ID NO: 115)	EGRFA Y (SEQ ID NO: 91)	KASQNV GTNVA (SEQ ID NO: 106)	SASYRY S (SEQ ID NO: 107)	QQYNSY PYT (SEQ ID NO: 108)

*Two productive V_K sequences (sequences 6 and 7 in Table 10; sequences 40 and 41 in Table11) were isolated from cell lines 3A8A12G7 and 3A8E12H8; the "V_K_G" sequences were prepared from clones made using "G" primer mix and "V_K_AD" sequences from clones made using "A" and "D" primer mixes. Accordingly, two antibodies with different kappa sequences are produced by these hybridomas.

Table 11. Nucleotide Sequence of the heavy chain and light chain variable regions (VH and VK)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-36	3A8A12G7*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49/ and SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 35)	GACATTGTGATGACACAGTCTCCATCCTCCCTGGCTATGT CAGTAGGACAGAAGGTCACTATGAGCTGCAAGTCCAGTC AGAGTGTTTTTAATAGTGGCAATCAAAAGAACTCTTTGGC CTGGTACCAGCAGAAACCAGGACAGTCTCCTAAACTTCT GGTATACTTTGCATCCACTAGGGAATCTGGGGTCCCTGA TCGCTTCATAGGCAGTGGATCTGGGACAGATTTCAGTCTT ACCATCAGCAGTGTGCAGGCTGAGGACCTGGCAGATTAC TTCTGTCAGGAACATTATACCACTCCTCCCACGTTCGGTA CTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 40)
ACI-36	3A8A12G7*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_G (SEQ ID NO: 50 and SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 35)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGGCTTGTACACAGTCATGGAAAAACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGTTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 41)
ACI-36	3A8E12H8*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49 and SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 35)	GACATTGTGATGACACAGTCTCCATCCTCCCTGGCTATGT CAGTAGGACAGAAGGTCACTATGAGCTGCAAGTCCAGTC AGAGTGTTTTTAATAGTGGCAATCAAAAGAACTCTTTGGC CTGGTACCAGCAGAAACCAGGACAGTCTCCTAAACTTCT GGTATACTTTGCATCCACTAGGGAATCTGGGGTCCCTGA TCGCTTCATAGGCAGTGGATCTGGGACAGATTTCAGTCTT ACCATCAGCAGTGTGCAGGCTGAGGACCTGGCAGATTAC TTCTGTCAGGAACATTATACCACTCCCTCCCACGTTCGGTA CTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 40)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-36	3A8E12H8*	SEQ ID NO: 46 and SEQ ID NO: 47	VK_G (SEQ ID NO: 50 and SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 35)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGGCTTGTACACAGTCATGGAAAAACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGTTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 41)
ACI-36	2B6A10C11	SEQ ID NO: 46/SEQ ID NO: 52 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGACTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTTAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 36)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCCTTGTACACAGTCATGGAAAAACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGTTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 42)
ACI-36	2B6G7A12	SEQ ID NO: 46/SEQ ID NO: 52 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGACTTCAGTGAAGATATCCTGTAAGGCT TCTGGATATACGTTCACTACACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTTAAGGGCAAGGCCACGTTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAACTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 36)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCCTTGTACACAGTCATGGAAAAACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGTTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 42)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-36	6H1A11C11	SEQ ID NO: 46 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATACACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CACGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 37)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCCTTCTACACAGTCATGGAAACACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGCTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 43)
ACI-36	6H1G6E6	SEQ ID NO: 46 and SEQ ID NO: 47	SEQ ID NO: 50 and SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAACTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATACACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACCGTGGTGGAACTACTTACAA CCAGAAGTTCAAGGGCAAGGCCACGTTGACTGTAGA CACGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCA AGTTACTACGCCGTGGGCTACTGGGGCCAAGGCAC CACTCTCACAGTCTCCTCA (SEQ ID NO: 37)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCCTTCTACACAGTCATGGAAACACCTATTTACATTGG TACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCAACCGGTTTTCTGGGGTCCCAGACAGG TTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTC TGCTCTCAAACTGCACATTTTCCGTACACGTTCGGAGGG GGGACCAAGCTGGAAATAAAA (SEQ ID NO: 43)
ACI-33	6C10E5E9C12	SEQ ID NO: 53/SEQ ID NO:54 and SEQ ID NO: 47	SEQ ID NO: 48/SEQ ID NO:49 and SEQ ID NO: 51	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGT GAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCACC CTCTGGATTCACTTTCAGTGACTATGGAATGCACTGG GTTCGTCAGGCTCCAGAGAAGGGACTGGAGTGGGT TGCATACATTAGTAGTGGCAGTAGTACCATCTACTAT GGAGACACAGTGAAGGGCCGATTCACCATCTCCAGA GACAATGCCAAGAACACCCTGTTCCTGCAAATGACC AGTCTGAGGTCTGAGGACACGGCCATGTATTACTGT GCAAGAAGGGGACAGCTCAGGCTACGCCTGTTTGCT TACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 38)	GACATTGTGATGACCCAGTCTCACAAATTCATGTCCACAT CAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAGT CAGGATGTGAGTACTGCTGTAGCCTGGTATCAACAGAAA CCAGGACAATCTCCTAAACTACTGATTTACTCGGCATCCT ACCGGTACACTGGAGTCCCTGATCGCTTCACTGGCAGTG GATCTGGGACGGATTTCACTTTCACCATCAGCAGTGTGC AGGCTGAAGACCTGGCAGTTTATTACTGTCAGCAACATTA TACTACTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGA GCTGAAA (SEQ ID NO: 44)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-33	6C10F9C12 A11	SEQ ID NO: 53/SEQ ID NO:54 and SEQ ID NO: 47	SEQ ID NO: 54 and SEQ ID NO: 51	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGT GAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCACC CTCTGGATTCACTTTCAGTGACTATGGAATGCACTGG GTTCGTCAGGCTCCAGAGAAGGGGACTGGAGTGGGT TGCATACATTAGTAGTGGCAGTAGTACCATCTACTAT GGAGACACAGTGAAGGGCCGATTCACCATCTCCAGA GACAATGCCAAGAACACCCTGTTCCTGCAAATGACC AGTCTGAGGTCTGAGGACACGGCCATGTATTACTGT GCAAGAAGGGGACAGCTCAGGCTACGCCTGTTTGCT TACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 38)	GACATTGTGATGACCCAGTCTCACAAATTCATGTCCACAT CAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAGT CAGGATGTGAGTACTGCTGTAGCCTGGTATCAACAGAAA CCAGGACAATCTCCTAAACTACTGATTTACTCGGCATCCT ACCGGTACACTGGAGTCCCTGATCGCTTCACTGGCAGTG GATCTGGGACGGATTTCACTTTCACCATCAGCAGTGTGC AGGCTGAAGACCTGGCAGTTTATTACTGTCAGCAACATTA TACTACTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGA GCTGAAA (SEQ ID NO: 44)
ACI-41	7C2(1)F10C 10D3	SEQ ID NO: 53/ SEQ ID NO:55 and SEQ ID NO: 47	SEQ ID NO: 49/SEQ ID NO:56/ SEQ ID NO: 57 and SEQ ID NO: 51	GAGGTGAAGCTGATGGAATCTGGAGGAGGCTTGGTA CACCCTGGGGCTTCTCTGAGACTCTACTGTGCAGCT TCTGGATTCACCTTTACTGATTACTACATGAGCTGGG TCCGCCAGCCTCCAGGGAAGGCACCTGAGTGGTTG GCTTTGATTAGAAACAAAGCTAATGGTTACACAACAG AGTATACTGCATCTGTTAAGGGTCGGTTCACCATCTC CAGAGATAATTCCCAAAACATCCTCTATCTTCAAATG AACACCCTGAGGGCTGAGGACAGTGCCACTTATTAC TGTGTAAAAGCTCTGGGACGTTACTTCGATGTCTGG GGCACAGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO: 39)	GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGT CAGTTGGAGAGAAGGTTACTATGAGCTGCAAGTCCAGTC AGAGCCTTTTATATAGTAGCAATCAAAAGAACTACTTGGC CTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCT GATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGA TCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCT CACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTTA TTACTGTCAGCAATATTATAGCTATCCATTCACGTTCGGC TCGGGGACAAAGTTGGAAATAAAA (SEQ ID NO: 45)
ACI-41	7C2(2)B9F1 1D5	SEQ ID NO: 53/ SEQ ID NO:55 and SEQ ID NO: 47	SEQ ID NO: 57 and SEQ ID NO: 51	GAGGTGAAGCTGATGGAATCTGGAGGAGGCTTGGTA CACCCTGGGGCTTCTCTGAGACTCTACTGTGCAGCT TCTGGATTCACCTTTACTGATTACTACATGAGCTGGG TCCGCCAGCCTCCAGGGAAGGCACCTGAGTGGTTG GCTTTGATTAGAAACAAAGCTAATGGTTACACAACAG AGTATACTGCATCTGTTAAGGGTCGGTTCACCATCTC CAGAGATAATTCCCAAAACATCCTCTATCTTCAAATG AACACCCTGAGGGCTGAGGACAGTGCCACTTATTAC TGTGTAAAAGCTCTGGGACGTTACTTCGATGTCTGG GGCACAGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO: 39)	GACATTGTGATGTCACAGTCTCCATCCTCCCTAGCTGTGT CAGTTGGAGAGAAGGTTACTATGAGCTGCAAGTCCAGTC AGAGCCTTTTATATAGTAGCAATCAAAAGAACTACTTGGC CTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCT GATTTACTGGGCATCCACTAGGGAATCTGGGGTCCCTGA TCGCTTCACAGGCAGTGGATCTGGGACAGATTTCACTCT CACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTTA TTACTGTCAGCAATATTATAGCTATCCATTCACGTTCGGC TCGGGGACAAAGTTGGAAATAAAA (SEQ ID NO: 45)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-35	A4-4A6-18			CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTTCT GAAGCCTGGGGCTTCAGTGAAACTGTCCTGCAAGGC TTCTGGCTACACCTTCACCAGCTACTGGATGCACTG GGTGAAGCAGAGGCCTGGACGAGGCCTTGAGTGGA TTGGAAGGATTGATCCTAATAGTGATCGTACTAAGTA CAATGAGAAGTTCAAGCGCAAGGCCACACTGACTGT AGACAAATCCTCCAGCACAGCCTACATGCAGCTCAG CAGCCTGACATCTGAGGACTCTGCGGTCTATTATTGT GCAAGGGATGATTACGCCTGGTTTGCTTACTGGGGC CAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 84)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCATTGTACATAGTAATGGAAACACCTATTTAGAATGG TACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATC TACAAACTTTCCAACCGATTTTCTGGGGTCCCAGACAGGT TCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGA TCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACT GCTTTCAAGGTTCACATGTTCCTCCGACGTTCGGTGGAG GCACCAAGCTGGAAATCAAA (SEQ ID NO: 85)
ACI-35	A6-1D2-12			CAGGTTACTCTGAAAGAGTCTGGCCCTGGGATATTG CAGTCCTCCCAGACCCTCAGTCTGACTTGTTCTTTCT CTGGGTTTTCACTGAGCACTTCTGGTATGGGTGTGA GCTGGATTCGTCAGCCTTCAGGAAAGGGTCTGGAGT GGCTGGCACACATTTACTGGGATGATGACAAGCGCT ATAACGCATCCCTGAAGAGCCGGCTCACAATCTCCA AGGATACCTCCAGAAACCAGGTATTCCTCAAGATCA CCTGTGTGGACACTGCAGATACTGCCACATACTACT GTGCTCGGTTACTGCGTCCTTATGCTTTGGACTACTG GGGTCAAGGAACCTCAGTCACCGTCTCCTCA (SEQ ID NO: 86)	AACATTTTGATGACACAGTCGCCATCATCTCTGGCTGTGT CTGCAGGAGAAAAGGTCACTATGAGCTGTAAGTCCAGTC AAAGTGTTTTATATCAGTTCAAATCAGAAGAACTACTTGGC CTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCT GATCTACTGGGCATCCACTAGGGAATCTGGTGTCCCTGA TCGCTTCACAGGCAGTGGATCTGGGACAGATTTTACTCTT ACCATCAGCAGTGTACAAGCTGAAGACCTGGCAGTTTATT ACTGTCTTCAATACCTCTCCTCGCTCACGTTCGGTGCTGG GACCAAGCTGGAGCTGAAA (SEQ ID NO: 87)
ACI-35	A4-2A1-18			GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATACACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACAATGGTGGTACTAGCTACAA CCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG TCTGACATCTGAGGACTCTGCAGTCTATTATTGTGTA AGAGAGGGGCGGTTTGCTTACTGGGGTCATGGGAC TCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCA CTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGTA AGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTATTG GTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGAT ACATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAG GTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACTGAG AATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTTTATTA CTGTATGCAACATCTAAAATCTCCGTACACGTTCGGAGG GGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 117)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-35	A4-2A1-40			GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATACACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACAATGGTGGTACTAGCTACAA CCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG TCTGACATCTGAGGACTCTGCAGTCTATTATTGTGTA AGAGAGGGGCGGTTTGCTTACTGGGGTCATGGGAC TCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTR*TGATGACTCAGGCTGCACCCTCTGTACCTGTC ACTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGT AAGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTATT GGTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGA TATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACA GGTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACTGA GAATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTTTATT ACTGTATGCAACATCTAAAATCTCCGTACACGTTCGGAGG GGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 110) R* = A or G
ACI-35	A4-4A6-48			GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATACACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACAATGGTGGTACTAGCTACAA CCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG TCTGACATCTGAGGACTCTGCAGTCTATTATTGTGTA AGAGAGGGGCGGTTTGCTTACTGGGGTCATGGGAC TCTGGTCACTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTACCTGTCA CTCCTGGAGAGTCAGTATCCATCTCCTGCAGGTCTAGTA AGAGTCTCCTGCATAGTAATGGCAACACTTACTTGTATTG GTTCCTGCAGAGGCCAGGCCAGTCTCCTCAGCTCCTGAT ATATCGGATGTCCAACCTTGCCTCAGGAGTCCCAGACAG GTTCAGTGGCAGTGGGTCAGGAACTGCTTTCACACTGAG AATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTTTATTA CTGTATGCAACATCTAAAATCTCCGTACACGTTCGGAGG GGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 119)
ACI-35	A6-2G5-08			CAGGTCCAGCTGAAGCAGTCTGGGGCTGAGCTGGT GAGGCCTGGGGCTTCAGTGAAACTGTCCTGCAAGG CTTCTGGCTACACTTTCACTGACTACTATATAAACTG GGTGAAGCAGAGGCCTGGACAGGGACTTGAGTGGA TTGCAAGGATTTATCCTGGAAGAGGTAATATTTACTA CAATGAGAAGTTCAAGGGCAAGGCCACACTGACTGC AGAAAAATCCTCCAGCACTGCCTACATGCAGCTCAG CAGCCTGACATCTGAGGACTCTGCTGTCTATTTCTGT GCAAGATTCTGGGACGTGACTTACTGGGGCCAAGG GACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 111)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCA GTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCA GAGCATTGTACATAGTAATGGAAACACCTATTTAGAATGG TTCCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATC TACAAAGTTTCCCAACCGATTTTCTGGGGTCCCAGACAGGT TCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGA TCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACT GCTTTCAAGGTTCACATGTTCCGTACACGTTCGGAGGGGG GGACCAAGCTGGAAATAAAA (SEQ ID NO: 112)

Vaccine	Hybridoma	VH Primer (mix)	VK Primer (mix)	VH	VK
ACI-35	A6-2G5-30			GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATTCACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACAATGGTGGTACTAGCTACCA CCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGTA AGAGAGGGAAGATTTGCTTACTGGGGCCAAGGGACT CTGGTCACTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACAT CAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGT CAGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAA CCAGGGCAATCTCCTAAAGCACTGATTTACTCGGCATCCT ACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAATGTGCA GTCTGAAGACTTGGCAGAGTATTTCTGTCAGCAATATAAC AGCTATCCGTACACGTTCGGAGGGGGGACCAAGCTGGA AATAAAA (SEQ ID NO: 114)
ACI-35	A6-2G5-41			GAGGTCCAGCTGCAACAATCTGGACCTGAGCTGGTG AAGCCTGGGGCTTCAGTGAAGATATCCTGTAAGGCT TCTGGATTCACGTTCACTGACTACTACATGAACTGGG TGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTG GAGATATTAATCCTAACAATGGTGGTACTAGCTACCA CCAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGA CAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAG CCTGACATCTGAGGACTCTGCAGTCTATTACTGTGTA AGAGAGGGAAGATTTGCTTACTGGGGCCAAGGGACT CTGGTCACTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACAT CAGTAGGAGACAGGGTCAGCGTCACCTGCAAGGCCAGT CAGAATGTGGGTACTAATGTAGCCTGGTATCAACAGAAA CCAGGGCAATCTCCTAAAGCACTGATTTACTCGGCATCCT ACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAATGTGCA GTCTGAAGACTTGGCAGAGTATTTCTGTCAGCAATATAAC AGCTATCCGTACACGTTCGGAGGGGGGACCAAGCTGGA AATAAAA (SEQ ID NO: 114)

*Two productive V_K sequences (sequences 6 and 7 in Table 10; sequences 40 and 41 in Table11) were isolated from cell lines 3A8A12G7 and 3A8E12H8; the "V_K_G" sequences were prepared from clones made using "G" primer mix and "V_K_AD" sequences from clones made using "A" and "D" primer mixes. Accordingly, two antibodies with different kappa sequences are produced by these hybridomas.

Table 12. Primers used for CDR sequencing of antibody variable regions

Subclone	Ab isotype	Primer sequences			SEQ ID NO
3A8A 12G7	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5' AD	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	48 49
			5' G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
3A8E 12H8	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5' AD	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	48 49
			5' G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
2B6A 10C11	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT	46 52
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
2B6G 7A12	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT	46 52
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6H1A11C11	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6H1G 6E6	IgG2b	VH primers	5'	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46

Subclone	Ab isotype	Primer sequences			SEQ ID NO
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6C10F9 C12A11	IgG3	VH primers	5'	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	53 54
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	54
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6C10E5E 9C12	IgG3	VH primers	5'	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	53 54
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	48 49
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
7C2(1)F10C 10D3	IgG2b	VH primers	5'	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT	53 55
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGGAGWCAGACACACISCTGYTATGGGT ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	56 49 57
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
7C2(2)B9F 11D5	IgG2b	VH primers	5'	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT	53 55
			3'	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		VK primers	5'	ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	57
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51

Subclone	Ab isotype	Primer sequences			SEQ ID NO
A6-2G5-08	IgG2a	VH primers	5'	GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
				GGGAATTCATGGAATGCAGCTGGGTCATTCTCTT	122
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGAAATGGAGCTGGGTCTTTTCTT	125
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTTC	127
			3'	CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAGTGGATAGACGGGTGG	132
				CCCAAGCTTCCAGGGACCAAGGGATAGATGATGG	133
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGGCCAATGGATAAACGATGG	135
		VK primers	5'	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-2A1-18	IgG2b	VH primers	5'	GGGAATTCATGGAATGGAGCTGGGTCATTCTCTT	136
				GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGAGCTGGGTCTTCCTCTT	138
				GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				ACTAGTCGACATGGGATGAGCTTATCATCCTCTT	140
			3'	CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGGATAAACGGGTGG	142
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGGTGG	143

Subclone	Ab isotype	Primer sequences			SEQ ID NO
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGATGG	145
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGGCCAGGGATAAACGGGTGG	146
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAGTGGATAAACGGTGG	148
		VK primers	5'	ACTAGTCGACATGGTGTCCACAGCTCAGTTCCTTG	149
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6-2G5-30	IgG2b	VH primers	5'	GGGAATTCATGAAATGGAGCTGGGTCTTCCTCTT	150
				GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTTATGGAATGGAGCTGGGTCTTCCTCTT	152
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127
				GGGAATTCATGGAATGCAGCTGGGTCATCCTCTT	153
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				ACTAGTCGACATGGGATGGAGCTATATCATCCTCTT	156
				ACTAGTCGACATGGGATGGAGCTTATCATCTTCTT	157
				ACTAGTCGACATGTAGATGTGGTTAAACTGGGT	158
			3'	CCCAAGCTTCCAGGGGCCAGGGGATAAACGGATGG	159
				CCCAAGCTTCCAGGGGCCAAGGGATAGACGGATGG	160
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGGTGG	161
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGATGG	162
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGATGG	163
				CCCAAGCTTCCAGGGGCCAATGGATAACGATGG	164
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGATGG	165
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166
				CCCAAGCTTCCAGGGACCAATGGATAACGATGG	167
				CCCAAGCTTCCAGGGACCATGGATAAACGGGTGG	168

Subclone	Ab isotype	Primer sequences			SEQ ID NO
		VK primers	5'	ACTAGTCGACATGGGCATCAAGATGAAGTCACATACTCTGG	169
				ACTAGTCGACATGGGCATCAAGATGAGTCACATACTCTGG	170
				ACTAGTCGACTGGGCATCAGATGAGTCACATACTCTGG	171
				ACTAGTCGACATGGGCATCAAGATGAAGTCACAGACCCAGG	172
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCTGG	173
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATATTCAGG	174
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-2A1-40	IgG2b	VH primers	5'	GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
				ACTAGTCGACATGGATGGAGCTTATCATCCTCTT	175
			3'	CCCAAGCTTCCAGGGACCAAGGGATAAACGGTGG	176
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGGTGG	177
				CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
		VK primers	5'	ACTAGTCGACATGAGGTACTCGGCTCAGTTCCTGGG	178
				ACTAGTCGACATGAGGTCCCCGGCTCAGTTCCTGGG	179
				ACTAGTCGACATGAGGACGTCGATTCAGTTCTTGGG	180
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6-2G5-41	IgG2b	VH primers	5'	GGGAATTCATGGAATGGACCTGGGTCATCCTCTT	181
				GGGAATTCATGGAATGCAGCTGGGTTTTCTCTT	120
				GGGAATTCATGGAATGCAGCTGGGTTATCCTCTT	182
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126
				GGGAATTCATGAATGGATCTGGGTTATTCTCTT	183

Subclone	Ab isotype	Primer sequences			SEQ ID NO
			3'	CCCAAGCTTCCAGGGACCAGGGGATAAACGGGTGG	184
				CCCAAGCTTCCAGGGACCAAGGGACGGGTGG	185
				CCCAAGCTTCCAGGGACCAATGGATAAACAGATGG	186
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGATGG	145
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGGTGG	187
		VK primers	5'	GGGAATTCATGGAGACACATTCCCAGGTCTTT	188
				GGGAATTCATGGAGTCACAGTCTCAGGTCTTT	189
				ACTAGTCGACATGGGCTTCAAGATGGAGTCACATTTTCAGG	190
				ACTAGTCGACATGGGCATCAAGATGAAGTCACATATTCAGG	191
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCAGG	192
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-4A6-48	IgG2b	VH primers	5'	ACTAGTCGACATGGGATGGAGCTTATCATGTTCTT	193
				ACTAGTCGACATGGGATGGAGCTTATCATGCTCTT	194
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGACCTGGGTTTTCCTCTT	195
				GGGAATTCATGGAATGGACCTGGGTCTTTCTCTT	196
				GGGAATTCATGAAATGGAGCTGGGTTATTCTCTT	197
				GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
			3'	CCCAAGCTTCCAGGGGCCAATGGATAGACGATGG	198
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGATGG	199
				CCCAAGCTTCCAGGGACCAAGGGATAGACGATGG	200
				CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144

Subclone	Ab isotype	Primer sequences			SEQ ID NO
		VK primers	5'	ACTAGTCGACATGATGTACCCGGCTCAGTTTCTGGG	201
				ACTAGTCGACATGAGGACTTCGATTCAGTTCTTGGG	202
				ACTAGTCTACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	203
				ACTAGTCGACATGAAGTTGTCTGTTAGGCTGTTGGTGCT	204
				ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3'	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4-4A6-18	IgG2b	VH primers	5'	ATGGGATGGAGCTRTATCATSYTCTT	205
				ATGAAGWTGTGGBTRAACTGGRT	206
				ATGGRATGGASCKKIRTCTTTMTCT	207
			3'	CCAGGGRCCARKGGATARACIGRTGG	208
		VK primers	5'	ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3'	ACTGGATGGTGGGAAGATGGA	215
A6-1D2-12	IgG2a	VH primers	5'	ATGAAATGCAGCTGGRTYATSTTCTT	216
				ATGGRCAGRCTTACWTYYTCATTCCT	217
				ATGATGGTGTTAAGTCTTCTGTACCT	218
			3'	CCAGGGRCCARKGGATARACIGRTGG	208

Subclone	Ab isotype	Primer sequences			SEQ ID NO
		VK primers	5'	ATGRAGWCACAKWCYCAGGTCTTT	219
				ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT	220
				ATGGGCWTCAAGATGRAGTCACAKWYYCWGG	221
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3'	ACTGGATGGTGGGAAGATGGA	215

Degenerate Codons:

R	= A or G	S	= C or G	D	= A or G or T
Y	= C or T	M	= A or C	H	= A or C or T
K	= G or T	W	= A or T	V	= A or G or C
				B	= C or G or T

Table 13. Longest isoform of human Tau (441aa), also called Tau40

Longest isoform of human Tau (441aa), also called Tau40 (SEQ ID NO: 67) Microtubule-associated protein tau isoform 2 [Homo sapiens] NCBI Reference Sequence: NP_005901.2	MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L (SEQ ID NO: 67)

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U.S. Patent No. 5,268,164,

U.S. Patent No. 5,506,206,

U.S. Patent No. 5,686,416

U.S. Patent No. 5,004,697

Claims:

1. An antibody, or a functional part thereof, which antibody recognizes and specifically binds to a phospho-epitope on the mammalian Tau protein or on a fragment thereof, wherein said antibody or antibody fragment has a high binding affinity to soluble and insoluble Tau protein, and modulates soluble and insoluble Tau levels.

2. The antibody of claim 1 or a functional fragment thereof, wherein said antibody or antibody fragment modulates soluble and insoluble Tau levels in the brain of a mammal.

3. The antibody of claim 2 or a functional fragment thereof, wherein said antibody or antibody fragment modulates soluble and insoluble Tau levels in brain cortex and/or hippocampus.

4. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of total soluble tau protein.

5. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of soluble phosphorylated tau protein.

6. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels of paired helical filaments containing hyperphosphorylated tau protein.

7. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment reduces the levels total soluble tau protein, soluble phosphorylated tau protein and pTau paired helical filaments.

8. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said mammal is a human.

9. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 10 nM.

10. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a dissociation constant of at least 5 nM, at least 2 nM; at least 1 nM, at least 500 pM,at least 300 pM, at least 200 pM, at least 100 pM.

11. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater; of 10^5 $M^{-1}s^{-1}$ or greater, of 10^6 $M^{-1}s^{-1}$ or greater.

12. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 4 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

13. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 3 nM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

14. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 2 nM and an association rate constant of 10^4 $M^{-1}s^{-1}$ or greater.

15. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 1 nM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

16. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a dissociation constant of at least 200 pM and an association rate constant of 10^5 $M^{-1}s^{-1}$ or greater.

17. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment has a high binding affinity with a

dissociation constant of at least 100 pM and an association rate constant of 10^6 $M^{-1}s^{-1}$ or greater.

18. The antibody of any one of the preceding claims or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position (pS214); Tau aa 393-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

19. The antibody of claim 12 or 13 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 15-20 with a phosphorylated Tyr at position 18 (Y18).

20. The antibody of claim 14 or 15 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 405-412 with a phosphorylated Ser at position 409 (pS409).

21. The antibody of claim 16 or 17 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 405-411 with a phosphorylated Ser at position 409 (pS409).

22. The antibody of claim 12 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396).

23. The antibody of claim 12 or a functional fragment thereof, wherein said antibody or antibody fragment binds to an epitope comprising Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404).

24. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains, particularly in sequence, a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, or an amino acid sequence at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at

least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, or an amino acid sequence at least 60%, particularly at least 70%, particularly at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, 70, 78, 89, 98, or an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, 115, or an amino acid sequence at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, 72, 80, 91, 100, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

25. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 70% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 90% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 60% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid

sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 80% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 60% identical thereto.

26. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 85% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 80% identical thereto; and/or a antibody domain which contains a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 85% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 85% identical thereto.

27. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, or an amino acid sequence at least 95% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto

28. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain (antibody domain) which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 90% identical thereto; a CDR2 with the amino acid

sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 90% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

29. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 95% identical thereto and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

30. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, or an amino acid sequence at least 98% identical thereto; a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, or an amino acid sequence at least 98% identical thereto;. and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, or an amino acid sequence at least 98% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20, or an amino acid sequence at least 90% identical thereto.

31. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, 24, 27, 28, 29, 32, a CDR2 with the

amino acid sequence shown in SEQ ID NO: 22, 25, 30, 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, 26, 31, 34, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, 15, 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, 16, 19, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, 17, 20.

32. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 21, or an amino acid sequence at least 76% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 22, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 23, or an amino acid sequence at least 66% identical thereto, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, a DR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66% identical thereto.

33. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 24, or an amino acid sequence at least 88% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 25, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 26, or an amino acid sequence at least 66% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 12, a CDR2 with the amino acid sequence shown in SEQ ID NO: 13, or an amino acid sequence at least 88% identical thereto,and a CDR3 with the amino acid sequence shown in SEQ ID NO: 14, or an amino acid sequence at least 66% identical thereto

34. The antibody of claim 33 or a functional fragment thereof, comprising a first binding domain wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 27, or an amino acid sequence at least 88% identical thereto.

35. The antibody of claim 33 or a functional fragment thereof, comprising a first binding domain wherein the CDR1 has the amino acid sequence shown in SEQ ID NO: 28, or an amino acid sequence at least 88% identical thereto.

36. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 29, a CDR2 with the amino acid sequence shown in SEQ ID NO: 30, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 31, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 15, a CDR2 with the amino acid sequence shown in SEQ ID NO: 16, or an amino acid sequence at least 94% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 17, or an amino acid sequence at least 36% identical thereto.

37. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 32, a CDR2 with the amino acid sequence shown in SEQ ID NO: 33, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 34, and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 18, a CDR2 with the amino acid sequence shown in SEQ ID NO: 19, or an amino acid sequence at least 95% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 20, or an amino acid sequence at least 63% identical thereto.

38. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 73, a CDR2 with the amino acid sequence shown in SEQ ID NO: 74, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 75, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 70, or an amino acid sequence at least 95%, particularly 98%, particularly 99% identical thereto, a CDR2 with the amino acid sequence shown in SEQ ID NO: 71, or an amino acid sequence at least 94%, 95%, 96%, 97%, 98%, or 99% identical thereto, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 72, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least

95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

39. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 81, a CDR2 with the amino acid sequence shown in SEQ ID NO: 82, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 83, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 78, a CDR2 with the amino acid sequence shown in SEQ ID NO: 79, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 80, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

40. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 93, a CDR2 with the amino acid sequence shown in SEQ ID NO: 94, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 95, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 90, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

41. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 101, a CDR2 with the amino acid sequence shown in SEQ ID NO: 102, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 103, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 98, a CDR2 with the amino acid sequence shown in SEQ ID NO: 99, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 100, or an amino acid sequence at least at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

42. The antibody of any one of the preceding claims or a functional fragment thereof, comprising a first binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 106, a CDR2 with the amino acid sequence shown in SEQ ID NO: 107, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 108, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs and/or a second binding domain which contains in sequence a CDR1 with the amino acid sequence shown in SEQ ID NO: 89, a CDR2 with the amino acid sequence shown in SEQ ID NO: 115, and a CDR3 with the amino acid sequence shown in SEQ ID NO: 91, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical to any one of the above CDRs.

43. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in

SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 85% identical thereto.

44. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 90% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

45. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain (antibody domain) which contains the amino acid sequence shown in SEQ ID NO: 6, 7, 8, 9, 10, 11, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, 5, or an amino acid sequence at least 91% identical thereto.

46. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69, 77, 116/92, 97, 105, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto, and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68, 76, 88, 96, 104, or an amino acid sequence at least 60%, at least 70%, at least 80%, particularly at least 85%, particularly at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,particularly at least 95%, particularly at least 96%, particularly at least 97%, particularly at least 98%, particularly at least 99% or 100% identical thereto.

47. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 or SEQ ID NO: 7, or an amino acid sequence at least 90% and 94%, respectively, identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1, or an amino acid sequence at least 91% identical thereto.

48. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2, or an amino acid sequence at least 90% identical thereto.

49. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain, which contains the amino acid sequence shown in SEQ ID NO: 9, or an amino acid sequence at least 95% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3, or an amino acid sequence at least 90% identical thereto.

50. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10, or an amino acid sequence at least 99% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4, or an amino acid sequence at least 89% identical thereto.

51. The antibody of any one of the preceding claims or a functional fragment thereof comprising a first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11, or an amino acid sequence at least 98% identical thereto; and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5, or an amino acid sequence at least 87% identical thereto.

52. The antibody of any one of the preceding claims or a functional fragment thereof comprising a

a. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 6 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or

b. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 7 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 1; or

c. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 8 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 2; or

d. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 9 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 3; or

e. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 10 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 4; or

f. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 11 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 5; or a

g. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 69 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 68; or a

h. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 77 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 76; or a

i. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 116 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a;

j. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 92 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 88; or a

k. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 97 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 6; or a

l. first binding domain which contains the amino acid sequence shown in SEQ ID NO: 105 and/or a second binding domain which contains the amino acid sequence shown in SEQ ID NO: 104.

53. The antibody of the preceding claim or a functional fragment thereof, which is polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody or a functional fragment thereof.

54. The antibody of any one of the preceding claims or a functional fragment thereof which is of the IgG2b, IgG2a or the IgG3 isotype.

55. A binding peptide or protein of the antibody according to any one of claims 1-54.

56. The binding peptide or protein or the antibody or functional fragment thereof according to any one of the preceding claims, wherein said binding peptide, protein antibody or antibody fragment recognizes and specifically binds to a phospho-epitope on the mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a phospho-epitope on aggregated Tau protein, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes.

57. A polynucleotide encoding the antibody any one of the preceding claims or a functional fragment thereof.

58. The polynucleotide of claim 57 comprising a nucleic acid molecule selected from the group consisting of

a. a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising the amino acid sequence as depicted in SEQ ID NOs: 35-45,;

b. a nucleic acid molecule comprising a nucleotide sequence that has at least 85% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

c. a nucleic acid molecule comprising a nucleotide sequence that has at least 90% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117;

d. a nucleic acid molecule comprising a nucleotide sequence that has at least 95% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

e. a nucleic acid molecule comprising a nucleotide sequence that has at least 98% sequence identity to the sequence shown in SEQ ID NOs: 35-45; SEQ ID NOs: 84-87, SEQ ID NO: 109-112 and 117-119;

f. a nucleic acid molecule comprising a nucleotide sequence the complementary strand of which hybridizes to the nuclei acid molecule of any of a) – f);

g. a nucleic acid molecule comprising a nucleotide sequence that deviates from the nucleotide sequence defined in any of a) – g) by the degeneracy of the genetic code:

wherein said nucleic acid molecule as defined in any of a) – g) recognizes and specifically binds to a phospho-epitope on a mammalian, particularly on the human Tau protein or on a fragment thereof, particularly to a pathological protein tau conformer, but does not bind to the corresponding unphosphorylated epitope and/or to non-related epitopes, wherein said antibody has a high binding affinity to aggregated Tau protein, and modulates soluble and insoluble Tau levels.

59. A pharmaceutical composition comprising a binding peptide or protein, an antibody or a functional fragment thereof or a polynucleotide according to any one of the preceding claims, or a combination thereof, in a therapeutically effective amount together with a pharmaceutically acceptable carrier.

60. The pharmaceutical composition of claim 59, which comprises at least two binding peptides or proteins, antibodies or functional fragments thereof or polynucleotides according to any one of the preceding claims, which recognize and bind to two different phospho-epitope on the mammalian Tau protein.

61. The pharmaceutical composition of claim 59 or 60, which comprises an additional antibody or a functional fragment thereof which regcongizes and binds to a different amyloidogenic protein or peptide.

62. An antibody or a functional fragment thereof, abinding peptide or protein, a polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof, for use in therapy, particularly for use in the treatment of neurodegenerative disorders such as tauopathies in a mammal, particularly a human in need of such a treatment.

63. An antibody or a functional fragment thereof, a binding peptide or protein,, or pharmaceutical composition according to the preceding claim for use in treatment or alleviation of cognitive deficits in a mammal, particularly a human suffering from such a deficit.

64. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim, wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an arrest in the progression of the cognitive deficits.

65. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim,

wherein the treatment or alleviation of cognitive deficits in a mammal, particularly a human, leads to an increase in the retention, particularly a complete restoration of cognitive memory capacity in the treated subject.

66. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or the pharmaceutical composition according to the preceding claim for use in the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

67. The antibody or a functional fragment thereof, a binding peptide or protein, polynucleotide, or pharmaceutical composition according to the preceding claim for use in the treatment of Alzheimer's Disease.

68. A method for the treatment of a neurodegenerative disease or disorder such as tauopathy comprising administering to an animal, particularly to a mammal, but especially to human, suffering from such a disease or disorder, an antibody or a functional fragment thereof, a binding peptide or protein, a polynucleotide or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

69. The method according to the preceding claim for the treatment of diseases and disorders which are caused by or associated with the formation of neurofibrillary

lesions, the predominant brain pathology in tauopathy comprising a heterogenous group of neurodegenerative diseases or disorders including diseases or disorders which show co-exsistance of tau and amyloid pathologies including, but not limited to, Alzheimer's Disease, Creutzfeldt-Jacob disease, Dementia pugilistica, Down's Syndrome, Gerstmann-Sträussler-Scheinker disease, inclusion-body myositis, and prion protein cerebral amyloid angiopathy, traumatic brain injury and further diseases or disorders which do not show a distinct amyloid pathology including, but not limited to, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, Non-Guamanian motor neuron disease with neurofibrillary tangles, argyrophilic grain dementia, corticobasal degeneration, diffuse neurofibrillary tangles with calcification, frontotemporal dementia with parkinsonism linked to chromosome 17, Hallevorden-Spatz disease, multiple system atrophy, Niemann-Pick disease, type C, Pallido-ponto-nigral degeneration, Pick's disease, progressive subcortical gliosis, progressive supranuclear palsy, Subacute sclerosing panencephalitis Tangle only dementia, Postencephalitic Parkinsonism, Myotonic dystrophy.

70. A method for inducing a passive immune response in an animal, particularly a mammal or a human, suffering from a neurodegenerative disorder such as tauopathy by administering to said animal or human an antibody or a functional fragment thereof, a binding peptide or protein or a pharmaceutical composition according to any one of the preceding claims, or a combination thereof.

71. A method of diagnosing a tau-protein-associated disease, disorder or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau protein into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau protein to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau protein in the sample or specific body part or area.

72. A method for diagnosing a predisposition to tau-protein-associated disease, disorder or condition in a patient comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the tau protein in a sample or *in situ* which includes the steps of

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient is suffering from or is at risk of developing an tau protein-associated disease or condition.

73. A method for monitoring minimal residual disease in a patient following treatment with an antibody or a pharmaceutical composition according to any one of the preceding claims, wherein said method comprises:

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex to a normal control value,

wherein an increase in the amount of said aggregate compared to a normal control value indicates that said patient still suffers from a minimal residual disease.

74. A method for predicting responsiveness of a patient being treated with an antibody or a pharmaceutical composition according to any one of the preceding claims comprising

a. bringing the sample or a specific body part or body area suspected to contain the tau antigen into contact with an antibody or a fragment thereof according to any one of the preceding claims, which peptide or fragment thereof binds an epitope of the tau protein;
b. allowing the antibody to bind to the tau antigen to form an immunological complex;
c. detecting the formation of the immunological complex; and
d. correlating the presence or absence of the immunological complex with the presence or absence of tau antigen in the sample or specific body part or area,
e. comparing the amount of said immunological complex before and after onset of the treatment,

wherein an decrease in the amount of said aggregate indicates that said patient has a high potential of being responsive to the treatment.

75. Test kits for detection and diagnosis of tau protein-associated diseases, disorders or conditions comprising an antibody according to any of the preceding claims.

76. Test kit according to the preceding claim comprising a container holding one or more antibodies or functional fragments thereof according to the present invention and instructions for using the antibodies for the purpose of binding to tau antigen to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of tau antigen.

77. An epitope selected from the group consisting of Tau aa 15-20 comprising a phosphorylated Tyr at position 18 (Y18), Tau aa 405-412 comprising a phosphorylated Ser at position 409 (pS409), Tau aa 405-411 comprising a phosphorylated Ser at position 409 (pS409); and Tau aa 208-218 comprising a phosphorylated Thr at position 212 (pT212) and a phosphorylated Ser at position (pS214), Tau aa 393-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 396-401 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 394-

400 comprising a phosphorylated Ser at position 396 (pS396), Tau aa 402-406 comprising a phosphorylated Ser at position 404 (pS404), and Tau aa 393-400 comprising a phosphorylated Ser at position 396 (pS396).

78. A cell line producing an antibody according to any of the preceding claims.

79. A cell line according to claim 78, which is hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079.

80. A cell line according to claim 78, which is hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081

81. A cell line according to claim 78, which is hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080.

82. A cell line according to claim 78, which is hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088.

83. A cell line according to claim 78, which is hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084.

84. A cell line according to claim 78, which is hybridoma cell line 2B6G7A12 deposited on March 10, 2010 as DSM ACC3087.

85. A cell line according to claim 78, which is hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086.

86. A cell line according to claim 78, which is hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085.

87. A cell line according to claim 78, which is hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082.

88. A cell line according to claim 78, which is hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083.

89. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10F9C12A11 deposited on August 25, 2010 as DSM ACC3079 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

90. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6C10E5E9C12 deposited on August 25, 2010 as DSM ACC3081 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 54 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

91. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1A11C11 deposited on August 25, 2010 as DSM ACC3080 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

92. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 6H1G6E6 deposited on August 25, 2010 as DSM ACC3088 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

93. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6A10C11 deposited on August 25, 2010 as DSM ACC3084 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

94. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 2B6G7A12 deposited on August 25, 2010 as DSM ACC3087 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 46 and SEQ ID NO: 52 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

95. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 3A8A12G7 deposited on August 25, 2010 as DSM ACC3086 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

96. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR

amplification of DNA of hybridoma cell line 3A8E12H8 deposited on August 25, 2010 as DSM ACC3085 using

a_1. a mix of primers comprising a 5'-primer of SEQ ID NO: 48 and SEQ ID NO: 49 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; or

a_2. a primer pair comprising a 5'-primer of SEQ ID NO: 50 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a primer pair comprising a 5'-primer of SEQ ID NO: 46 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

97. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(1)F10C10D3 deposited on August 25, 2010 as DSM ACC3082 using

a. a mix of primers comprising a 5'-primer of SEQ ID NO: 49; SEQ ID NO: 56 and SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

98. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line 7C2(2)B9F11D5 deposited on August 25, 2010 as DSM ACC3083 using

a. a pair of primers comprising a 5'-primer of SEQ ID NO: 57 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain;

b. a mix of primers comprising a 5'-primer of SEQ ID NO: 53 and SEQ ID NO: 55 and a 3'-primer of SEQ ID NO: 47 for amplification of a second binding domain.

99. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139 using

a. a primer pair comprising a 5'-primer of SEQ ID NO: 149 and a 3'-primer of SEQ ID NO: 51 for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, and 140 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 134, and 141-148, for amplification of a second binding domain.

100. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 169-174 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 124, 127, and 150-158 and a 3'-primer selected from the group consisting of SEQ ID NOs: 130, and 159-168, for amplification of a second binding domain.

101. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 178, 179 and 180 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 127, 139, 154, 155, and 175 and a 3'-primer selected from the group consisting of SEQ ID NOs: 128, 129, 147, 176, and 177, for amplification of a second binding domain.

102. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 51 and 188-192 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 120, 124, 126, 181, 182 and 183 and a 3'-primer selected from the group consisting of SEQ ID NOs: 144, 145 and 184-187, for amplification of a second binding domain.

103. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 50 and 201-204 and a 3'-primer of SEQ ID NO: 51, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 121, 137, 151 and 193-197 and a 3'-primer selected from the group consisting of SEQ ID NOs: 131, 141, 144, 166, 198, 199 and 200, for amplification of a second binding domain.

104. An antibody or a functional fragment thereof according to claim 1 which is encoded by a polynucleotide located on a nucleotide fragment that can be obtained by PCR amplification of DNA of hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141 using

a. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 209-214, and 219-221 a 3'-primer of SEQ ID NO: 215, for amplification of a first binding domain; and/or

b. a mix of primers comprising a 5'-primer selected from the group consisting of SEQ ID NOs: 216, 217 and 218 and a 3'-primer of SEQ ID NOs: 208, for amplification of a second binding domain.

105. The antibody or a functional fragment thereof of any one of the preceding claims, wherein said antibody is an antibody of the IgG2a, IgG2b or the IgG3 isotype, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody or a fully human antibody.

106. A binding fragment or protein or an antibody according to any one of the preceding claims.

107. A cell line that produces the antibody of any one of claims 1-54, 56 or of any one of claims 89 – 105.

108. A cell line comprising the polynucleotide of claim 57 or 58.

109. A method of producing the antibody of any one of claims 1-54, 56 or of any one of claims 89 – 105, comprising the step of culturing the cell line of claim 106 in a suitable cultivation medium.

110. A method of producing an antibody according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , comprising the step of culturing the cell line of any one of claims 78-88 in a suitable cultivation medium.

111. The method of claim 109 or 110, further comprising the step of purifying the antibody from the cell line or cultivation medium.

112. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in the treatment of tauopathies, a group of tau protein associated diseases and disorders.

113. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in treating or alleviating the symptoms associated with tauopathies, a group of tau protein associated diseases and disorders.

114. Use of the antibody including any functional parts thereof according to any one of claims 1-54, 56 or of any one of claims 89 – 105 , in the preparation of a medicament for use in retaining or increasing cognitive memory capacity in a mammal suffering from a tauopathy.

115. A cell line according to claim 78, which is hybridoma cell line A4-4A6-48 deposited on August 30, 2011 as DSM ACC3136.

116. A cell line according to claim 78, which is hybridoma cell line A6-2G5-30 deposited on August 30, 2011 as DSM ACC3137.

117. A cell line according to claim 78, which is hybridoma cell line A6-2G5-41 deposited on August 30, 2011 as DSM ACC3138.

118. A cell line according to claim 78, which is hybridoma cell line A4-2A1-18 deposited on August 30, 2011 as DSM ACC3139.

119. A cell line according to claim 78, which is hybridoma cell line A4-2A1-40 deposited on August 30, 2011 as DSM ACC3140.

120. A cell line according to claim 78, which is hybridoma cell line A6-1D2-12 deposited on September 6, 2011 as DSM ACC3141.

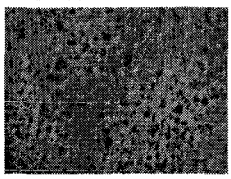
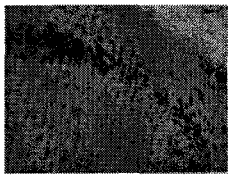
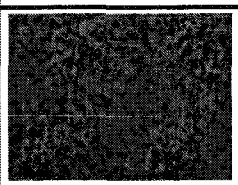
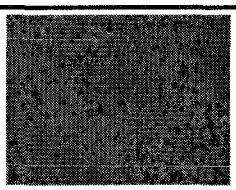
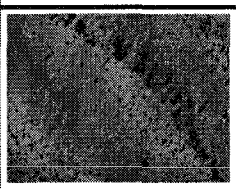
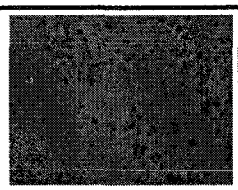
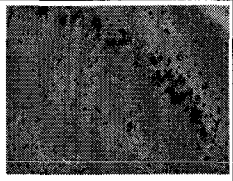
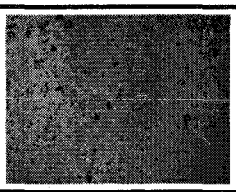
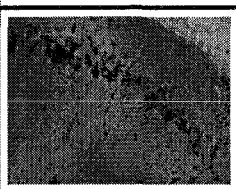
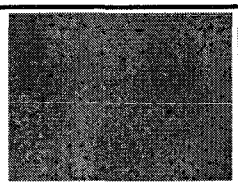
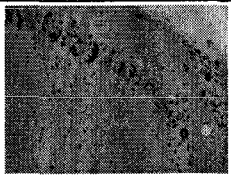
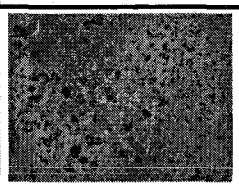
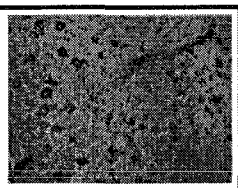
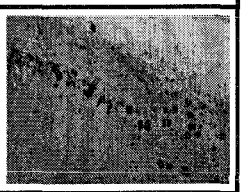
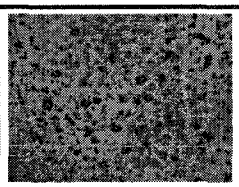
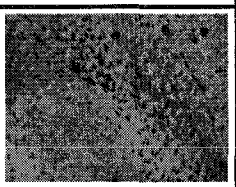
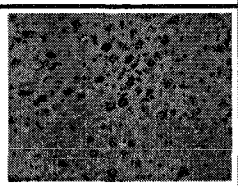
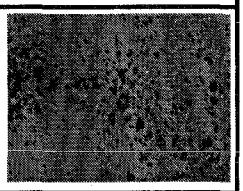
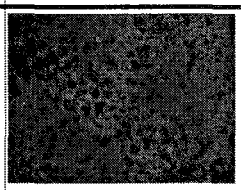
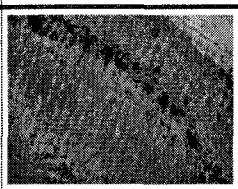
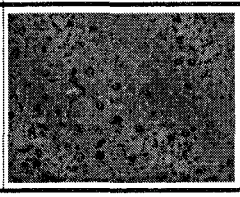
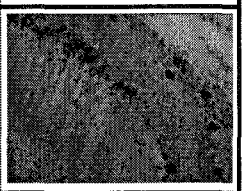
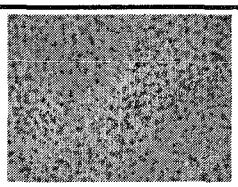
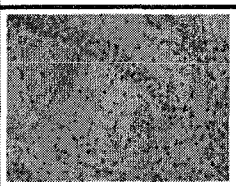
Clone	Cortex	Hippocampus (CA1)	Clone	Cortex	Hippocampus (CA1)
6C10F9 C12A11			6C10E5 E9C12		
6H1A11 C11			6H1G6E 6		
2B6A10 C11			2B6G7A 12		
3A8A12 G7			3A8E12 H8		
7C2(1) F10C10 D3			7C2(2) B9F11D 5		
Control AT100 antibody			***Control*** PG5 antibody		
Control No primary antibody					

FIGURE 1-1

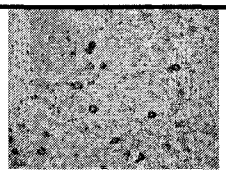
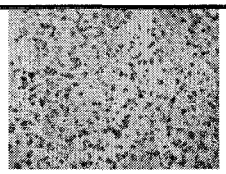
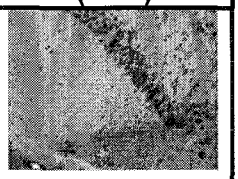
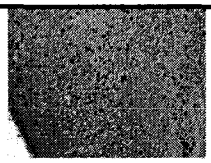
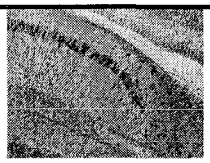
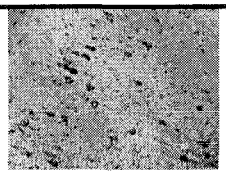
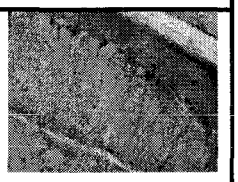
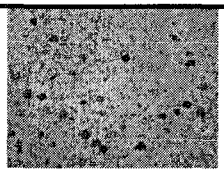
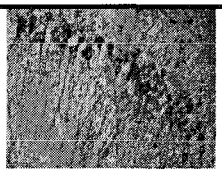
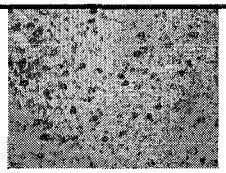
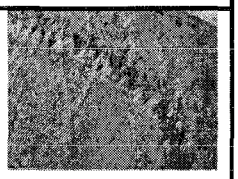
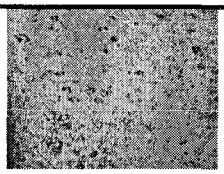
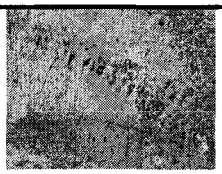
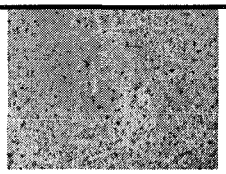
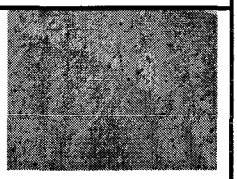
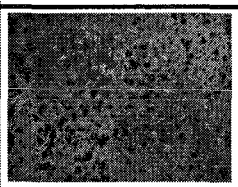
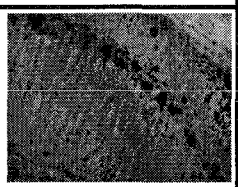
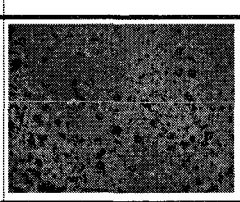
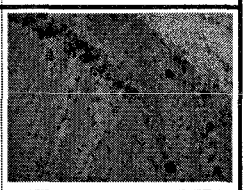
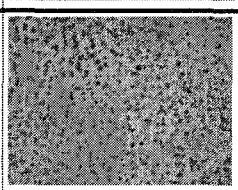
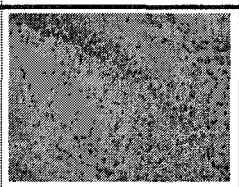
Clone	Cortex	Hippocampus (CA1)	Clone	Cortex	Hippocampus (CA1)
A4-2A1-18			A6-2G5-08		
A4-4A6-48			A6-2G5-30		
A4-2A1-40			A6-2G5-41		
A6-1D2-12			A4-4A6-18		
Control AT100 antibody			***Control*** PG5 antibody		
Control No primary antibody					

FIGURE 1-2

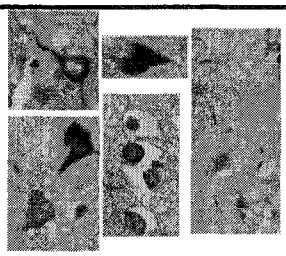
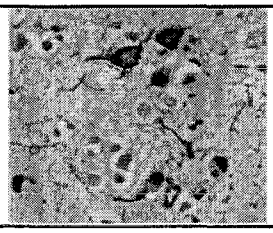
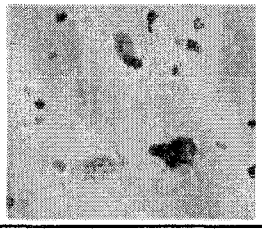
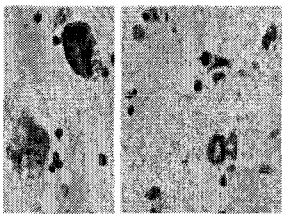
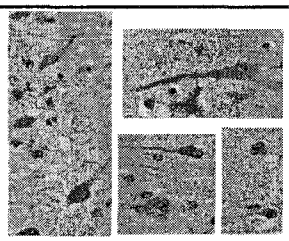
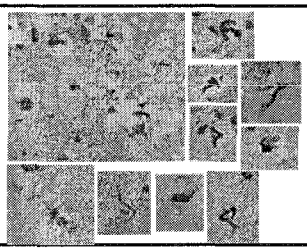
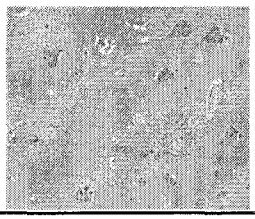
Diagnosis	Region	ACI-3A8-Ab1 TAUPIR staining
AD	Hippocampus	
FAD	Anterior hippocampus	
AGD	Anterior hippocampus	
PSP	Pallidum	
FTDP-17	Frontal cortex	
CBD	Frontal cortex	
Healthy control	Hippocampus	

FIGURE 2

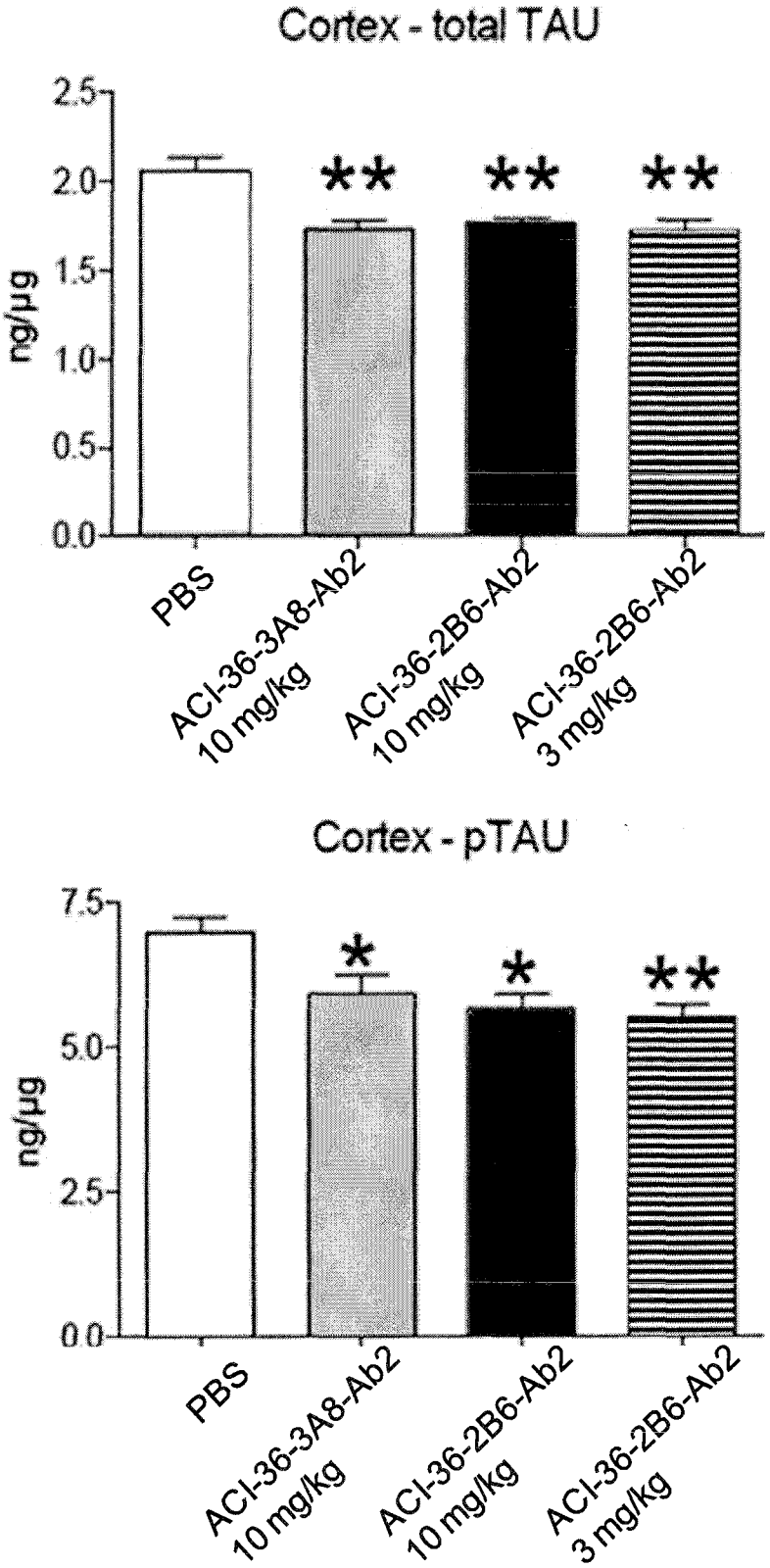

Cortex - total TAU
ng/µg
2.5
2.0
1.5
1.0
0.5
0.0
**
**
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg
Cortex - pTAU
ng/µg
7.5
5.0
2.5
0.0
*
*
**
PBS
ACI-36-3A8-Ab2 10 mg/kg
ACI-36-2B6-Ab2 10 mg/kg
ACI-36-2B6-Ab2 3 mg/kg

FIGURE 3

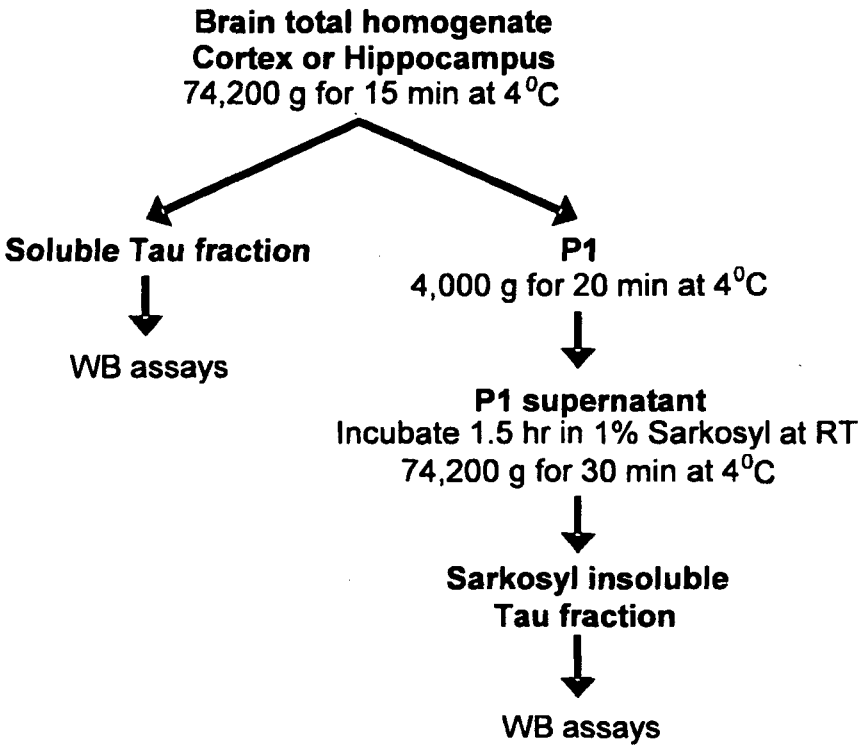


FIGURE 4-1

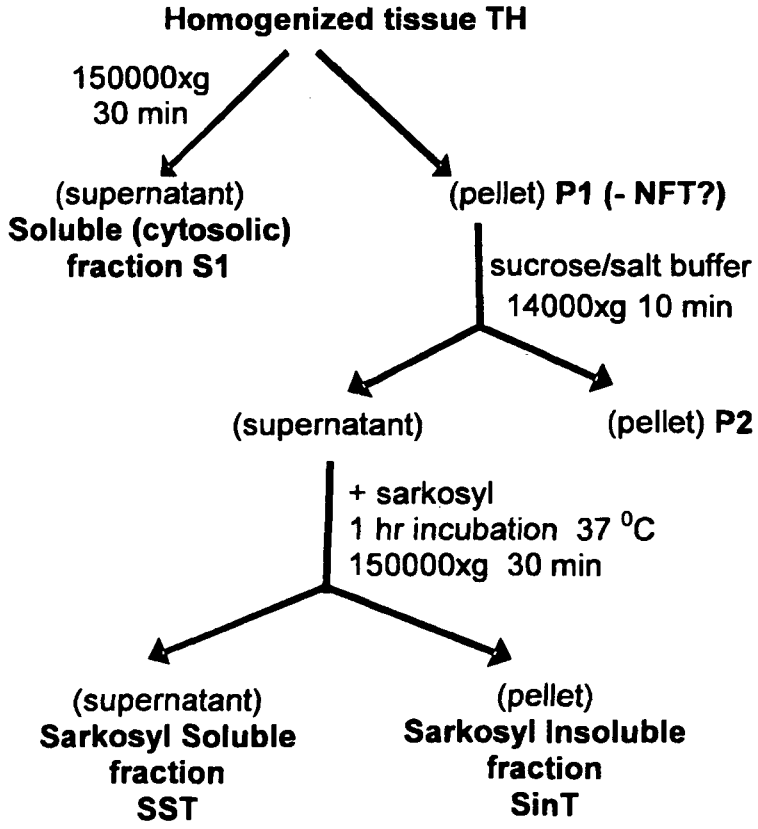


FIGURE 4-2

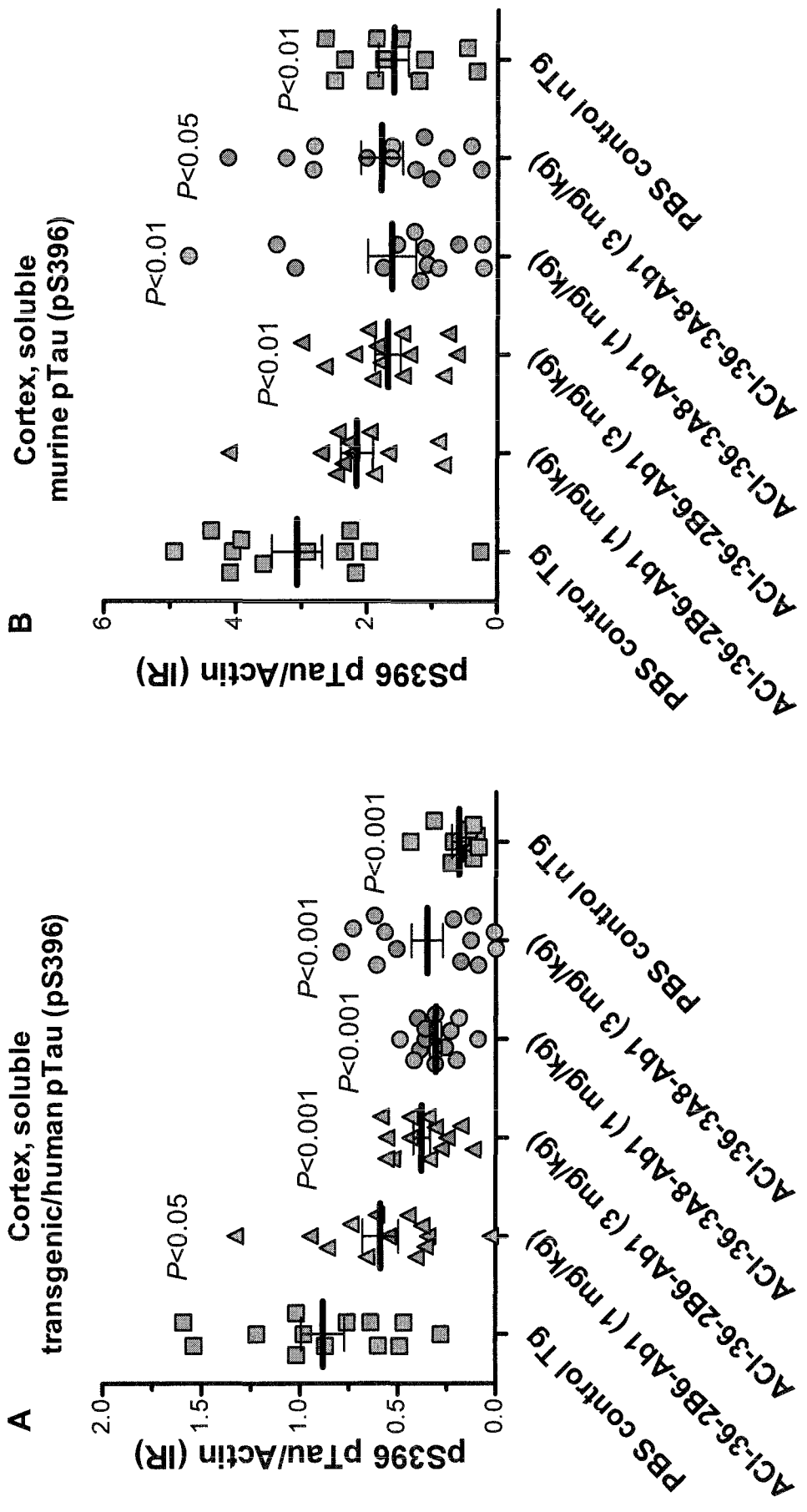

A
Cortex, soluble
transgenic/human pTau (pS396)
pS396 pTau/Actin (IR)
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
P<0.001
P<0.001
P<0.001
PBS control nTg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control Tg
B
Cortex, soluble
murine pTau (pS396)
pS396 pTau/Actin (IR)
6
4
2
0
P<0.01
P<0.05
P<0.01
P<0.01
PBS control nTg
ACI-36-3A8-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-2B6-Ab1 (1 mg/kg)
PBS control Tg

FIGURE 5-1

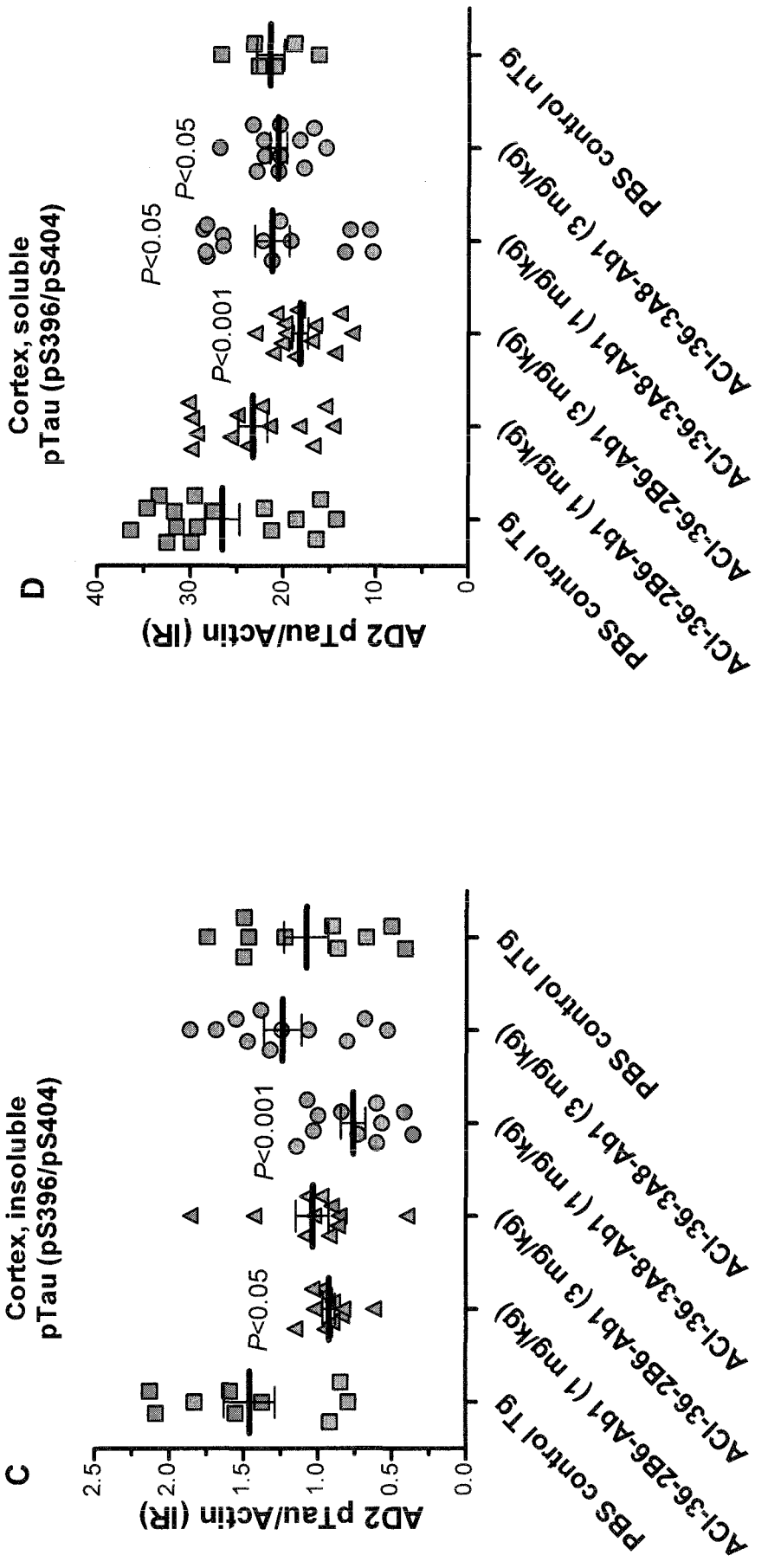

C
Cortex, insoluble
pTau (pS396/pS404)
AD2 pTau/Actin (IR)
2.5
2.0
1.5
1.0
0.5
0.0
P<0.05
P<0.001
PBS control nTg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control Tg
D
Cortex, soluble
pTau (pS396/pS404)
AD2 pTau/Actin (IR)
40
30
20
10
0
P<0.05
P<0.001
P<0.05
PBS control nTg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control Tg

FIGURE 5-2

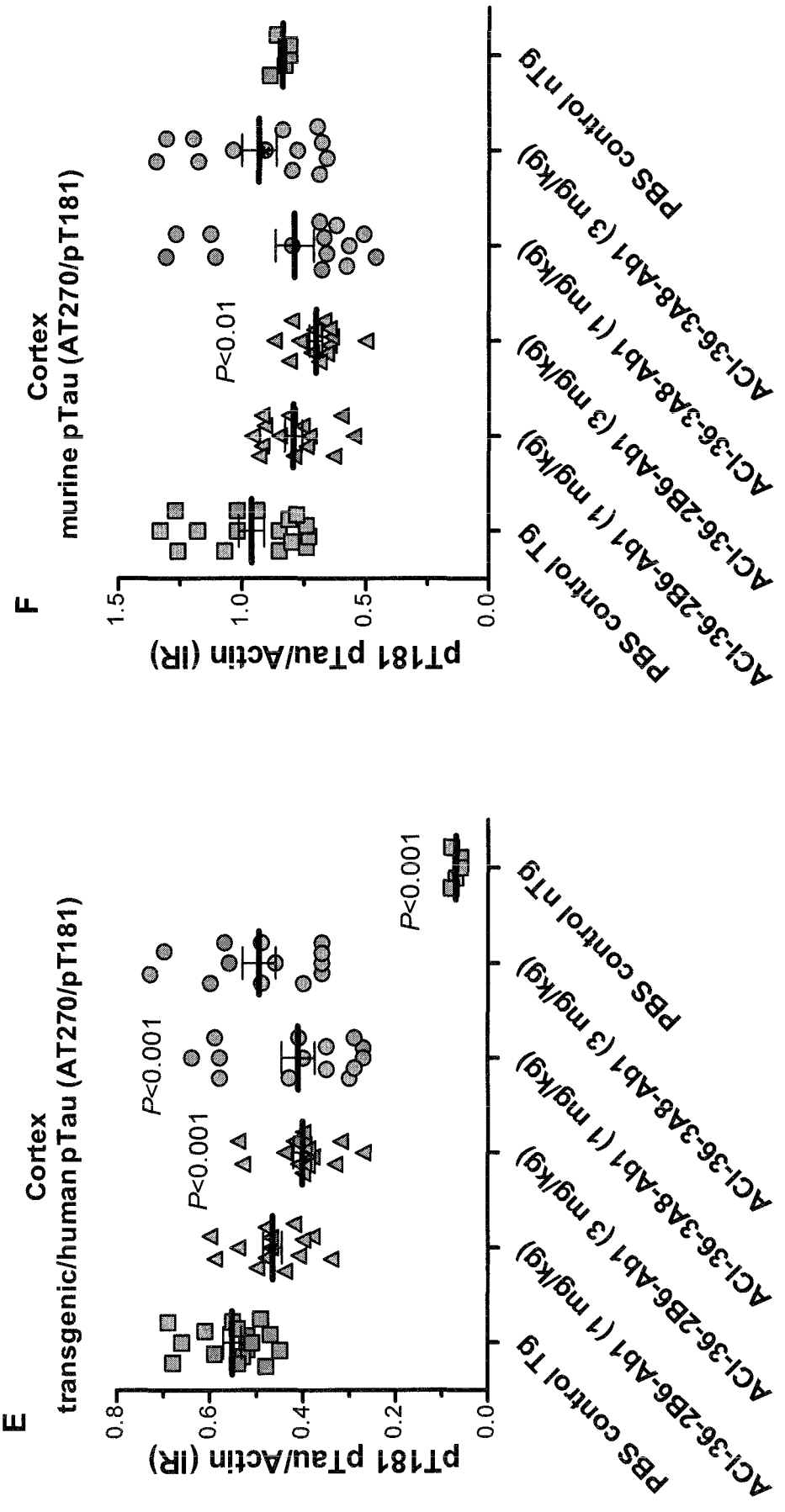

E
Cortex
transgenic/human pTau (AT270/pT181)
pT181 pTau/Actin (IR)
0.0
0.2
0.4
0.6
0.8
P<0.001
P<0.001
P<0.001
PBS control TG
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTG
F
Cortex
murine pTau (AT270/pT181)
pT181 pTau/Actin (IR)
0.0
0.5
1.0
1.5
P<0.01
PBS control TG
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTG

FIGURE 5-3

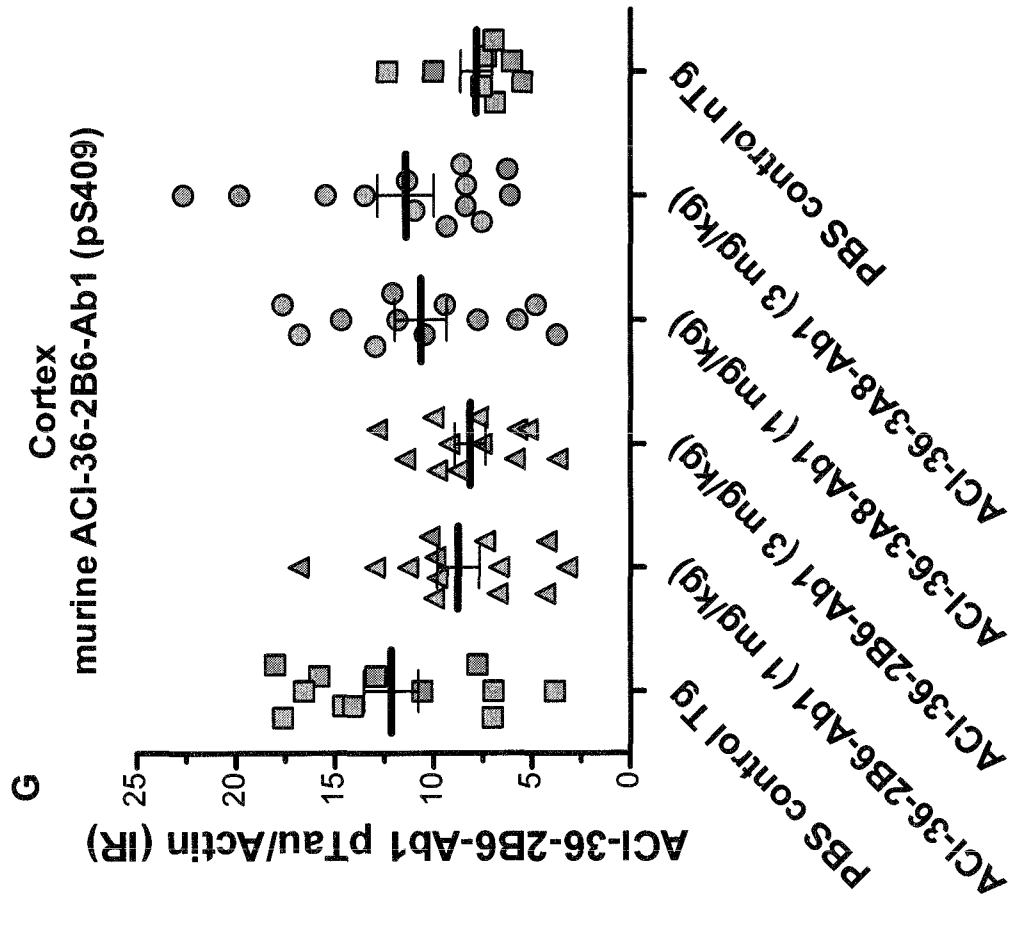

G
Cortex
murine ACI-36-2B6-Ab1 (pS409)
ACI-36-2B6-Ab1 pTau/Actin (IR)
25
20
15
10
5
0
PBS control TG
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-4

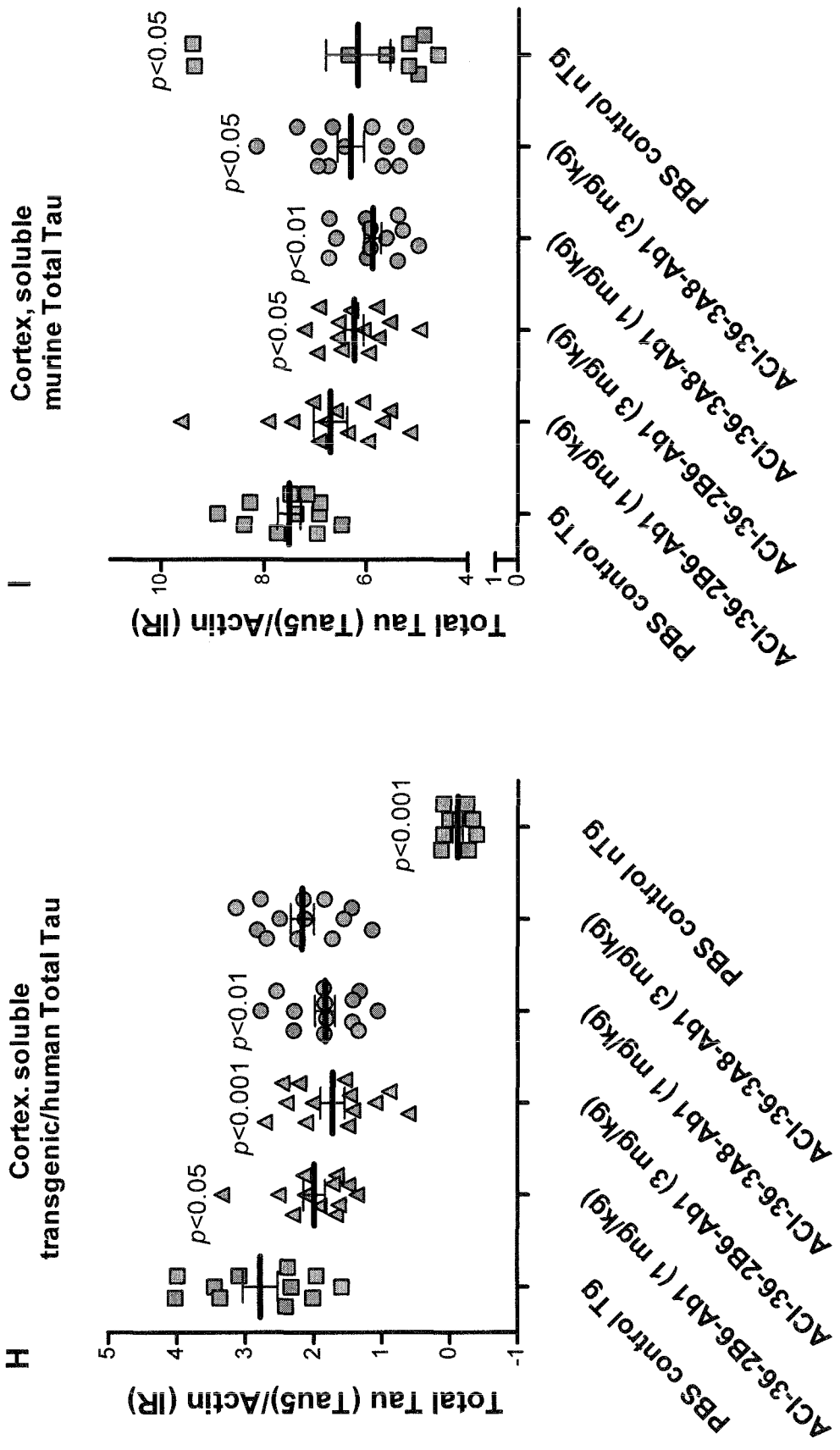

H
Cortex. soluble
transgenic/human Total Tau
Total Tau (Tau5)/Actin (IR)
p<0.05
p<0.001
p<0.01
p<0.001
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg
I
Cortex, soluble
murine Total Tau
Total Tau (Tau5)/Actin (IR)
p<0.05
p<0.05
p<0.01
p<0.05
PBS control Tg
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)
PBS control nTg

FIGURE 5-5

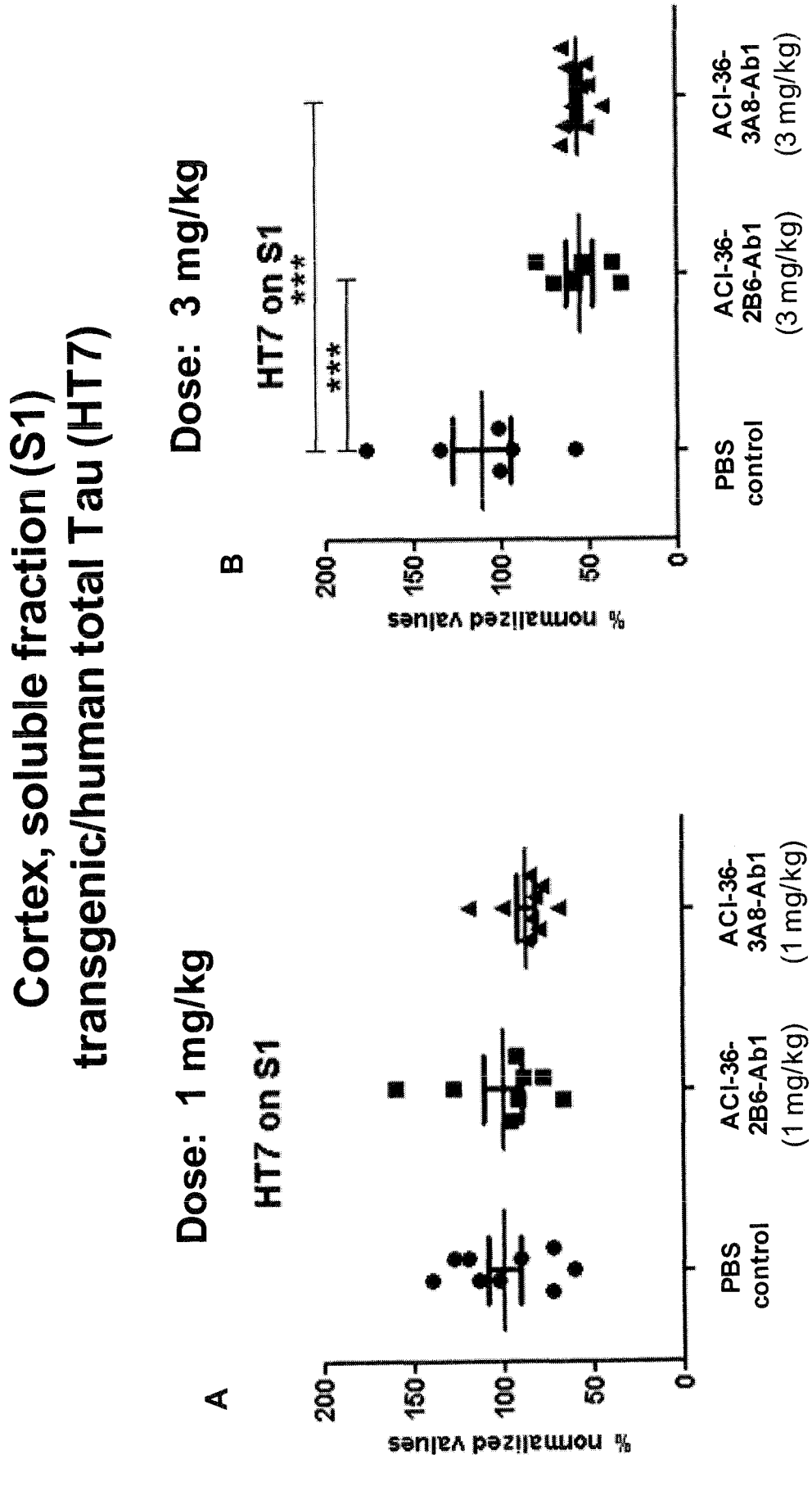

Cortex, soluble fraction (S1)
transgenic/human total Tau (HT7)
A
Dose: 1 mg/kg
HT7 on S1
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
B
Dose: 3 mg/kg
HT7 on S1
***
***
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-1

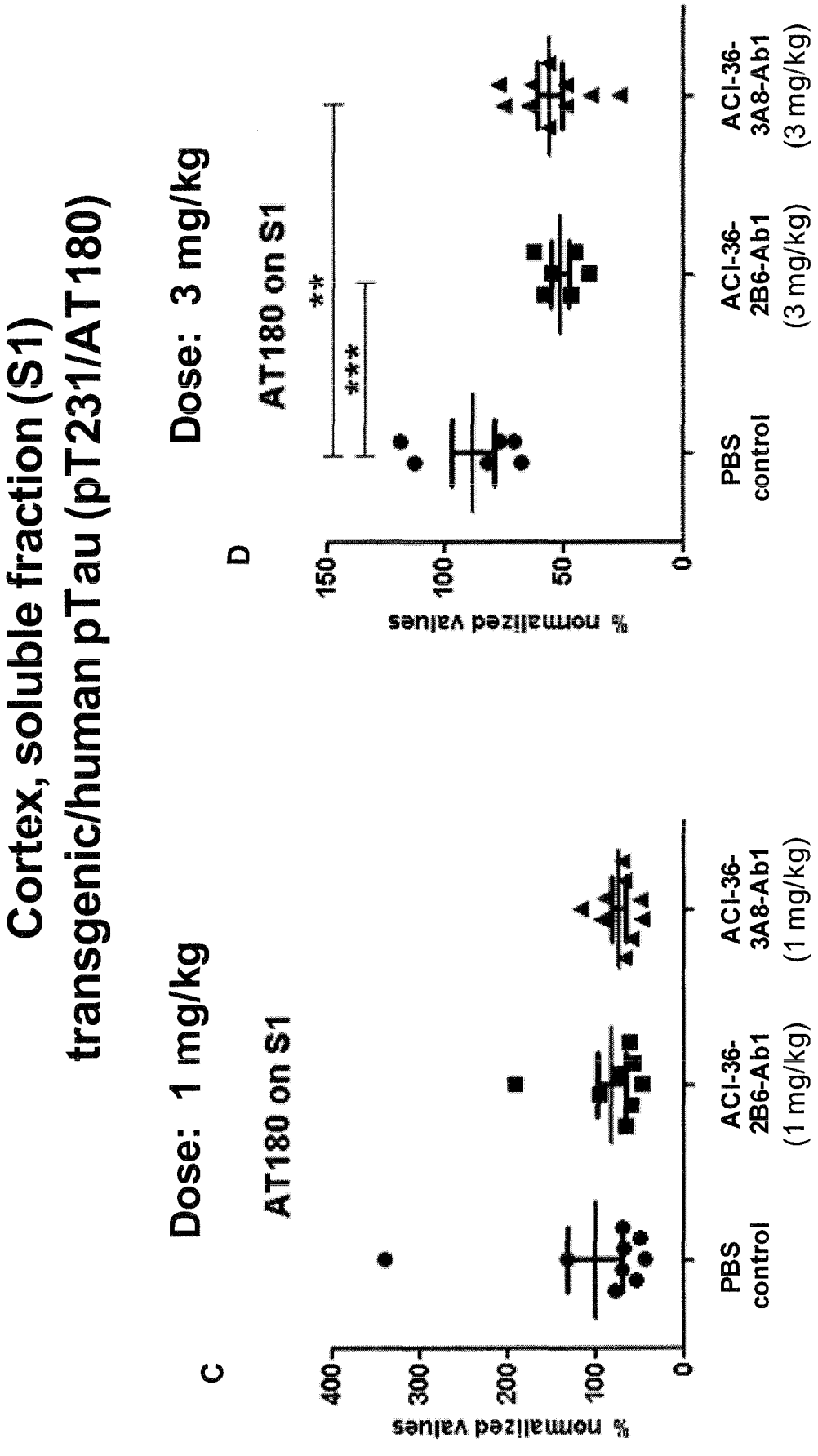

Cortex, soluble fraction (S1)
transgenic/human pTau (pT231/AT180)
C
Dose: 1 mg/kg
AT180 on S1
% normalized values
400
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
D
Dose: 3 mg/kg
AT180 on S1
**
***
% normalized values
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-2

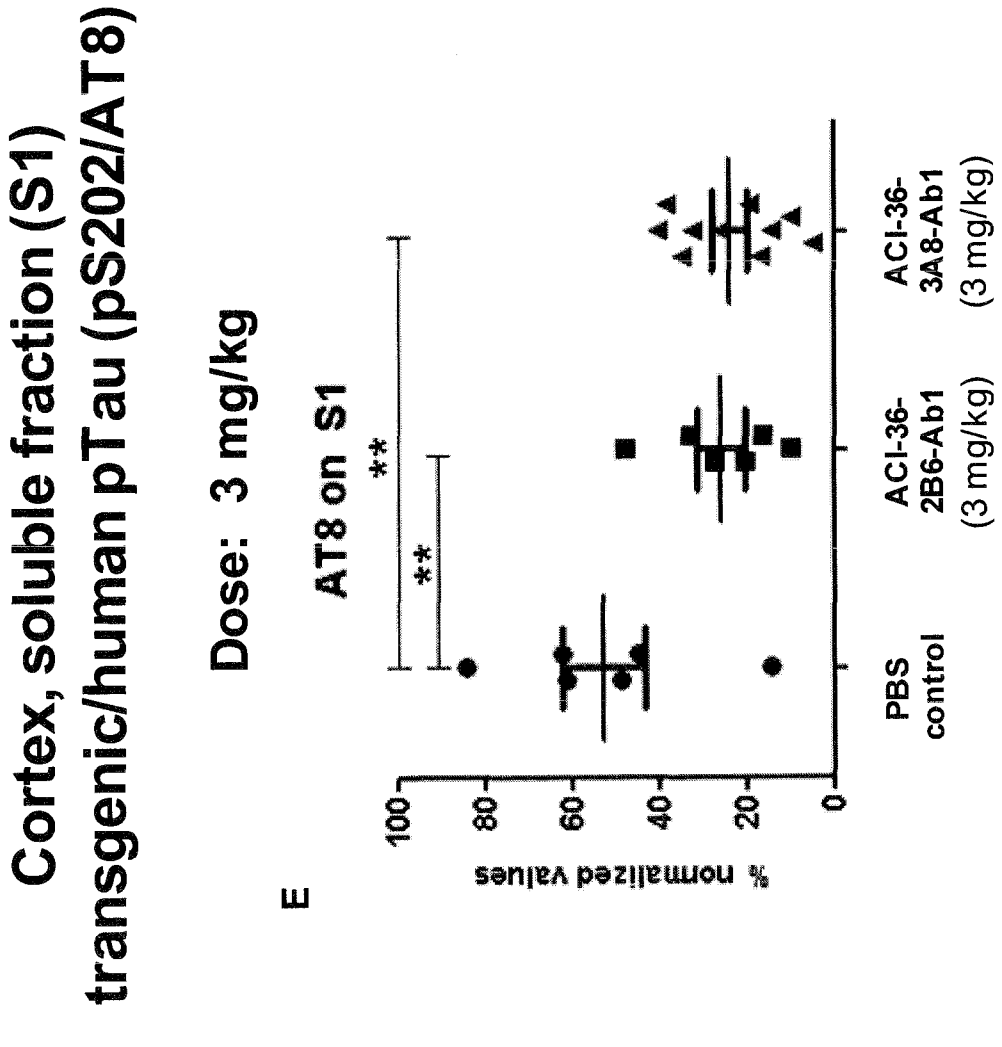

Cortex, soluble fraction (S1)
transgenic/human pTau (pS202/AT8)
Dose: 3 mg/kg
E
AT8 on S1
**
**
% normalized values
100
80
60
40
20
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-3

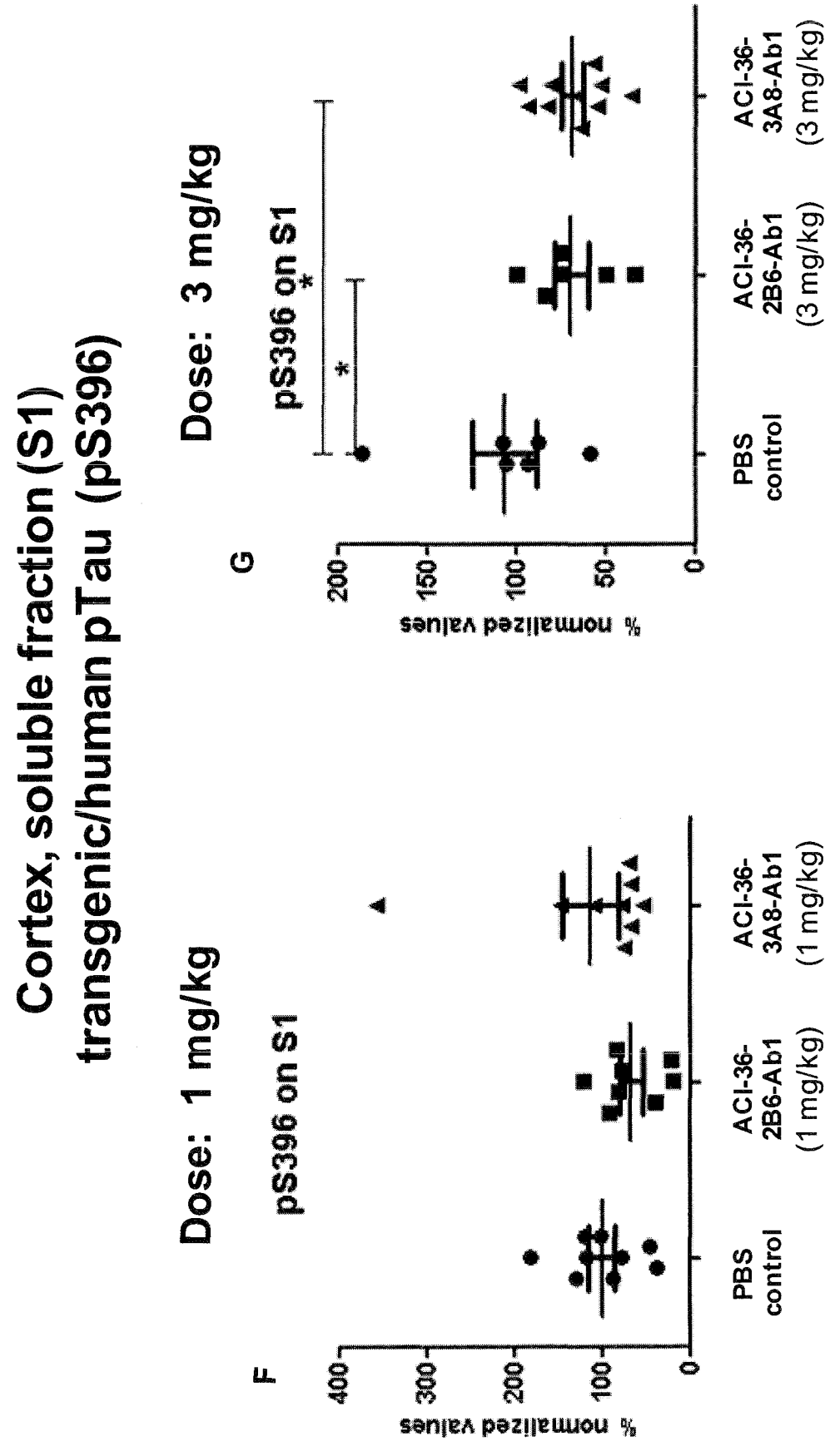

Cortex, soluble fraction (S1)
transgenic/human pTau (pS396)
F
Dose: 1 mg/kg
pS396 on S1
% normalized values
400
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
G
Dose: 3 mg/kg
pS396 on S1
*
*
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-4

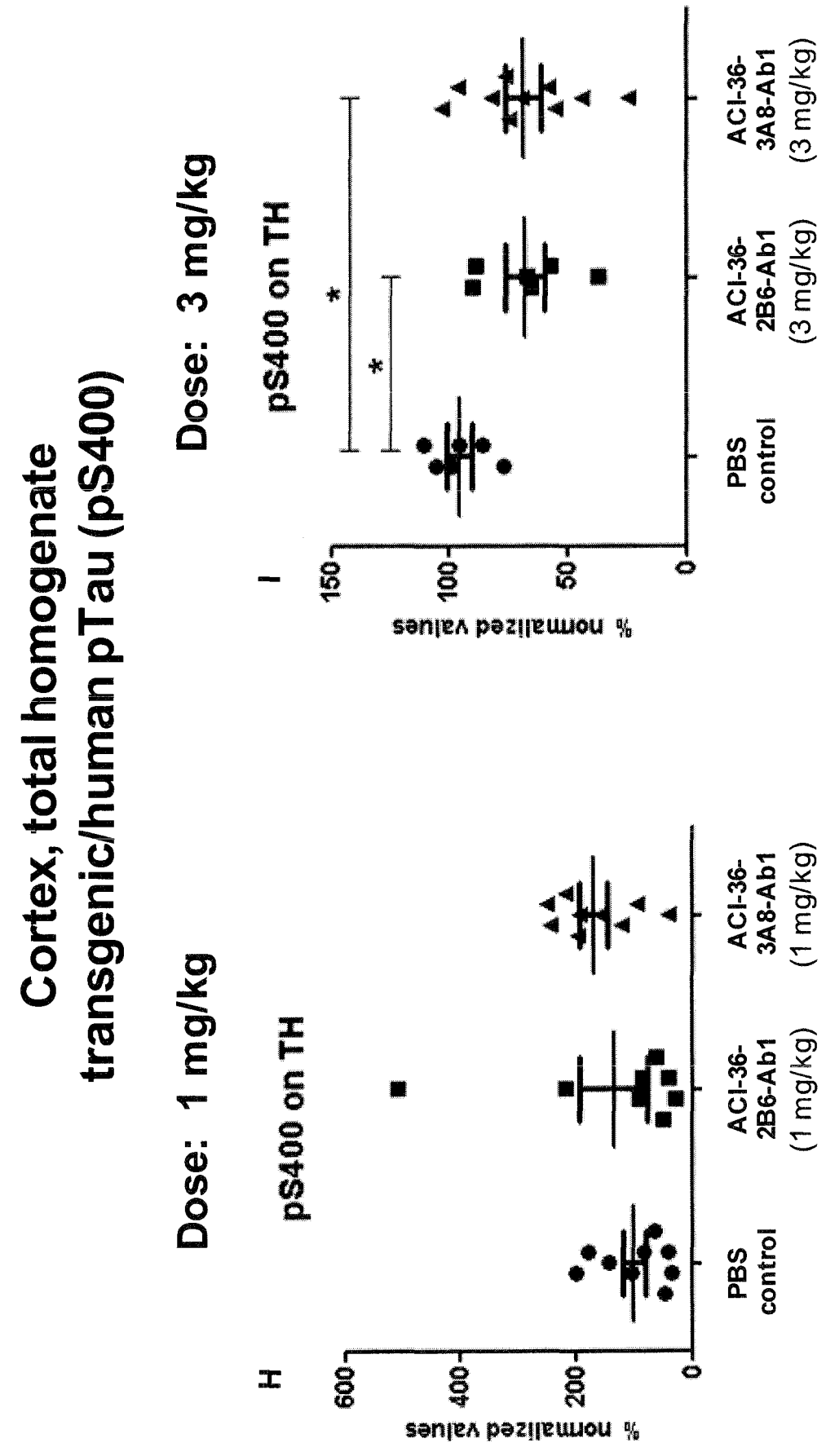

Cortex, total homogenate
transgenic/human pTau (pS400)
Dose: 1 mg/kg
Dose: 3 mg/kg
H
I
pS400 on TH
pS400 on TH
% normalized values
% normalized values
600
400
200
0
150
100
50
0
*
*
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-5

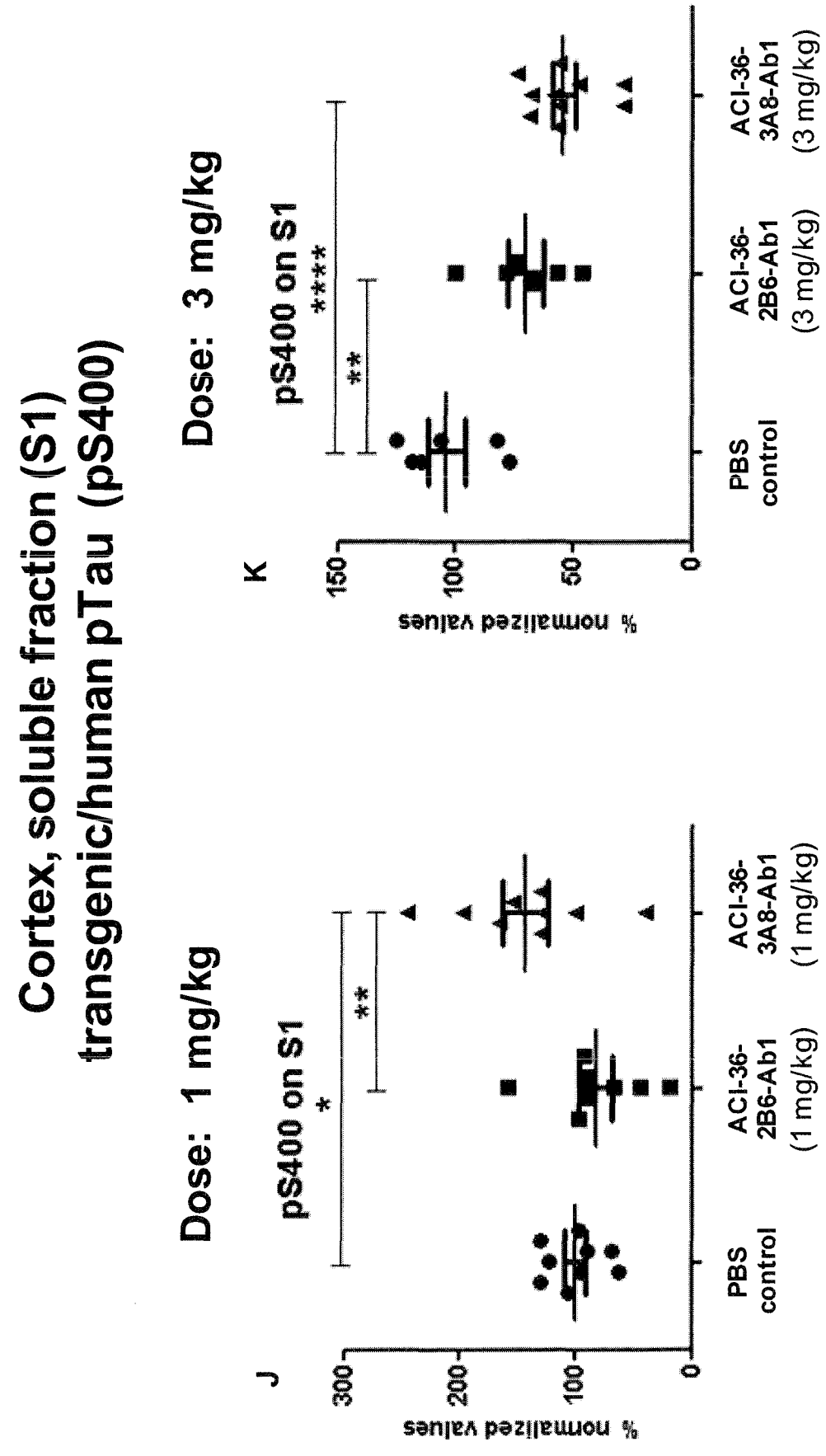

Cortex, soluble fraction (S1)
transgenic/human pTau (pS400)
Dose: 1 mg/kg
pS400 on S1
J
% normalized values
300
200
100
0
*
**
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
pS400 on S1
K
% normalized values
150
100
50
0
**
****
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-6

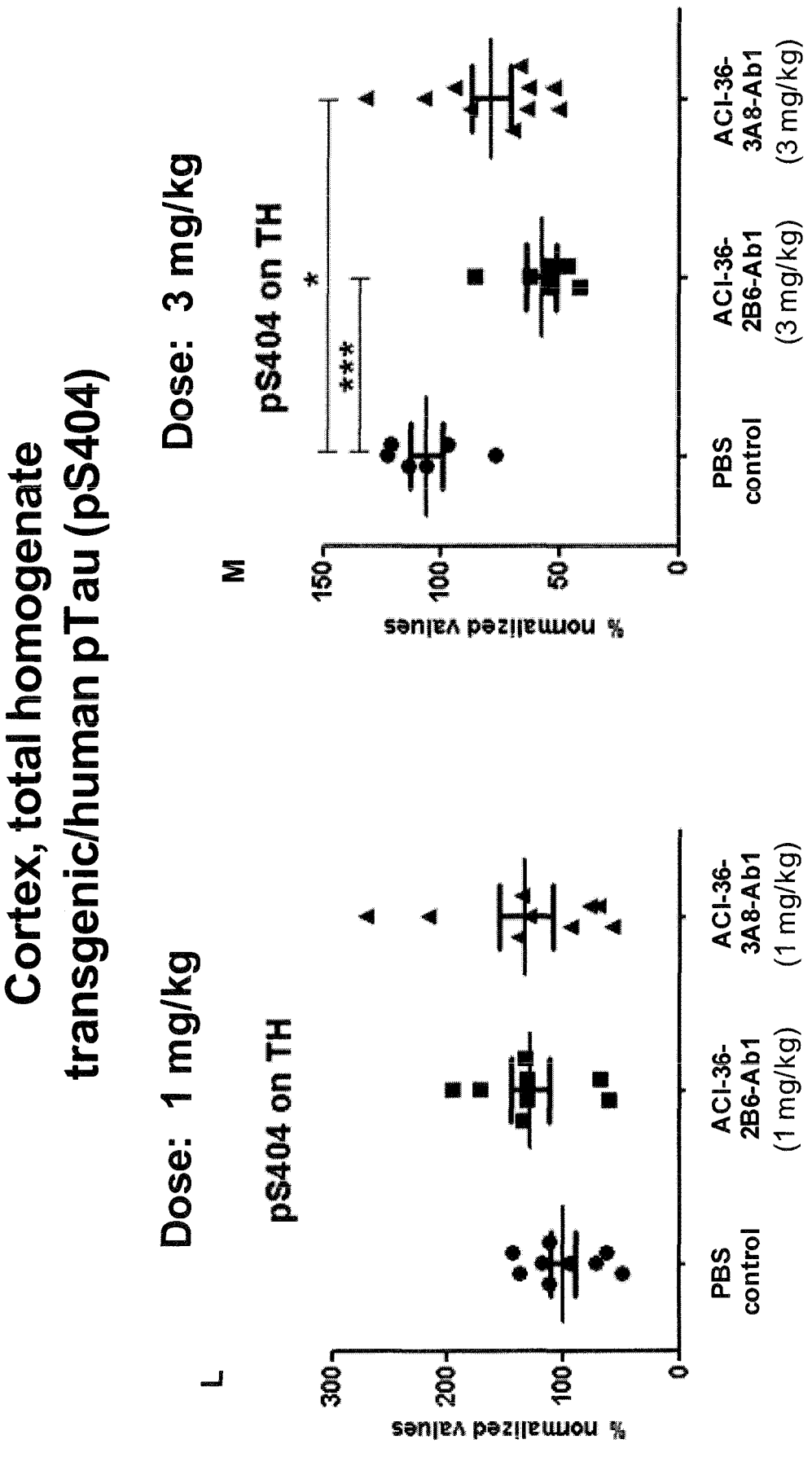

Cortex, total homogenate
transgenic/human pTau (pS404)
Dose: 1 mg/kg
pS404 on TH
L
% normalized values
300
200
100
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
pS404 on TH
M
% normalized values
150
100
50
0
***
*
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-7

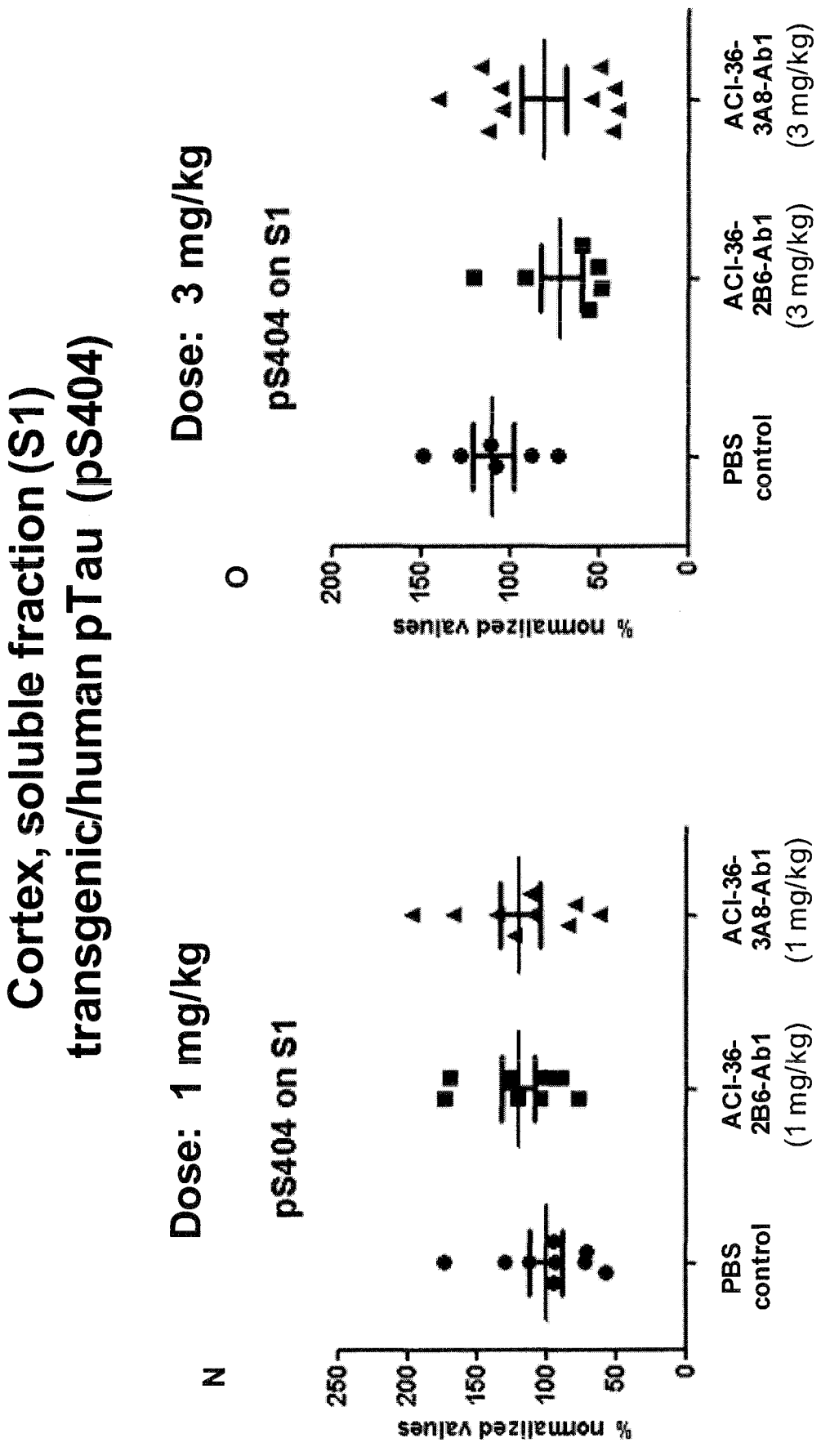

Cortex, soluble fraction (S1)
transgenic/human pTau (pS404)
Dose: 1 mg/kg
pS404 on S1
% normalized values
250
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (1 mg/kg)
ACI-36-3A8-Ab1 (1 mg/kg)
Dose: 3 mg/kg
pS404 on S1
% normalized values
200
150
100
50
0
PBS control
ACI-36-2B6-Ab1 (3 mg/kg)
ACI-36-3A8-Ab1 (3 mg/kg)

FIGURE 6-8

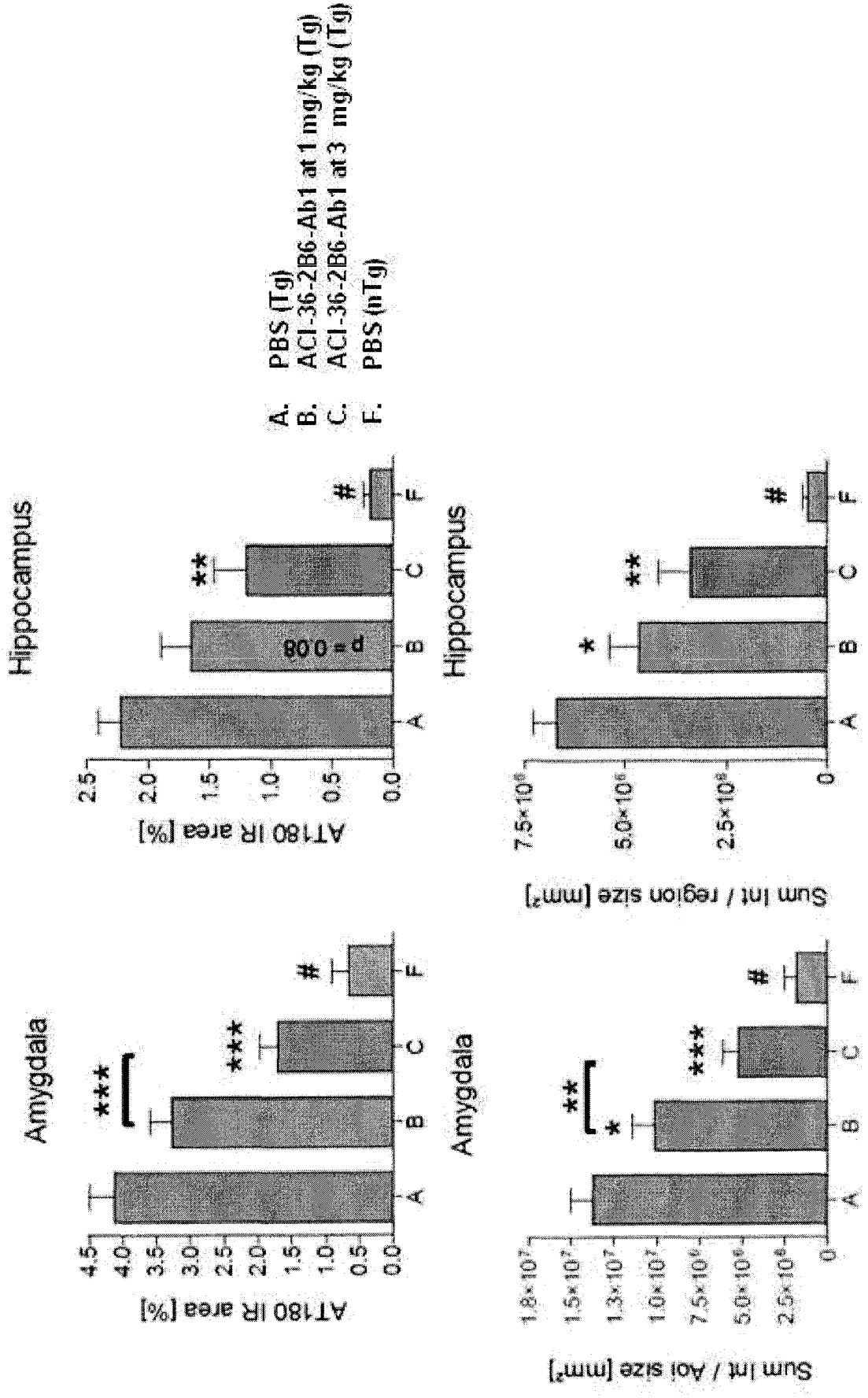

A. PBS (Tg)
B. ACI-36-2B6-Ab1 at 1 mg/kg (Tg)
C. ACI-36-2B6-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)
Hippocampus
AT180 IR area [%]
p = 0.08
Amygdala
AT180 IR area [%]
Hippocampus
Sum Int / region size [mm²]
Amygdala
Sum Int / Aoi size [mm²]

FIGURE 7

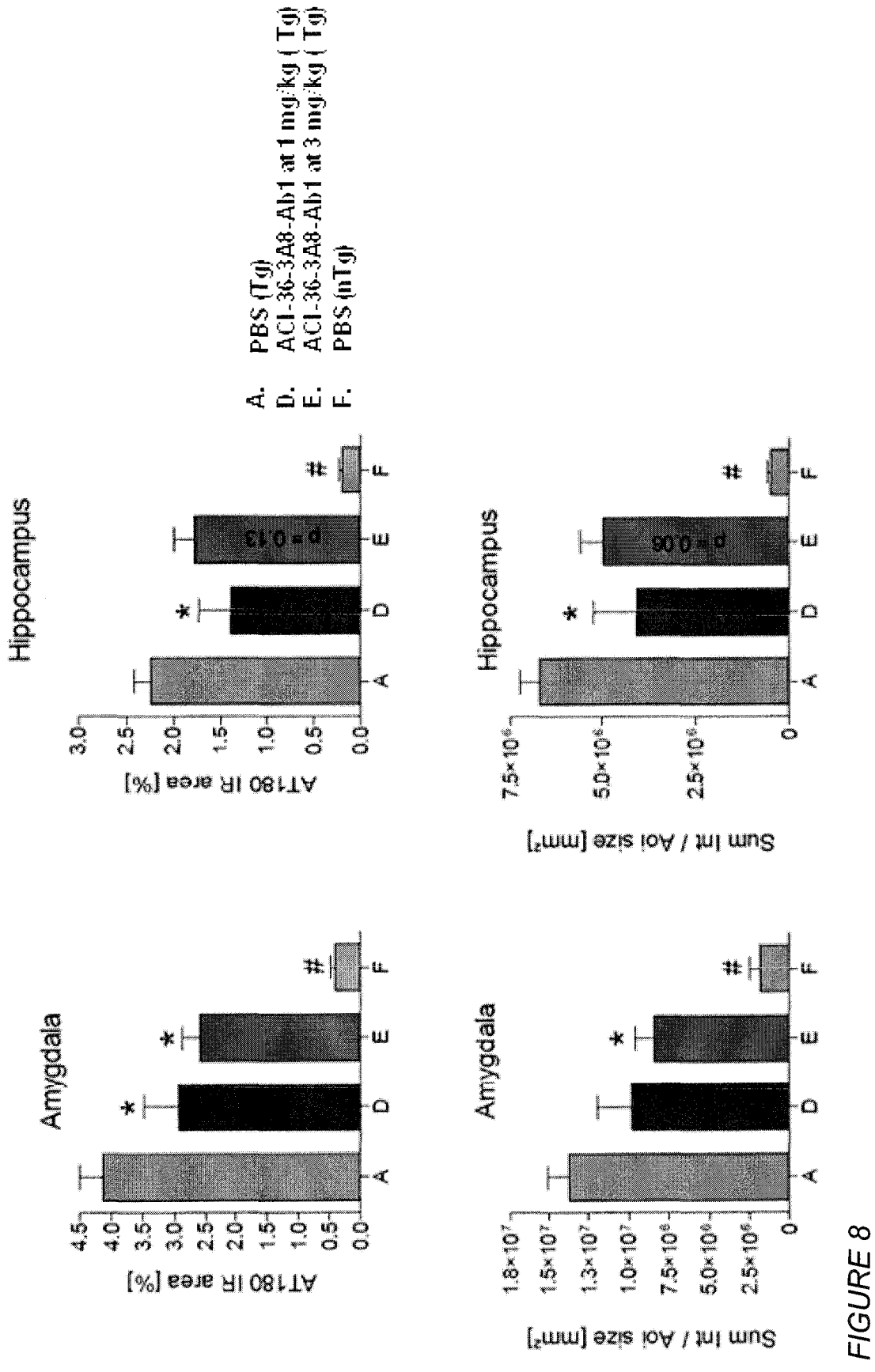
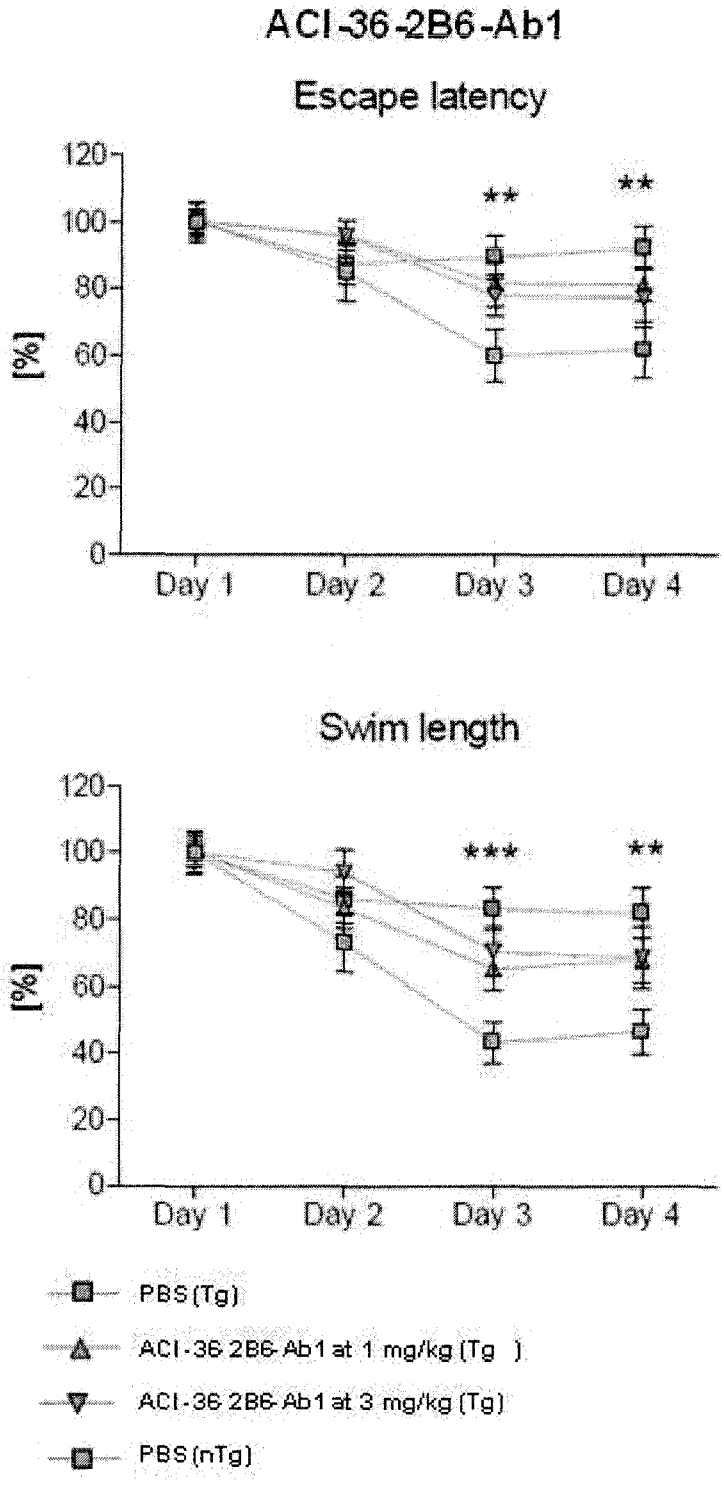
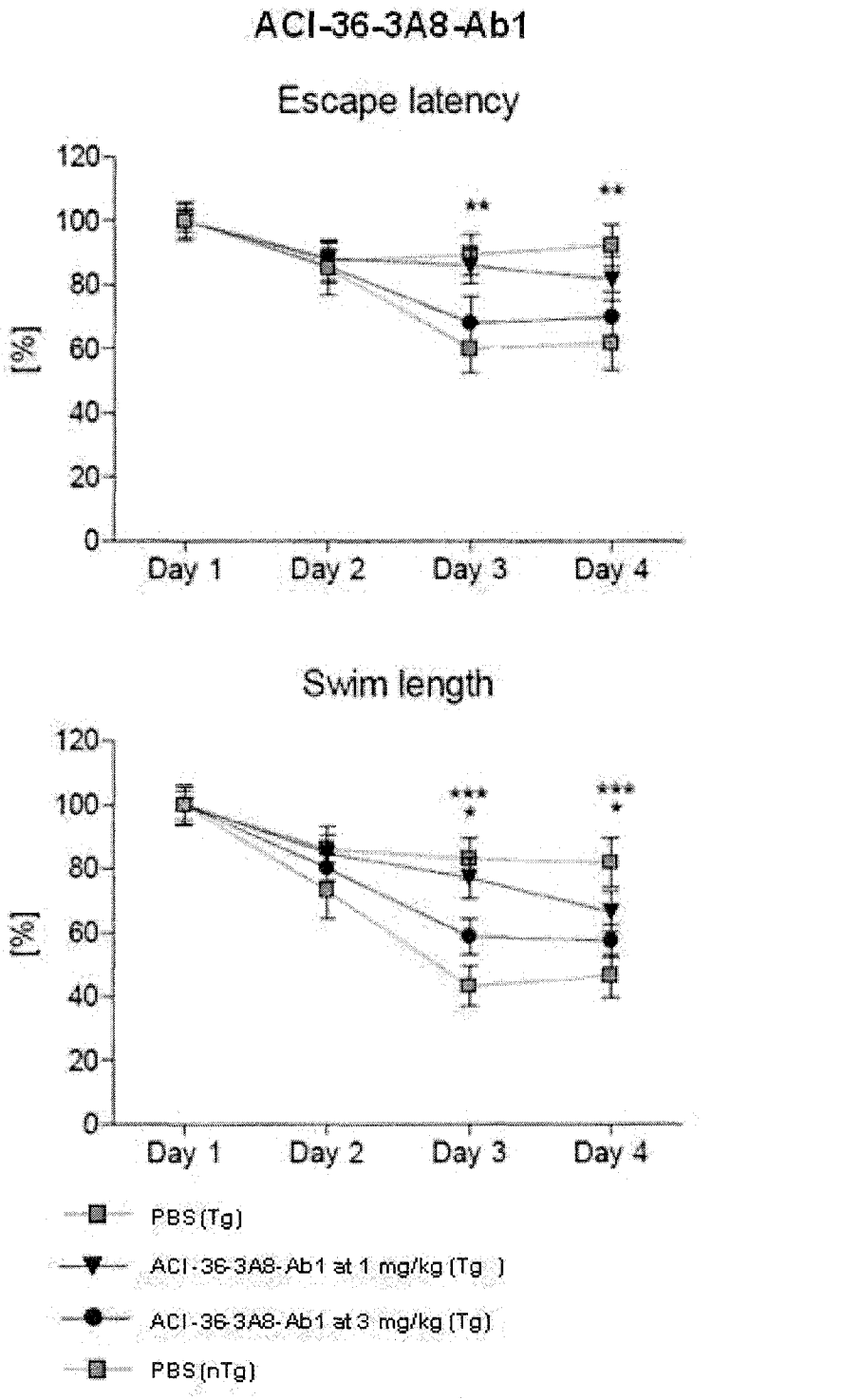

A. PBS (Tg)
D. ACI-36-3A8-Ab1 at 1 mg/kg (Tg)
E. ACI-36-3A8-Ab1 at 3 mg/kg (Tg)
F. PBS (nTg)
Hippocampus
AT180 IR area [%]
p = 0.13
Hippocampus
Sum Int / Aoi size [mm²]
p = 0.06
Amygdala
AT180 IR area [%]
Amygdala
Sum Int / Aoi size [mm²]

FIGURE 8



	Escape latency			Swim length		
	Day 2	Day 3	Day 4	Day 2	Day 3	Day 4
A vs B	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs C	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.01

FIGURE 9



	Escape latency			Swim length		
	Day 2	Day 3	Day 4	Day 2	Day 3	Day 4
A vs D	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
A vs E	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.05	P < 0.05
A vs F	P > 0.05	P<0.01	P<0.01	P > 0.05	P<0.001	P<0.001

FIGURE 10

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(71) Applicants *(for all designated States except US)*: **AC IMMUNE S.A.** [CH/CH]; EPFL-PSE Building B, CH-1015 Lausanne (CH). **KATHOLIEKE UNIVERSITEIT LEUVEN** [BE/BE]; KU Leuven Research & Development, Waaistraat 6, Box 5105, B-3000 Leuven (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PFEIFER, Andrea** [DE/CH]; Route de Fenil 16A, CH-1806 St-Légier (CH). **MUHS, Andreas** [DE/CH]; Avenue des Cerisiers 39b, CH-1009 Pully (CH). **VAN LEUVEN, Fred** [BE/BE]; Hertogenlaan 18, B-3210 Linden (BE). **PIHLGREN, Maria** [SE/CH]; Chemin du Tessin 6B, CH-1052 Mont-sur-Lausanne (CH). **ADOLFSSON, Oskar** [IS/CH]; Chemin de Larry 16, CH-1038 Bercher (CH).

(74) Agent: **WERNER BASTIAN**; Vossius & Partner, Siebertstrasse 4, 81675 München (DE).

[Continued on next page]

(54) Title: PHOSPHOSPECIFIC ANTIBODIES RECOGNISING TAU

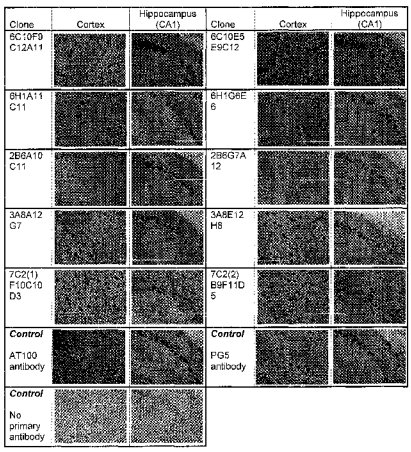


FIGURE 1-1

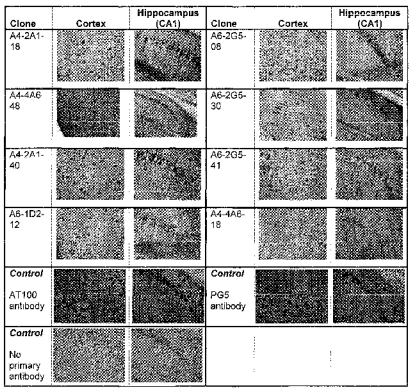


FIGURE 1-2

(57) Abstract: The present invention relates to methods and compositions for the therapeutic and diagnostic use in the treatment of diseases and disorders which are caused by or associated with neurofibrillary tangles. In particular, the invention relates to antibodies, which specifically recognize and bind to phosphorylated pathological protein tau-conformers and to methods and compositions involving said antibodies for the therapeutic and diagnostic use in the treatment of tauopathies including Alzheimer's Disease (AD).

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEE G ET AL: "Phosphorylation of tau by fyn: implications for Alzheimer's disease", JOURNAL OF NEUROSCIENCE, THE SOCIETY, WASHINGTON, DC, US, vol. 24, no. 9, 3 March 2004 (2004-03-03), pages 2304-2312, XP002350398, ISSN: 1529-2401, DOI: 10.1523/JNEUROSCI.4162-03.2004 the whole document page 2305, column 1 - column 2 figures 1-2,5-7 ----- -/--	1-120

[X] Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2012	03/04/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Jenkins, Gareth

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	K. BHASKAR ET AL: "Tyrosine phosphorylation of tau accompanies disease progression in transgenic mouse models of tauopathy", NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY, vol. 36, no. 6, 1 October 2010 (2010-10-01), pages 462-477, XP55016663, ISSN: 0305-1846, DOI: 10.1111/j.1365-2990.2010.01103.x the whole document page 475, column 1, paragraph 1	1-120
X	US 2008/050383 A1 (SIGURDSSON EINAR [US] ET AL) 28 February 2008 (2008-02-28) the whole document claims 1,6,31,37,38 page 11; sequence 6	1-120
X	WO 2010/106127 A2 (AC IMMUNE SA [CH]; PFEIFER ANDREA [CH]; MUHS ANDREAS [CH]; PIHLGREN MA) 23 September 2010 (2010-09-23) the whole document claims 1-62 pages 44,67 sequence 2	1-120
X,P	WO 2010/115843 A2 (AC IMMUNE SA [CH]; LEUVEN K U RES & DEV [BE]; PFEIFER ANDREA [CH]; MUH) 14 October 2010 (2010-10-14) the whole document page 52; table 1 page 77; table 10 claims 1-97	1-120
X	US 2005/221391 A1 (DAVIES PETER [US]) 6 October 2005 (2005-10-06) the whole document paragraph [0081]	1-120
X	US 2002/086009 A1 (ISHIGURO KOICHI [JP] ET AL) 4 July 2002 (2002-07-04) the whole document claims 1,2,7	1-120

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JICHA: "cAMP-dependent protein kinase phosphorylations on tau in Alzheimer's disease", JOURNAL OF NEUROSCIENCE, vol. 19, no. 17, 1 January 1999 (1999-01-01), page 7486, XP55022354, ISSN: 0270-6474 the whole document abstract table 1 figure 1	1-120
X,P	THOMAS VANHELMONT ET AL: "Serine-409 phosphorylation and oxidative damage define aggregation of human protein tau in yeast", FEMS YEAST RESEARCH, vol. 10, no. 8, 1 December 2010 (2010-12-01), pages 992-1005, XP55022356, ISSN: 1567-1356, DOI: 10.1111/j.1567-1364.2010.00662.x the whole document abstract table 1	1-120
X	WO 98/22120 A1 (WISTAR INST [US]; UNIV PENNSYLVANIA [US]; OTVOS LASZLO [US]; HOFFMANN) 28 May 1998 (1998-05-28) the whole document claims 1,7,8,18,26	1-120
X	EP 2 210 901 A1 (IMMUNAS PHARMA INC [JP]; NAT CT FOR GERIATRICS AND GERO [JP]) 28 July 2010 (2010-07-28) the whole document claims 2,9 sequences 3,15,17,19	1-120
X,P	WO 2010/144711 A2 (UNIV NEW YORK [US]; SIGURDSSON EINAR M [US]) 16 December 2010 (2010-12-16) the whole document claim 1 table 1	1-120

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOFFMANN R ET AL: "Unique Alzheimer's disease paired helical filament specific epitopes involve double phosphorylation at specific sites", BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 36, no. 26, 1 July 1997 (1997-07-01), pages 8114-8124, XP002625812, ISSN: 0006-2960, DOI: 10.1021/BI970380+ the whole document abstract tables 1,2	1-120
X	LICHTENBERG-KRAAG B ET AL: "'Phosphorylation-dependent epitopes of neurofilament antibodies on tau protein and relationship with Alzheimer tau", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC; US, vol. 89, no. 12, 1 June 1992 (1992-06-01), pages 5384-5388, XP002226003, ISSN: 0027-8424, DOI: 10.1073/PNAS.89.12.5384 the whole document figure 7	1-120
X	FRANCOIS TORREILLES ET AL: "Binding specificity of monoclonal antibody AD2: influence of the phosphorylation state of tau", MOLECULAR BRAIN RESEARCH, vol. 78, 1 January 2000 (2000-01-01), pages 181-185, XP55022594, the whole document figure 2B	1-120
X	L OTVOS ET AL: "Monoclonal Antibody PHF4 Recognizes Tau Protein Phosphorylated at Serine Residues 396 and 404", JOURNAL OF NEUROSCIENCE RESEARCH, vol. 39, 1 January 1994 (1994-01-01), pages 669-673, XP55022593, the whole document table 1 figure 1	1-120

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DAVID SINGER ET AL: "Immuno-PCR-based quantification of multiple phosphorylated tau-epitopes linked to Alzheimerâ s disease", ANALYTICAL AND BIOANALYTICAL CHEMISTRY, SPRINGER, BERLIN, DE, vol. 395, no. 7, 11 October 2009 (2009-10-11), pages 2263-2267, XP019756726, ISSN: 1618-2650, DOI: 10.1007/S00216-009-3208-8 the whole document table 1	1-120
X	DAVID SINGER ET AL: "Characterization of Phosphorylation Dependent Antibodies To Study the Phosphorylation Status of the Tau Protein", INTERNATIONAL JOURNAL OF PEPTIDE RESEARCH AND THERAPEUTICS ; FORMERLY KNOWN AS LETTERS IN PEPTIDE SCIENCE, KLUWER ACADEMIC PUBLISHERS, DO, vol. 11, no. 4, 1 December 2005 (2005-12-01), pages 279-289, XP019287635, ISSN: 1573-3904 the whole document table 1 figure 2	1-120
X	ZHENG-FISCHHOEFER QINGYI ET AL: "Sequential phosphorylation of Tau by glycogen synthase kinase-3beta and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation", EUROPEAN JOURNAL OF BIOCHEMISTRY, BLACKWELL PUBLISHING, BERLIN, DE, vol. 252, no. 3, 1 March 1998 (1998-03-01) , pages 542-552, XP002202096, ISSN: 0014-2956, DOI: 10.1046/J.1432-1327.1998.2520542.X the whole document figure 10	1-120
X,P	WO 2011/013034 A1 (PFIZER VACCINES LLC [US]; SMITH III GEORGE JOSEPH [US]; WILLS KENNETH) 3 February 2011 (2011-02-03) the whole document claim 1 page 8 pages 18-20 page 82; table 5	1-120

3 -/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZEMLAN F P ET AL: "MONOCLONAL ANTIBODY PHF-9 RECOGNIZES PHOSPHORYLATED SERINE 404 OF TAU PROTEIN AND LABELS PAIRED HELICAL FILAMENTS", JOURNAL OF NEUROSCIENCE RESEARCH, WILEY-LISS, US, vol. 46, no. 1, 1 October 1996 (1996-10-01), pages 90-97, XP000646724, ISSN: 0360-4012, DOI: 10.1002/(SICI)1097-4547(19961001)46:1<90::AID-JNR11>3.0.CO;2-G the whole document table 1 figure 3 -----	1-120
X	RODER H M ET AL: "PHOSPHORYLATION-DEPENDENT MONOCLONAL TAU ANTIBODIES DO NOT RELIABLYREPORT PHOSPHORYLATION BY EXTRACELLULAR SIGNAL-REGULATED KINASE 2 AT SPECIFIC SITES", JOURNAL OF BIOLOGICAL CHEMISTRY, THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC, US, vol. 272, no. 7, 14 February 1997 (1997-02-14), pages 4509-4515, XP000979104, ISSN: 0021-9258, DOI: 10.1074/JBC.272.34.21616 the whole document tables 1,2 -----	1-120
X	HIRATA-FUKAE C ET AL: "Levels of soluble and insoluble tau reflect overall status of tau phosphorylation in vivo", NEUROSCIENCE LETTERS, LIMERICK, IE, vol. 450, no. 1, 23 January 2009 (2009-01-23), pages 51-55, XP025800599, ISSN: 0304-3940, DOI: 10.1016/J.NEULET.2008.11.023 [retrieved on 2008-11-13] the whole document figure 1 -----	1-120
X	US 2008/220449 A1 (VASAN SARA [US] ET AL) 11 September 2008 (2008-09-11) the whole document paragraph [0071] example 6 ----- -/--	1-120

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. ODDO ET AL: "Reduction of Soluble Abeta and Tau, but Not Soluble Abeta Alone, Ameliorates Cognitive Decline in Transgenic Mice with Plaques and Tangles", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 281, no. 51, 1 January 2006 (2006-01-01), pages 39413-39423, XP55022863, ISSN: 0021-9258, DOI: 10.1074/jbc.M608485200 the whole document page 39417 -----	1-120

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/067604

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 19, 36, 50, 79, 80, 89, 90(completely); 1-18, 24-31, 43-45, 53-78, 105-114(partially)

   The claims in so far as they relate to the epitope comprising Tau 15-20, pY18.

2-5. claims: 1-18, 20-35, 37-49, 51-78, 81-88, 91-114(all partially)

   The claims in so far as they relate to the epitopes comprising residue pS409, pT212+pS214, pS396, pS404, respectively.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/067604

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COMPOSICIÓN FARMACÉUTICA

La presente invención se refiere a métodos y composiciones para el uso terapéutico y de diagnóstico en el tratamiento de enfermedades y trastornos que son causados por ovillos neurofibrilares o están relacionados con ellos. En particular, la invención se refiere a anticuerpos, que específicamente reconocen y se unen a conformadores de proteína tau patológicos fosforilados y con métodos y composiciones que incluyen dichos anticuerpos para el uso terapéutico y diagnóstico en el tratamiento de tauopatías entre las que encuentra la enfermedad de Alzheimer (EA).

Los ovillos neurofibrilares y los hilos de neurópilo (HN) son las características neuropatológicas distintivas principales de la enfermedad de Alzheimer (EA). Están compuestos por la proteína tau asociada a microtúbulos que ha sufrido modificaciones postraduccionales, que incluyen la fosforilación, la desamidación y la isomerización en residuos de asparaginilo o aspartilo. Se originan por el agregado de proteína tau hiperfosforilada y sus conformadores. La EA comparte esta patología con muchas tauopatías neurodegenerativas, en especial, con tipos específicos de demencia frontotemporal (DFT).

La proteína tau es una proteína altamente soluble, "naturalmente no plegada" que se une con avidez a los microtúbulos (MT) para promover su unión y estabilidad. Los MT son de suma importancia para la integridad citoesquelética de las neuronas, y por lo tanto, para la formación y funcionamiento adecuados de los circuitos neuronales, por ende, para el aprendizaje y la memoria. La unión de la proteína tau con el MT es controlada por fosforilación y desfosforilación dinámicas, según lo demuestran en especial las células *in vitro* y no neuronales. Debido a la gran cantidad de sitios de fosforilación posibles (>80), la contribución exacta de cada uno y la identidad de las quinasas responsables continúan en gran medida sin definirse *in vivo*.

En un cerebro con EA, la patología tau se desarrolla con posterioridad a una patología amiloide, y por lo tanto es probable que en respuesta a ella, lo que constituye la esencia de la hipótesis de la cascada amiloide. Esto se basa en los estudios en pacientes con EA y síndrome de Down y son indicados en ellos, y son corroborados por estudios en ratones transgénicos con patología tau y amiloide combinadas ((Lewis et al., 2001; Oddo et al., 2004; Ribe et al., 2005; Muyllaert et al, 2006; 2008; Terwel et al, 2008).

El preciso momento oportuno de ambas patologías en pacientes humanos con EA así como también los mecanismos que unen patología amiloide y tau continúan siendo desconocidos, pero se propone que involucran una activación de vías de señalización neuronal que actúan en GSK3 y cdk5 o a través de ellas como las "tau quinasas" principales (revisado por Muyllaert et al, 2006, 2008).

La hipótesis de que la tauopatía no es un efecto colateral inocente sino un ejecutor patológico principal en EA se basa en las observaciones genéticas, patológicas y experimentales sólidas que se corroboran entre sí por completo:

- en los casos de EA genética de aparición precoz que se deben a mutaciones en el precursor de la proteína amiloide (PPA) o presenilina, la causa patogénica obligada es la acumulación amiloide, pero invariablemente la patología comprende tauopatía colateral, idéntica a la de los casos de EA esporádica de aparición tardía;
- la gravedad en la disfunción cognitiva y en la demencia se corresponde con la tauopatía, no con la patología amiloide, ejemplificado últimamente a través de varios estudios clínicos de fase 1 y 2 que incluyen estudios por imágenes PIB–PET para amiloide e identifican muchos "falsos positivos"; individuos cognitivamente normales con una alta carga de amiloide cerebral;
- en DFT genético, la tauopatía es provocada por tau mutante y causa neurodegeneración en forma directa, sin patología amiloide;

- en modelos de ratón experimental, los defectos cognitivos ocasionados por patología amiloide son mitigados casi por completo a través de la ausencia de proteína tau (Roberson et al, 2007).

Los diversos argumentos relacionados fundamentan la hipótesis de que la proteína tau ejerce una función principal en la dispersión cognitiva en EA y tauopatías neurodegenerativas relacionadas.

Un nuevo tratamiento destacado de EA es a través de inmunoterapia pasiva con mAbs específicos para limpiar péptidos amiloides y sus agregados que se supone son neurotóxicos o sinaptotóxicos.

De la forma propuesta aquí, se prevé la inmunoterapia que apunta a la patología tau para contrarrestar los conformadores de proteína tau patológicos conocidos o que supuestamente causan disfunción sináptica y neurodegeneración. Se propone que la patología amiloide producida y los agregados intraneuronales de proteína tau hiperfosforilada actúen en forma sinérgica en la cascada cognitiva y degenerativa de eventos patológicos que provocan desde trastorno cognitivo leve (TCL) hasta demencia grave de EA. Por lo tanto, la combinación de medicación dirigida por tau con la medicación dirigida por amiloide (o cualquier otra) constituye el tratamiento de preferencia y esencialmente el más eficaz para EA, en contraposición a la actual monoterapia.

Otros enfoques terapéuticos que tienen como objetivo la proteína tau son escasos y comprenden principalmente:

- los inhibidores de las quinasas que se cree aumentan la fosforilación de tau a niveles patológicos;
- los compuestos que bloquean la agregación citoplásmica de proteína tau hiperfosforilada.

Estos enfoques sufren varios inconvenientes de especificidad y eficacia, un problema que comparten con intentos para modificar el metabolismo del PPA y del

amiloide, donde todo ello hace hincapié en la importancia de una búsqueda continua de opciones de tratamientos adicionales, incluso la inmunoterapia contra tau.

No se ha dedicado prácticamente ningún esfuerzo por definir –y mucho menos poner como objetivo– los conformadores de tau patológicos *in vivo*. En el ensayo clínico Aβ42 de fase II, la patología de ovillo no parecer estar bien considerada ni analizada en demasiada profundidad (Nicoll et al., 2003; Masliah et al., 2005). Por otro lado, el amiloide con objetivo en la inmunoterapia experimental en un modelo de ratón preclínico con patología tipo EA combinada demostró también un efecto en la patología tau aunque los agregados de tau persistieron (Oddo et al., 2004).

Han surgido algunas dudas sobre la posibilidad de abordar la proteína tau intracelular por inmunoterapia. Fueron encontradas por el estudio experimental más reciente en un modelo de ratón de tauopatía (Asuni et al., 2007). Demostraron una reducción en la patología de ovillo y mejoras funcionales por medio de la vacunación con un fosfopéptido derivado de proteína tau. Estos datos corroboran informes previos de α–sinucleína con objetivo en la inmunoterapia en un modelo de enfermedad de Parkinson (EP) y de cuerpos de Lewy (Masliah et al., 2005, 2011) y de superóxido dismutasa en un modelo de esclerosis lateral amiotrófica (ELA) (Urushitiani et al., 2007). Estas enfermedades son ejemplos en los que las proteínas intracelulares llevan a defectos sinápticos y neurodegeneración a través de mecanismos aún no comprendidos por completo. Por otro lado, la proteína tau recombinante de longitud completa producida en bacterias y aislada de ella no parece ser adecuada como vacuna, aunque los adyuvantes empleados, es decir toxina pertussis y completos de Freund, podrían haber contribuido al resultado negativo del estudio (Rosenmann et al., 2006).

Existe una necesidad insatisfecha de inmunoterapias pasivas y/o activas que funcionen para contrarrestar los conformadores de proteína patológicos que

se sabe –o presume– causan trastornos neurodegenerativos, tales como la patología amiloide en EA provocada, por ejemplo, por agregados intraneuronales de proteína tau hiperfosforilada típicos para la EA como amiloide.

Esta necesidad insatisfecha podría satisfacerse dentro del alcance de la presente invención mediante la provisión de proteínas de unión que reconozcan y se unan a fosfoepítopes patológicos de la proteína tau. En particular, la presente invención provee anticuerpos específicos contra fosfoepítopes lineales y conformacionales, simples y complejos, en proteína tau, en particular, en proteína tau agregada que son considerados responsables de la sinaptotoxicidad y neurotoxicidad en tauopatías, incluso en EA.

Conforme a ello, la presente invención se refiere en una forma de realización a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, cuyo péptido de unión o proteína o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular a un fosfo–epítope en la proteína Tau agregada, en particular a un confórmero de proteína tau patológico pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo tiene una alta afinidad de unión con la proteína tau soluble e insoluble y modula los niveles de la proteína tau soluble e insoluble, en particular en el cerebro, en particular con una constante de disociación de al menos 10 nM, en particular de al menos 8 nM, en particular de al menos 5 nM, en particular de al menos 2 nM, en particular de al menos 1 nM, en particular de al menos 500 pM, en particular de al menos 400 pM, en particular de al menos 300 pM, en particular de al menos 200 pM, en particular de al menos 100 pM, en particular de al menos 50 pM.

En una segunda forma de realización, la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo tiene una constante de velocidad de asociación de 10^4 $M^{-1}s^{-1}$ o mayor, en particular de entre 3 – 5 x 10^4 $M^{-1}s^{-1}$ o mayor, en particular de 10^5 $M^{-1}s^{-1}$ o mayor; en particular de 2 – 9 x 10^5 $M^{-1}s^{-1}$ o mayor; en particular de 10^6 $M^{-1}s^{-1}$ o mayor, en particular de 1 – 4 x 10^6 $M^{-1}s^{-1}$ o mayor, en particular de 10^7 $M^{-1}s^{-1}$ o mayor.

En una tercera forma de realización, la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 4 nM y una constante de velocidad de asociación de 10^5 $M^{-1}s^{-1}$ o mayor, en particular una constante de disociación de al menos 3 nM y una constante de velocidad de asociación de 10^6

$M^{-1}s^{-1}$ o mayor, en particular una constante de disociación de al menos 2 nM y una constante de velocidad de asociación de 10^4 $M^{-1}s^{-1}$ o mayor, en particular una constante de disociación de al menos 1 nM y una constante de velocidad de asociación de 10^5 $M^{-1}s^{-1}$ o mayor, en particular una constante de disociación de al menos 200 pM y una constante de velocidad de asociación de 10^5 $M^{-1}s^{-1}$ o mayor, en particular una constante de disociación de al menos 100 pM y una constante de velocidad de asociación de 10^6 $M^{-1}s^{-1}$ o mayor.

Una forma de realización (4) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo se une con un epítope en un mamífero, en particular en la proteína humana Tau tal como se indica en SEC ID NO: 67, seleccionada del grupo que consiste en Tau aa 15–20 que comprende Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende Ser fosforilada en la posición 409 (pS409); y Tau aa 208–218 que comprende Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición 214 (pS214).

Una forma de realización (5) se refiere al péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, en donde dicho péptido se une con un epítope en un mamífero, en particular en la proteína Tau humana,

pero especialmente la proteína Tau humana tal como se indica en SEC ID NO: 67, que comprende Tau aa 15–20 con Tyr fosforilada en la posición 18 (Y18).

Una forma de realización (6) se refiere al péptido de unión o anticuerpo de cualquiera o las formas de realización precedentes, en donde dicho péptido se une con un epítope en un mamífero, en particular en la proteína Tau humana, pero especialmente la proteína Tau humana tal como se indica en SEC ID NO: 67, que comprende Tau aa 405–412 con Ser fosforilada en la posición 409 (pS409).

Una forma de realización (7) se refiere al péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, en donde dicho péptido se une con un epítope en un mamífero, en particular en la proteína Tau humana, pero especialmente la proteína Tau humana tal como se indica en SEC ID NO: 67, que comprende Tau aa 405–411 con Ser fosforilada en la posición 409 (pS409).

Una forma de realización (8) se refiere al péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, en donde dicho péptido se une con un epítope en un mamífero, en particular en la proteína Tau humana, pero especialmente la proteína Tau humana tal como se indica en SEC ID NO: 67, que comprende Tau aa 208–218 con Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición 214 (pS214).

En otra forma de realización (9), la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un

confórmero de proteína tau patológica pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene, en particular en secuencia, una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, o una secuencia de aminoácidos al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, o una secuencia de aminoácidos al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, o una secuencia de aminoácidos al menos 60%, en particular al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, 70, 78, 89, 98, o una secuencia de aminoácidos al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, 71,

79, 90, 99, 115, o una secuencia de aminoácidos al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, 72, 80, 91, 100, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

Una forma de realización (10) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: SEC ID NO: 21, 24, 27, 28, 29, 32, 73, 81, o una secuencia de aminoácidos al menos 85% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, 74, 82, o una secuencia de aminoácidos al menos 95% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, 75, 83, o una secuencia de aminoácidos al menos 80% idéntica; y/o un dominio de anticuerpos que

contiene una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, 70, 78, o una secuencia de aminoácidos al menos 95% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, 71, 79, o una secuencia de aminoácidos al menos 85% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, 72, 80, o una secuencia de aminoácidos al menos 85% idéntica.

Una forma de realización (11) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, 73, 81, o una secuencia de aminoácidos al menos 90% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, 74, 82, o una secuencia de aminoácidos al menos 95% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, 75, 83, o una secuencia de aminoácidos al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, 70, 78, o una secuencia de aminoácidos al menos 95% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, 71, 79, o una secuencia de aminoácidos al menos 95% idéntica y una

CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, 72, 80, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (12) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión (dominio de anticuerpo) que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, 73, 81, o una secuencia de aminoácidos al menos 90% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, 74, 82, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, 75, 83, o una secuencia de aminoácidos al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, 70, 78, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, 71, 79, o una secuencia de aminoácidos al menos 95% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, 72, 80, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (13) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes

funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácidos al menos 98% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, o una secuencia de aminoácidos al menos 95% idéntica; y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, o una secuencia de aminoácidos al menos 95% idéntica y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (14) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no

relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácidos al menos 98% idéntica; una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, o una secuencia de aminoácidos al menos 98% idéntica; y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, o una secuencia de aminoácidos al menos 98% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (15) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, o 106, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, 25, 30, 33, 74, 82, 94, 102, o 107, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, 26, 31, 34, 75, 83, 95, 103, o 108, y/o un segundo

dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, 15, 18, 70, 78, 89, o 98, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, 16, 19, 71, 79, 90, 99, o 115, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, 17, 20, 72, 80, 91, o 100.

Una forma de realización (16) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 21, o una secuencia de aminoácidos al menos 76%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 22, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 23, o una secuencia de aminoácidos al menos 66%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica, y/o un segundo dominio de unión que contiene en secuencia una

CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, o una secuencia de aminoácidos al menos 88%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, o una secuencia de aminoácidos al menos 66%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica.

Una forma de realización (17) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 24, o SEC ID NO: 27, o SEC ID NO: 28, o una secuencia de aminoácidos al menos 88%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 25, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% y una CDR3 con la secuencia de aminoácidos

mostrada en SEC ID NO: 26, o una secuencia de aminoácidos al menos 66%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 12, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 13, o una secuencia de aminoácidos al menos 88%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 14, o una secuencia de aminoácidos al menos 66%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica.

Una forma de realización (18) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (15) que comprende un primer dominio de unión, en donde la CDR1 tiene la secuencia de aminoácidos mostrada en SEC ID NO: 27, o una secuencia de aminoácidos al menos 88% idéntica.

Una forma de realización (19) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (15), que comprende un primer dominio de unión en donde la CDR1 tiene la secuencia de aminoácidos

mostrada en SEC ID NO: 28, o una secuencia de aminoácidos al menos 88% idéntica.

Una forma de realización (20) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 29, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 30, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 31, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 15, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 16, o una secuencia de aminoácidos al menos 94%, 95%, 96%, 97%, 98%, o 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 17, o una secuencia de aminoácidos al menos 36%, en particular al menos 40%, en particular al menos 50%, en particular al menos 60%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos

90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica.

Una forma de realización (21) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 32, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 33, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 34, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 18, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 19, o una secuencia de aminoácidos al menos 95%, 96%, 97%, 98% o 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 20, o una secuencia de aminoácidos al menos 63%, en particular al menos 70%, en particular al menos 75%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, en particular al menos 95%, en particular al menos 98%, en particular al menos 99% idéntica.

Una forma de realización (22) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 73, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 74, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 75, o una secuencia de aminoácidos al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 70, o una secuencia de aminoácidos al menos 95%, en particular 98%, en particular 99% idéntica, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 71, o una secuencia de aminoácidos al menos 94%, 95%, 96%, 97%, 98%, o 99% idéntica, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 72, o una secuencia de aminoácidos al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en

particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

Una forma de realización (23) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 81, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 82, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 83, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 78, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 79, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 80, o una secuencia de aminoácidos al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos

96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

Una forma de realización (24) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 93, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 94, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 95, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de las anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 89, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 90, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 91, o una secuencia de aminoácidos al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%,

en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de las anteriores CDRs.

Una forma de realización (25) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 101, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 102, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 103, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de las anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 98, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 99, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 100, o una secuencia de aminoácidos al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al

menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de las anteriores CDRs.

Una forma de realización (26) de la presente invención se refiere a un péptido de unión o una proteína una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 106, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 107, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 108, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de las anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia una CDR1 con la secuencia de aminoácidos mostrada en SEC ID NO: 89, una CDR2 con la secuencia de aminoácidos mostrada en SEC ID NO: 115, y una CDR3 con la secuencia de aminoácidos mostrada en SEC ID NO: 91, o una secuencia de aminoácidos al menos 60%, al menos 70%, al menos

80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de las anteriores CDRs.

En otra forma de realización (27), la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácidos al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácidos al menos 85% idéntica.

En una forma de realización (28), la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un

confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácidos al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácidos al menos 91% idéntica.

Una forma de realización (29) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión (dominio de anticuerpo) que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácidos al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácidos al menos 91% idéntica.

En otra forma de realización (30), la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las

formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 69, 77, 116/92, 97, 105, o una secuencia de aminoácidos en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 68, 76, 88, 96, 104, o una secuencia de aminoácidos al menos 80%, en particular al menos 85%, en particular al menos 86%, en particular al menos 87%, en particular al menos 88%, en particular al menos 89%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

Una forma de realización (31) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se

une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6 o SEC ID NO: 7, o una secuencia de aminoácidos al menos 90% y 94%, respectivamente, idénticas; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1, o una secuencia de aminoácidos al menos 91% idéntica.

Una forma de realización (32) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 8, o una secuencia de aminoácidos al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 2, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (33) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la

proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 9, o una secuencia de aminoácidos al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 3, o una secuencia de aminoácidos al menos 90% idéntica.

Una forma de realización (34) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 10, o una secuencia de aminoácidos al menos 99% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 4, o una secuencia de aminoácidos al menos 89% idéntica.

Una forma de realización (35) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y

específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 11, o una secuencia de aminoácidos al menos 98% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 5, o una secuencia de aminoácidos al menos 87% idéntica.

Una forma de realización (36) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 69, o una secuencia de aminoácidos al menos 98% o 99% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 68, o una secuencia de aminoácidos al menos 90%, 91%, 92% o 93% idéntica.

Una forma de realización (37) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes

funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 77, o una secuencia de aminoácidos al menos 93%, 94% o 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 76, o una secuencia de aminoácidos al menos 88%, 89% o 90% idéntica.

Una forma de realización (38) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 116, 92 ó 118, o una secuencia de aminoácidos al menos 93%, 94% o 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 88, o una secuencia de aminoácidos al menos 90%, 91%, 92% o 93% idéntica.

Una forma de realización (39) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 97, o una secuencia de aminoácidos al menos 99% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 96, o una secuencia de aminoácidos al menos 86%, 87%, 88%, 89% o 90% idéntica.

Una forma de realización (40) de la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular con un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 105, o una secuencia de aminoácidos al menos 98% o 99% idéntica; y/o un

segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 104, o una secuencia de aminoácidos al menos 88%, 89% o 90% idéntica.

En otra forma de realización (41), la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con las formas de realización (22) – (24), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 21–34 y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 12–20.

Una forma de realización (42) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (31), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 21–23 y SEC ID NOs: 24–26, respectivamente, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 12–14.

Una forma de realización (43) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (32), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 27, 25, 26 y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 12–14.

Una forma de realización (44) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes

funcionales, de acuerdo con la forma de realización (33), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 28, 25 y 26, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 12–14.

Una forma de realización (45) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (34), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 29–31, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 15–17.

Una forma de realización (46) de la presente invención se refiere a un péptido de unión o proteíona o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (35), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 32–34, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 18–20.

Una forma de realización (47) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (27), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 73–75, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 70–72.

Una forma de realización (48) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes

funcionales, de acuerdo con la forma de realización (27), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 81–83, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 78–80.

Una forma de realización (49) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (27), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 101–103, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 98–100.

Una forma de realización (50) de la presente invención se refiere a un péptido de unión o proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con la forma de realización (27), en donde dicho primer dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 89, 115, y 91, y dicho segundo dominio de unión contiene las CDRs tal como se indica en SEC ID NOs: 106–108.

En otra forma de realización más (51), la presente invención se refiere a un péptido de unión o una proteína o una de sus partes funcionales, en particular a un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, en particular un péptido de unión o anticuerpo de cualquiera de las formas de realización precedentes, cuyo péptido de unión o anticuerpo reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular un confórmero de proteína tau patológica, pero, en una forma de realización, no se une con el

correspondiente epítope no fosforilado y/o con epítopes no relacionados en donde dicho péptido de unión o anticuerpo comprende

a. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1; o
b. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 7 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 1; o
c. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 8 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 2; o
d. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 9 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 3; o
e. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 10 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 4; o
f. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 11 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 5; o
g. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 69 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 68; o
h. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 77 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 76; o

i. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 116 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 88; o

j. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 92 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 88; o

k. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 97 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 6; o

l. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 105 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEC ID NO: 104.

En una forma de realización (52) de la invención, el péptido de unión de cualquiera de las formas de realización precedentes es un anticuerpo, en particular un anticuerpo del isotipo IgG2a, IgG2b o IgG3, en particular un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo completamente humano.

Una forma de realización (48) de la invención se refiere a un polinucleótido que codifica el péptido de unión de cualquiera de las formas de realización precedentes.

En una forma de realización (53), dicho polinucleótido comprende una molécula de ácido nucleico seleccionada del grupo que consiste en

a. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que codifica un polipéptido que comprende la secuencia de aminoácidos tal como se indica en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

b. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 85% de identidad de secuencia con la secuencia mostrada en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

c. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 90% de identidad de secuencia con la secuencia mostrada en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

d. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 95% de identidad de secuencia con la secuencia mostrada en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

e. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 98% de identidad de secuencia con la secuencia mostrada en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

f. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 99% de identidad de secuencia con la secuencia mostrada en SEC ID NOs: 35–45, SEC ID NOs: 84–87, SEC ID NO: 109–112 y 117;

g. una molécula de ácido nucleico que comprende una secuencia de nucleótidos, cuya cadena complementaria se hibrida en la molécula de ácido nucleico de cualquiera de a) – f);

h. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que se desvía de la secuencia de nucleótidos definida en cualquiera de a) – g) por degeneración del código genético,

en donde dicha molécula de ácido nucleico tal como se define en cualquiera de a) – h) reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular en la proteína humana Tau tal como se indica en SEC ID NO: 67, seleccionada del grupo que consiste en Tau aa 15–20 que comprende Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende Ser fosforilada en la posición 409 (pS409); y Tau aa 208–218 que comprende Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición 214 (pS214), Tau aa 393–401, que comprende Ser fosforilada en la posición 396 (pS396), Tau aa 396–401 que comprende Ser fosforilada en la posición 396 (pS396), Tau aa 394–400 que comprende Ser fosforilada en la posición 396 (pS396), Tau aa 402–406 que comprende Ser fosforilada en la posición 404 (pS404), y Tau aa 393–400 que comprende Ser fosforilada en la posición 396 (pS396), en donde, en una forma de realización, dicho péptido de unión tiene una alta afinidad de unión con una constante de disociación de al menos 10 nM, en particular de al menos 8 nM, en particular de al menos 5 nM, en particular de al menos 2 nM, en particular de al menos 1 nM, en particular de al menos 500 pM, en particular de al menos 400 pM, en particular de al menos 300 pM, en particular de al menos 200 pM, en particular de al menos 100 pM, en particular de al menos 50 pM y/o tiene una constante de velocidad de asociación de 10^4 $M^{-1}s^{-1}$ o mayor, en particular de entre 3 – 5 x 10^4 $M^{-1}s^{-1}$ o mayor, en particular de 10^5 $M^{-1}s^{-1}$ o mayor; en particular de 6 – 9 x 10^5 $M^{-1}s^{-1}$ o mayor; en particular de 10^6 $M^{-1}s^{-1}$ o mayor, en particular de 1 – 4 x 10^6 $M^{-1}s^{-1}$ o mayor, en particular de 10^7 $M^{-1}s^{-1}$ o mayor, pero, en una forma de realización, no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados.

En diversas formas de realización (54) de la invención, se provee un péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos, que sea capaz de reconocer específicamente y de unirse a un fosfoepítope en un mamífero, en particular en la proteína humana tau, en particular una proteína tau asociada a microtúbulos, en particular una proteína tau hiperfosforilada y asociada a microtúbulos agregados como la presente en los filamentos helicoidales pareados (FHP), que son las estructuras predominantes en ovillos neurofibrilares, hilos de neurópilo y neuritis distrófica, pero en una forma de realización, no se une con el epítope no fosforilado correspondiente y/o con epítopes no relacionados.

En una forma de realización específica (55) de la invención, la proteína humana tau es la proteína humana tau mostrada en la SEC ID NO: 67.

De esta manera, los péptidos de unión y los anticuerpos de acuerdo con una cualquiera de las formas de realización anteriores pueden ser usados (56) para reducir los niveles de proteína tau soluble total, en particular, de proteína tau fosforilada soluble, en el cerebro, en particular, en la corteza cerebral y/o hipocampo de un mamífero o de un ser humano que contenga niveles aumentados de proteína tau soluble y/o proteína tau fosforilada soluble.

Los péptidos de unión y los anticuerpos de acuerdo con una cualquiera de las formas de realización anteriores también pueden ser usados (57) para reducir los niveles de filamentos helicoidales pareados que contienen proteína tau hiperfosforilada (pTau PHF) en el cerebro, en particular, en la corteza cerebral y/o hipocampo de un mamífero o de un ser humano que contenga niveles aumentados de dichos filamentos helicoidales pareados de proteína tau (pTau PHF).

La reducción en el nivel de proteína tau soluble total y/o proteína tau fosforilada soluble y/o filamentos helicoidales pareados de pTau (pTau PHF) en el cerebro, en particular en la corteza cerebral y/o hipocampo de un mamífero o de un ser humano que contenga niveles aumentados de dichas variantes de proteínas tau, que contribuyen a enfermedades, trastornos o estados asociados con la proteína tau en tales mamíferos o seres humanos, puede llevar a una mejora y/o mitigación de los síntomas asociados con dichas enfermedades, trastornos o estados asociados con la proteína tau (58).

Por lo tanto, los péptidos de unión y los anticuerpos de acuerdo con una cualquiera de las formas de realización anteriores pueden ser usados (59) en terapia, en particular, en terapia con seres humanos, para retardar o detener el avance de una enfermedad, trastorno o estado asociado con la proteína tau.

Los péptidos de unión y los anticuerpos de acuerdo con una cualquiera de las formas de realización anteriores pueden ser usados (60), además, en terapia, en particular, en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas que incluyen el razonamiento, el juicio situacional, la capacidad de la memoria, el aprendizaje, la navegación espacial, etc.

En una forma de realización (61), la invención se refiere a los péptidos de unión y a los anticuerpos de acuerdo con una cualquiera de las formas de realización anteriores para su uso en terapia, en particular, para su uso en el tratamiento de tauopatías, un grupo de enfermedades y trastornos asociados con la proteína tau, o para mitigar los síntomas asociados con tauopatías.

En una forma de realización (62), la invención se refiere a los péptidos de unión y a anticuerpos de acuerdo con una cualquiera de las formas de realización

anteriores para mantener o aumentar la capacidad de la memoria cognitiva en un mamífero que sufre de una tauopatía.

En una forma de realización específica (63) de la invención, los péptidos de unión y los anticuerpos que comprenden al menos una o la totalidad de las CDRs de cadena ligera de anticuerpos ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 de acuerdo con lo dado por las SEC ID NOs: 25, 26, 27 y SEC ID NOs: 21, 22, 23, respectivamente, y/o al menos una o la totalidad de las CDRs de cadena pesada de anticuerpos ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 de acuerdo con lo dado por las SEC ID NOs: 12, 13, 14, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

En otra forma de realización específica (64) de la invención, los anticuerpos que comprenden la cadena ligera de anticuerpos ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 de acuerdo con lo dado por las SEC ID NO: 8 y SEC ID NOs: 6, 7, respectivamente, y/o la cadena pesada de anticuerpos ACI–36–2B6–Ab1, ACI–36–2B6–Ab12, ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 de acuerdo con lo dado por las SEC ID NO: 1 y SEC ID NO: 2, respectivamente, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

En otra forma de realización específica (65) de la invención, los péptidos de unión y los anticuerpos que comprenden al menos una o la totalidad de las CDRs de cadena ligera de anticuerpos ACI–33–6C10–Ab1 y ACI–33–6C10–Ab2 de acuerdo con lo dado por las SEC ID NOs: 29, 30, 31, y/o al menos una o la totalidad de las CDRs de cadena pesada de anticuerpos ACI–33–6C10–Ab1 y ACI–33–6C10–Ab2 de acuerdo con lo dado por las SEC ID NOs: 15, 16, 17, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

En otra forma de realización específica (66) de la invención, los péptidos de unión y los anticuerpos que comprenden al menos una o la totalidad de las CDRs de cadena ligera de anticuerpos ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 de acuerdo con lo dado por las SEC ID NOs: 32, 33, 34, y/o al menos una o la totalidad de las CDRs de cadena pesada de anticuerpos ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 de acuerdo con lo dado por las SEC ID NOs: 18, 19, 20, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

En otra forma de realización específica (67) de la invención, los péptidos de unión y los anticuerpos que comprenden al menos una o la totalidad de las CDRs de cadena ligera de anticuerpos ACI–35–2A1–Ab1; ACI–35–2A1–Ab2; ACI–35–4A6–Ab1; ACI–35–4A6–Ab2; ACI–35–1D2–Ab1; ACI–35–2G5–Ab1; de acuerdo con lo dado por las SEC ID NOs: 73–75, 81–83, 93–95, 101–103, 106–108, y/o al

menos una o la totalidad de las CDRs de cadena pesada de anticuerpos ACI–35–2A1–Ab1; ACI–35–2A1–Ab2; ACI–35–4A6–Ab1; ACI–35–4A6–Ab2; ACI–35–1D2–Ab1; ACI–35–2G5–Ab1; de acuerdo con lo dado por las SEC ID NOs: 70–72, 78–80, 89–91, 98–100, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

En otra forma de realización específica (68) de la invención, los péptidos de unión y los anticuerpos que comprenden al menos una o la totalidad de las CDRs de cadena ligera de anticuerpos ACI–35–2G5–Ab2; ACI–35–2G5–Ab3 de acuerdo con lo dado por las SEC ID NOs: 106–108, y/o al menos una o la totalidad de las CDRs de cadena pesada de anticuerpos ACI–35–2G5–Ab2; ACI–35–2G5–Ab3; de acuerdo con lo dado por las SEC ID NOs: 89, 115 y 91, son empleados en terapia, en particular en terapia con seres humanos, para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas entre las que se incluyen el razonamiento, el juicio situacional, la memoria, el aprendizaje, la navegación espacial, etc.

La unión de los péptidos o anticuerpos de acuerdo con las formas de realización anteriores en ovillos de tau y pTau en el cerebro puede ser determinada mediante la aplicación de una prueba de inmunorreactividad de proteína de partes elegidas de cerebro y por transferencia Western de homogenatos de tejido cerebral, respectivamente, de acuerdo con lo descripto en los Ejemplos.

En otra forma de realización (69), la presente invención provee una composición farmacéutica que comprende un péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos, en una cantidad efectiva desde el punto de vista terapéutico junto con un portador aceptable desde el punto de vista farmacéutico.

En una forma de realización (70), el péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido, o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos, es empleado en terapia, en particular en terapia con humanos para el tratamiento o mitigación de los síntomas de enfermedades o trastornos asociados con la proteína tau que incluyen trastornos neurodegenerativos tales como las tauopatías.

De esta forma, los péptidos de unión, los anticuerpos y/o las composiciones farmacéuticas de acuerdo con una cualquiera de las formas de realización anteriores pueden ser usados (71) para retardar o detener el avance de una enfermedad, trastorno o estado asociado a la proteína tau, mediante la administración de dichos péptidos de unión, anticuerpos y/o composiciones farmacéuticas a una animal, en particular a un mamífero, en particular a un ser humano, que sufra de una enfermedad o estado como este.

Además, los péptidos de unión, los anticuerpos y/o las composiciones farmacéuticas de acuerdo con una cualquiera de las formas de realización anteriores pueden ser usados (72) para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados a la proteína tau, como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas que incluyen el

razonamiento, el juicio situacional, la capacidad de memoria, el aprendizaje, la navegación espacial, etc., mediante la administración de dichos péptidos de unión, anticuerpos y/o composiciones farmacéuticas a una animal, en particular a un mamífero, en particular a un ser humano, que sufra de una enfermedad o estado como este.

En una forma de realización (73), el péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional del él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos, es usado en el tratamiento de enfermedades y trastornos que son provocados por la formación de lesiones neurofibrilares o están asociados con ella, donde la patología cerebral predominante en tauopatía comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia con patologías tau y amiloide que incluyen, sin que la enumeración sea taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis

supranuclear progresiva, panencefalitis esclerosante subaguda, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica.

En una forma de realización (74), el péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellas, es usado en el tratamiento de la enfermedad de Alzheimer.

En una forma de realización (75) de la invención, se provee un método para modular los niveles de proteína tau soluble y/o insoluble, en particular en el cerebro, en particular en la corteza cerebral y/o en el hipocampo de un animal, en particular un mamífero o un ser humano, que comprende la administración a dicho animal, en particular a dicho mamífero o ser humano, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellas.

En un aspecto, la modulación se refiere a la reducción de los niveles de la proteína tau soluble, en particular de la proteína tau fosforilada soluble, en el cerebro, en particular en la corteza cerebral y/o el hipocampo, de un animal, en particular un mamífero o un ser humano que contiene niveles incrementados de la proteína tau soluble y/o la proteína tau fosforilada soluble.

En una forma de realización (76) de la invención, se provee un método para reducir los niveles de la proteína tau insoluble, en particular de filamentos helicoidales pareados que contienen proteína tau hiperfosforilada (pTau PHF), en el cerebro, en particular en la corteza cerebral y/o en el hipocampo de un animal, en particular un mamífero o un ser humano que contenga niveles aumentados de la proteína tau insoluble, en particular de los filamentos helicoidales pareados de

proteína tau (pTau PHF), que comprende la administración a dicho animal, en particular a dicho mamífero o ser humano, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellas.

En una forma de realización (77), la presente invención se refiere a un método para retardar o detener el avance de una enfermedad, trastorno o estado asociado con la proteína tau en un animal, en particular de un mamífero o de un ser humano, que comprende la administración a dicho animal, en particular a dicho mamífero o ser humano, que sufre de una enfermedad o estado como este, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos.

En una forma de realización (78), la presente invención se refiere a un método para mejorar o mitigar los síntomas asociados con enfermedades, trastornos o estados asociados con la proteína tau, como ser, por ejemplo, el deterioro o la pérdida de funciones cognitivas que incluyen el razonamiento, el juicio situacional, la capacidad de memoria, el aprendizaje, la navegación espacial, etc., en un animal, en particular de un mamífero o de un ser humano, que comprende la administración a dicho animal, en particular a dicho mamífero o ser humano, que sufre de una enfermedad o estado como este, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos.

En una forma de realización (79), la presente invención se refiere a un método para conservar o aumentar la capacidad de memoria cognitiva en un mamífero que sufre de una tauopatía.

En aun otra forma de realización (80) de la invención, se provee un método para el tratamiento de una enfermedad o trastorno asociado con la proteína tau que incluye una enfermedad o un trastorno neurodegenerativo como ser una tauopatía que comprende la administración a un animal, en particular a un mamífero, pero en especial a un ser humano, que sufre de una enfermedad o estado como este, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos.

En una forma de realización (81) de la invención, se provee un método para el tratamiento de enfermedades y trastornos que son causados por la formación de lesiones neurofibrilares o están asociados con ella, donde la patología cerebral predominante en tauopatía comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con

calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, panencefalitis esclerosante subaguda, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica, método que comprende la administración a una animal, en particular a una mamífero, pero en especial a un ser humano, que sufre de una enfermedad o trastorno como estos, del péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos.

En otra forma de realización (82) de la invención, se provee un método para inducir una respuesta inmune pasiva en un animal, en particular en un mamífero o en un ser humano, que sufre de un trastorno neurodegenerativo como ser una tauopatía mediante la administración a dicho animal o ser humano del péptido de unión o de una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte funcional de él, o un polinucleótido o una composición farmacéutica, de acuerdo con una cualquiera de las formas de realización anteriores, o una combinación de ellos.

En otra forma de realización más (83) de la invención, se proporciona un método para diagnosticar una enfermedad, trastorno o condición asociados con la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes, a un epítope de la proteína tau en una muestra o in situ que incluye las etapas de

a. poner en contacto la muestra o una parte del cuerpo específica o área corporal sospechada de contener la proteína tau con un péptido de unión o uno de sus fragmentos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las reivindicaciones precedentes, en donde dicho péptido de unión o anticuerpo o su fragmento se une con un epítope de la proteína tau;
b. permitir que dicho péptido de unión, en particular dicho anticuerpo, en particular dicho anticuerpo monoclonal o una de sus partes funcionales, se una con la proteína tau para formar un complejo inmunológico;
c. detectar la formación del complejo inmunológico; y
d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área específica del cuerpo.

En otra forma de realización más (84) de la invención, se proporciona un método para diagnosticar una predisposición a una enfermedad, trastorno o condición asociado a la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes, a un epítope de la proteína tau en una muestra o in situ, que incluye las etapas de

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que dicho péptido de unión, en particular dicho anticuerpo, en particular dicho anticuerpo monoclonal o una de sus partes funcionales, se una con el antígeno tau para formar un complejo inmunológico;
c. detectar la formación del complejo inmunológico; y
d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo;
e. comparar la cantidad de dicho complejo inmunológico con un valor de control normal;

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente padece o está en riesgo de desarrollar una enfermedad o condición asociada a la proteína tau.

En una forma de realización (85) de la invención, se proporciona un método para controlar una enfermedad residual mínima en un paciente después del tratamiento con el péptido de unión o una de sus partes funcionales, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, o un polinucleótido, o una composición farmacéutica, de acuerdo con cualquiera de las formas de realización precedentes, en donde dicho método comprende:

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con el péptido de unión o una de sus partes funcionales, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que dicho péptido de unión, en particular dicho anticuerpo, en particular dicho anticuerpo monoclonal o una de sus partes funcionales, se una con el antígeno tau para formar un complejo inmunológico;
c. detectar la formación del complejo inmunológico; y
d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,
e. comparar la cantidad de dicho complejo inmunológico con un valor de control normal,

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente todavía padece de una enfermedad residual mínima.

En una forma de realización (86), se proporciona un método para predecir la respuesta de un paciente en tratamiento con el péptido de unión o una de sus partes funcionales, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, o un polinucleótido, o una composición farmacéutica, de acuerdo con cualquiera de las formas de realización precedentes, que comprende

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales de acuerdo con cualquiera de las formas de realización precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;
b. permitir que dicho péptido de unión, en particular dicho anticuerpo, en particular dicho anticuerpo monoclonal o una de sus partes funcionales, se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y
d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,
e. comparar la cantidad de dicho complejo inmunológico antes y después del inicio del tratamiento,

en donde una disminución de la cantidad de dicho agregado indica que dicho paciente tiene un alto potencial de responder al tratamiento.

En otra forma de realización (87), la invención se refiere a un kit de ensayo para detección y diagnosis de enfermedades, trastornos o condiciones asociados con la proteína tau que comprende un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes.

En una forma de realización (88), dicho kit de ensayo comprende un recipiente que lleva uno o varios péptidos de unión o sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales, de acuerdo con cualquiera de las formas de realización precedentes e instrucciones de uso de los péptidos de unión o anticuerpos a los fines de unirse con el antígeno tau para formar un complejo inmunológico y detectar la formación del complejo inmunológico de modo que la presencia o la ausencia del complejo inmunológico se correlacione con la presencia o ausencia del antígeno tau.

En otra forma de realización más (89), la presente invención se refiere a un epítope seleccionado del grupo que consiste en Tau aa 15–20 de proteína tau humana mostrada en SEC ID NO: 67 que comprende Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende Ser fosforilada en la posición

409 (pS409), Tau aa 405–411 que comprende Ser fosforilada en la posición 409 (pS409); y Tau aa 208–218 que comprende Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición 214 (pS214).

En una forma de realización (90), dicho epítope consiste en Tau aa 15–20 con Tyr fosforilada en la posición 18 (Y18).

En una forma de realización (91), dicho epítope consiste en Tau aa 405–412 con Ser fosforilada en la posición 409 (pS409).

En una forma de realización (92), dicho epítope consiste en Tau aa 405–411 con Ser fosforilada en la posición 409 (pS409).

En otra forma de realización (93), la invención se refiere a una línea celular que produce un péptido de unión o uno de sus fragmentos activos, en particular un anticuerpo, en particular un anticuerpo monoclonal o una de sus partes funcionales de acuerdo con cualquiera de las formas de realización precedentes.

En una forma de realización (94), la invención se refiere a una línea celular que es la línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079.

En una forma de realización (95), la invención se refiere a una línea celular que es la línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081.

En una forma de realización (96), la invención se refiere a una línea celular que es la línea celular de hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080.

En una forma de realización (97), la invención se refiere a una línea celular que es la línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088.

En una forma de realización (98), la invención se refiere a una línea celular que es la línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084.

En una forma de realización (99), la invención se refiere a una línea celular que es la línea celular de hibridoma 2B6G7A12 depositada el 10 de marzo de 2010 como DSM ACC3087.

En una forma de realización (100), la invención se refiere a una línea celular que es la línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086.

En una forma de realización (101), la invención se refiere a una línea celular que es la línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085.

En una forma de realización (102), la invención se refiere a una línea celular que es la línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082.

En una forma de realización (103), la invención se refiere a una línea celular que es la línea celular de hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083.

En una forma de realización (103a), la invención se refiere a una línea celular, que es una línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136.

En una forma de realización (103b), la invención se refiere a una línea celular, que es una línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137.

En una forma de realización (103c), la invención se refiere a una línea celular, que es una línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138.

En una forma de realización (103d), la invención se refiere a una línea celular, que es una línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139.

En una forma de realización (103e), la invención se refiere a una línea celular, que es una línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140.

En una forma de realización (103e), la invención se refiere a una línea celular, que es una línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141.

En una forma de realización (104), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 54 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 53 y SEC ID NO: 54 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (105), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede

obtener por amplificación por PCR de ADN de línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081 usando

a. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 48 y SEC ID NO: 49 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 53 y SEC ID NO: 54 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (106), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o
b. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (107), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o
b. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (108), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y SEC ID NO: 52 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (109), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 2B6G7A12 depositada el 25 de agosto de 2010 como DSM ACC3087 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y SEC ID NO: 52 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (110), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086 usando

a_1. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 48 y SEC ID NO: 49 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (111), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085 usando

a_1. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 48 y SEC ID NO: 49 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 50 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 46 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (112), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082 usando

a. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 49; SEC ID NO: 56 y SEC ID NO: 57 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión;

b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 53 y SEC ID NO: 55 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (113), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 57 y un cebador 3’ de SEC ID NO: 51 para la amplificación de un primer dominio de unión;
b. una mezcla de cebadores que comprende un cebador 5’ de SEC ID NO: 53 y SEC ID NO: 55 y un cebador 3’ de SEC ID NO: 47 para la amplificación de un segundo dominio de unión.

En una forma de realización (114), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139 usando

a. un par de cebadores que comprende un cebador 5’ de SEC ID NO: 149 y un cebador 3’ de SEC ID NO: 51 para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 120, 123, 124, 136, 137, 138, 139 y 140 y un cebador 3’ seleccionado del grupo que consiste en SEC ID NOs: 131, 134 y 141–148, para amplificación de un segundo dominio de unión.

En una forma de realización (115), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 51 y 169–174 y un cebador 3’ de SEC ID NO: 51, para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 124, 127, y 150–158 y un cebador 3’ seleccionado del grupo que consiste en SEC ID NOs: 130, y 159–168, para amplificación de un segundo dominio de unión.

En una forma de realización (116), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 178, 179 y 180 y un cebador 3’ de SEC ID NO: 51, para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 121, 127, 139, 154, 155, y 175 y un cebador 3’ seleccionado del grupo que consiste en SEC ID NOs: 128, 129, 147, 176, y 177, para amplificación de un segundo dominio de unión.

En una forma de realización (117), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 51 y 188–192 y un cebador 3’ de SEC ID NO: 51, para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 120, 124, 126, 181, 182 y 183 y un cebador 3’ seleccionado del grupo que consiste en SEC ID NOs: 144, 145 y 184–187, para amplificación de un segundo dominio de unión.

En una forma de realización (118), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 50 y 201–204 y un cebador 3’ de SEC ID NO: 51, para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 121, 137, 151 y 193–197 y un cebador 3’ seleccionado del grupo que consiste en SEC ID NOs: 131, 141, 144, 166, 198, 199 y 200, para amplificación de un segundo dominio de unión.

En una forma de realización (119), la invención se refiere a un anticuerpo monoclonal o una de sus partes funcionales que comprende un dominio de cadena liviana (VL) y/o un dominio de cadena pesada (VH), que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 209–214, y 219–221 y un cebador 3’ de SEC ID NO: 215, para amplificación de un primer dominio de unión; y/o
b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEC ID NOs: 216, 217 y 218 y un cebador 3’ de SEC ID NOs: 208, para amplificación de un segundo dominio de unión.

En una forma de realización (120), el anticuerpo de acuerdo con cualquiera de las formas de realización precedentes puede ser un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado, un anticuerpo humano, un anticuerpo de camélido, un diacuerpo, o un anticuerpo modificado o sometido a ingeniería.

En una forma de realización (121), el péptido de unión o su parte funcional puede ser un fragmento que comprende una cadena pesada y/o una cadena liviana, en particular una cadena pesada tal como se indica en SEC ID NOs: 1–5 y/o una cadena liviana tal como se indica en SEC ID NOs: 6–11, en particular un fragmento Fab o $F(ab')_2$.

En una forma de realización específica (122), la invención se refiere a una cadena pesada tal como se indica en SEC ID NOs: 1–5.

En otra forma de realización específica (123), la invención se refiere a una cadena liviana tal como se indica en SEC ID NOs: 6–11.

En una forma de realización (124), la invención proporciona un método para producir los péptidos de unión o anticuerpos de cualquiera de las formas de realización precedentes, que comprende la etapa de cultivar la línea celular de cualquiera de las formas de realización precedentes en un medio de cultivo apropiado y, opcionalmente, purificar los péptidos de unión o anticuerpo de la línea celular o medio de cultivo.

Breve descripción de las figuras y de las secuencias

FIGURAS

La Figura 1 muestra un anticuerpo unido a una fosfo–tau en partes del cerebro de ratones biGT (tau bigénico) mediante el uso de TAUPIR.

La Figura 2 muestra un anticuerpo unido a una fosfo–tau en partes del cerebro de pacientes con EA y tauopatía mediante el uso de TAUPIR con anticuerpo ACI–36–3A8–Ab1.

La Figura 3 muestra el efecto del tratamiento con anticuerpo anti–tau después de estudio *in vivo* de una (1) semana en epítope pTau aT231 mediante el uso de MSD.

La Figura 4 representa un diagrama que demuestra cómo fueron preparados los cerebros para fracciones de proteína tau solubles e insolubles en sarcosilo (SinT).

La Figura 5 muestra los resultados de la transferencia Western de epítope pTau después de un tratamiento con anticuerpo antiTau en un estudio *in vivo* de un (1) mes (Figuras 5A, 5B, 5C, 5G, 5H, 5I) o de tres (3) meses (Figuras 5D, 5E, 5F).

La Figura 6 muestra los resultados de la transferencia Western de epítope pTau después del tratamiento con anticuerpo antiTau para un estudio *in vivo* de tres (3) meses mediante el uso de ratones bigénicos biGT.

La Figura 7 muestra IHC después del tratamiento con anticuerpo antiTau con ACI–36–2B6–Ab1 en un estudio *in vivo* de tres (3) meses.

La Figura 8 muestra IHC después del tratamiento con anticuerpo antiTau con ACI–36–3A8–Ab1 en un estudio *in vivo* de tres (3) meses.

La Figura 9 muestra los resultados del test de Morris Water–Maze después del tratamiento con anticuerpo antiTau con ACI–36–2B6–Ab1 en un estudio *in vivo* de tres (3) meses.

La Figura 10 muestra los resultados del test de Morris Water–Maze después del tratamiento con anticuerpo antiTau con ACI–36–3A8–Ab1 en un estudio *in vivo* de tres (3) meses.

SECUENCIAS

SEC ID NO: 1 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–3A8–Ab1 producido por la línea celular de hibridoma 3A8A12G7.

SEC ID NO: 2 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–2B6–Ab1 producido por la línea celular de hibridoma 2B6A10C11.

SEC ID NO: 3 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 4 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 5 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 6 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–Ab1$_{VK–AD}$ y ACI–36–3A8–Ab2$_{VK–AD}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 7 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–Ab1$_{VK–G}$ y ACI–36–3A8–Ab2$_{VK–G}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 8 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–2B6–Ab1 y ACI–36–2B6–Ab2 producido por la línea celular de hibridoma 2B6A10C11 y 2B6G7A12, respectivamente.

SEC ID NO: 9 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 10 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 11 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 12 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–3A8–Ab1, ACI–36–3A8–Ab2, ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 13 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–3A8–

Ab1, ACI–36–3A8–Ab2, ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 14 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–3A8–Ab1, ACI–36–3A8–Ab2, ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 15 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 16 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 17 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 18 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 19 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–41–7C2–

Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 20 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 21 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–$Ab1_{VK–AD}$ y ACI–36–3A8–$Ab2_{VK–AD}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 22 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–$Ab1_{VK–AD}$ y ACI–36–3A8–$Ab2_{VK–AD}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 23 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–$Ab1_{VK–AD}$ y ACI–36–3A8–$Ab2_{VK–AD}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 24 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–$Ab1_{VK–G}$ y ACI–36–3A8–$Ab2_{VK–G}$ producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 25 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–$Ab1_{VK–G}$, ACI–36–3A8–$Ab2_{VK–G}$, ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma

3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 26 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–Ab1$_{VK–G}$, ACI–36–3A8–Ab2$_{VK–G}$, ACI–36–2B6–Ab1, ACI–36–2B6–Ab2, ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 3A8A12G7, 3A8E12H8, 2B6A10C11, 2B6G7A12, 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 27 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–2B6–Ab1 y ACI–36–2B6–Ab2 producido por la línea celular de hibridoma 2B6A10C11 y 2B6G7A12, respectivamente.

SEC ID NO: 28 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 29 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 30 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 31 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–33–6C10–

Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 32 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 33 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 34 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 35 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 36 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–2B6–Ab1 y ACI–36–2B6–Ab2 producido por la línea celular de hibridoma 2B6A10C11 y 2B6G7A12, respectivamente.

SEC ID NO: 37 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 38 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–33–6C10–Ab2 y ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 39 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 40 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 41 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–3A8–Ab1 y ACI–36–3A8–Ab2 producido por la línea celular de hibridoma 3A8A12G7 y 3A8E12H8, respectivamente.

SEC ID NO: 42 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–2B6–Ab1 y ACI–36–2B6–Ab2 producido por la línea celular de hibridoma 2B6A10C11 y 2B6G7A12, respectivamente.

SEC ID NO: 43 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–36–6H1–Ab1 y ACI–36–6H1–Ab2 producido por la línea celular de hibridoma 6H1A11C11 y 6H1G6E6, respectivamente.

SEC ID NO: 44 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–33–6C10–Ab2 y

ACI–33–6C10–Ab1 producido por la línea celular de hibridoma 6C10E5E9C12 y 6C10F9C12A11, respectivamente.

SEC ID NO: 45 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–41–7C2–Ab1 y ACI–41–7C2–Ab2 producido por la línea celular de hibridoma 7C2(1)F10C10D3 y 7C2(2)B9F11D5, respectivamente.

SEC ID NO: 46 – 57 representa secuencias de nucleótidos de cebadores delanteros e inversos VH/VK

SEC ID NO: 58 representa la secuencia de aminoácidos de Tau 379–408 [pS396, pS404]

SEC ID NO: 59 representa la secuencia de aminoácidos de Tau 5–20 [pY18]

SEC ID NO: 60 representa la secuencia de aminoácidos de Tau 206–221 [pT212, pS214]

SEC ID NO: 61 representa la secuencia de aminoácidos de Tau 196–211 [pS202, pT205]

SEC ID NO: 62 representa la secuencia de aminoácidos de Tau 393–408 [pS396, pS404]

SEC ID NO: 63 representa la secuencia de aminoácidos de Tau 401–418 [pS404, pS409]

SEC ID NO: 64 representa la secuencia de aminoácidos de Tau 200–216 [pS202+ pT205 & pT212+pS214]

SEC ID NO: 65 representa la secuencia de aminoácidos de Tau 407–418 [pS409]

SEC ID NO: 66 representa la secuencia de aminoácidos de Tau 399–408 [pS404]

SEC ID NO: 67 representa la secuencia de aminoácidos de la isoforma más larga de Tau humana (441aa), también denominada Tau40

SEC ID NO: 68 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–4A6–Ab1 producido por la línea celular de hibridoma A4–4A6–18.

SEC ID NO: 69 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab1 producido por la línea celular de hibridoma A4–4A6–18.

SEC ID NO: 70 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 71 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 72 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 73 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 74 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 75 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab1

SEC ID NO: 76 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–1D2–Ab1 producido por la línea celular de hibridoma A6–1D2–12.

SEC ID NO: 77 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–1D2–Ab1 producido por la línea celular de hibridoma A6–1D2–12.

SEC ID NO: 78 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–1D2–Ab1.

SEC ID NO: 79 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–1D2–Ab1.

SEC ID NO: 80 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–1D2–Ab1

SEC ID NO: 81 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–1D2–Ab1

SEC ID NO: 82 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–1D2–Ab1

SEC ID NO: 83 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–1D2–Ab1

SEC ID NO: 84 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–4A6–Ab1 producido por la línea celular de hibridoma A4–4A6–18.

SEC ID NO: 85 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab1 producido por la línea celular de hibridoma A4–4A6–18.

SEC ID NO: 86 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–1D2–Ab1 producido por la línea celular de hibridoma A6–1D2–12.

SEC ID NO: 87 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–1D2–Ab1 producido por la línea celular de hibridoma A6–1D2–12.

SEC ID NO: 88 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2A1–Ab1, ACI–35–2A1–Ab2, y ACI–35–4A6–Ab2, respectivamente, producido por la línea celular de hibridoma A4–2A1–18, A4–2A1–40 y A4–4A6–48, respectivamente.

SEC ID NO: 89 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2A1–Ab1, ACI–35–2A1–Ab2, ACI–35–4A6–Ab2, ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente.

SEC ID NO: 90 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2A1–Ab1, ACI–35–2A1–Ab2, y ACI–35–4A6–Ab2, respectivamente.

SEC ID NO: 91 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2A1–Ab1, ACI–35–2A1–Ab2, ACI–35–4A6–Ab2, ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente.

SEC ID NO: 92 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab2 producido por la línea celular de hibridoma A4–2A1–40

SEC ID NO: 93 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab2.

SEC ID NO: 94 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab2.

SEC ID NO: 95 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab2.

SEC ID NO: 96 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–Ab1 producido por la línea celular de hibridoma A6–2G5–08.

SEC ID NO: 97 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–Ab1 producido por la línea celular de hibridoma A6–2G5–08.

SEC ID NO: 98 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 99 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 100 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 101 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 102 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 103 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–Ab1.

SEC ID NO: 104 representa la secuencia de aminoácidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente, producido por la línea celular de hibridoma A6–2G5–30 y A6–2G5–41, respectivamente.

SEC ID NO: 105 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente, producido por la línea celular de hibridoma A6–2G5–30 y A6–2G5–41, respectivamente.

SEC ID NO: 106 representa la secuencia de aminoácidos de la CDR1 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente.

SEC ID NO: 107 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente.

SEC ID NO: 108 representa la secuencia de aminoácidos de la CDR3 de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente.

SEC ID NO: 109 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2A1–Ab1, ACI–35–2A1–Ab2, y ACI–35–4A6–Ab2, respectivamente, producido por la línea celular de hibridoma A4–2A1–18, A4–2A1–40 y A4–4A6–48, respectivamente.

SEC ID NO: 110 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab2 producido por la línea celular de hibridoma A4–2A1–40.

SEC ID NO: 111 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–AB1 producido por la línea celular de hibridoma A6–2G5–08.

SEC ID NO: 112 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB1 producido por la línea celular de hibridoma A6–2G5–08.

SEC ID NO: 113 representa la secuencia de nucleótidos de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente, producido por la línea celular de hibridoma A6–2G5–30 y A6–2G5–41, respectivamente.

SEC ID NO: 114 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3, respectivamente, producido por la línea celular de hibridoma A6–2G5–30 y A6–2G5–41, respectivamente.

SEC ID NO: 115 representa la secuencia de aminoácidos de la CDR2 de la región variable de cadena pesada (VH) de anticuerpo monoclonal ACI–35–2G5–AB2 y ACI–35–2G5–AB3.

SEC ID NO: 116 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab1producido por la línea celular de hibridoma A4–2A1–18.

SEC ID NO: 117 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–2A1–Ab1 producido por la línea celular de hibridoma A4–2A1–18.

SEC ID NO: 118 representa la secuencia de aminoácidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab2 producido por la línea celular de hibridoma A4–4A6–48.

SEC ID NO: 119 representa la secuencia de nucleótidos de la región variable de cadena liviana (VK) de anticuerpo monoclonal ACI–35–4A6–Ab2 producido por la línea celular de hibridoma A4–4A6–48.

SEC ID NO: 120 – 221 representa secuencias de nucleótidos de cebadores delanteros e inversos VH/VK.

Definiciones de los términos

Los términos "polipéptido", "péptido" y "proteína", de la forma empleada en la presente, son indistintos y son definidos de manera que hagan referencia a una biomolécula compuesta por aminoácidos unidos por una unión de péptido.

Los términos "péptidos" o "péptido de unión" son utilizados en la presente en forma indistinta y hacen referencia a cadenas de aminoácidos (típicamente L–aminoácidos) cuyos carbonos alfa están unidos a través de uniones de péptido formadas por una reacción de condensación entre el grupo carboxilo del carbono alfa de un aminoácido y el grupo amino del carbono alfa de otro aminoácido. El aminoácido terminal en un extremo de la cadena (es decir, la terminal de amino) tiene un grupo amino libre, mientras que el aminoácido terminal en el otro extremo de la cadena (es decir, la terminal carboxi) tiene un grupo carboxilo libre. Como tal, el término "amino terminal" (que se abrevia N–terminal) hace referencia al grupo amino alfa libre en el aminoácido en la terminal amino del péptido, o al grupo amino alfa (grupo imino cuando participa en una unión de péptido) de un aminoácido en cualquier otro lugar dentro del péptido. De forma similar, el término "terminal carboxi" (que se abrevia C–terminal) hace referencia al grupo carboxilo libre en el aminoácido en la terminal carboxi de un péptido, o al grupo carboxilo de

un aminoácido en cualquier otro lugar dentro del péptido. Un péptido de unión puede constituir anticuerpos tales como anticuerpos policlonales o monoclonales, anticuerpos humanos o humanizados, diacuerpos, anticuerpos de camélidos, etc, o partes funcionales de él de acuerdo con lo definido en la presente.

Los términos "fragmento de él", "su fragmento" o "fragmento" de la forma utilizada en la presente, hacen referencia a un fragmento del péptido funcional que tiene esencialmente la misma actividad (biológica) que los péptidos definidos en la presente (por ejemplo, de acuerdo con lo mostrado en las SEC ID NOs 59–66 en la tabla 1, respectivamente), es decir que dichos fragmentos aún son capaces de suscitar una respuesta inmune muy específica, en particular específica respecto de su conformación, en un organismo, pero en particular dentro de un animal, en particular de un mamífero o de un ser humano, que es muy efectiva y capaz de evitar o mitigar tauopatías o los síntomas asociados con tauopatías. En particular, dichos fragmentos aún contienen el o los fosfoepítopes patológicos específicos del péptido tau, de acuerdo con lo utilizado y definido en la presente.

Típicamente, los aminoácidos que conforman un péptido están numerados en orden, comenzando en la terminal de amino y aumentando en la dirección hacia la terminal carboxi del péptido. De esta manera, cuando se dice que un aminoácido "sigue" a otro, ese aminoácido está ubicado más cerca de la terminal carboxi del péptido que el aminoácido precedente.

El término "residuo" es utilizado en la presente para hacer referencia a un aminoácido que es incorporado en un péptido por una unión de amida. Como tal, el aminoácido puede presentarse en forma natural como aminoácido o, a menos que se lo limite de otra forma, puede abarcar análogos conocidos de aminoácidos naturales que funcionan de una forma similar a los aminoácidos que se presentan en forma natural (es decir, los miméticos de aminoácidos). Además, un mimético

de unión de amida incluye modificaciones estructurales de péptido, que resultan conocidas para los expertos en la técnica.

La expresión "que esencialmente consiste/n en" es empleada en la presente de manera que quede excluido cualquier elemento que sustancialmente altere las propiedades esenciales de los péptidos a los que hace referencia la frase. De esta manera, la descripción de un péptido "que esencialmente consiste en..." excluye cualquier sustitución, agregado o eliminación de aminoácido que sustancialmente altere la actividad biológica de ese péptido.

Además, el experto en la técnica sabrá reconocer que, de acuerdo con lo mencionado con anterioridad, cada sustitución, eliminación o agregado que altere, agregue o elimine un solo aminoácido o un pequeño porcentaje de aminoácidos (típicamente menos del 5%, más típicamente menos del 1%) en una secuencia codificada, es una variante modificada en forma conservadora donde las alteraciones dan como resultado la sustitución de un aminoácido con un aminoácido similar desde el punto de vista químico. Las tablas de sustituciones conservadoras que proveen aminoácidos similares en cuanto a su función resultan bien conocidas en la técnica. Los siguientes seis grupos contienen, cada uno de ellos, aminoácidos que son sustituciones conservadoras entre sí:

1) alanina (A), serina (S), treonina (T);

2) ácido aspártico (D), ácido glutámico (E);

3) asparagina (N), glutamina (Q);

4) arginina (R), lisina (K);

5) isoleucina (I), leucina (L), metionina (M), valina (V); y

6) fenilalanina (F); tirosina (Y), triptófano (W).

Las expresiones "aislado" o "biológicamente puro" hacen referencia al material que está sustancial o esencialmente libre de componentes que por lo general lo acompañan de la forma que se encuentra en su estado original. De

esta manera, los péptidos descriptos en la presente no contienen materiales que en forma normal estén asociados con su entorno *in situ*. En general, los péptidos inmunogénicos aislados descriptos en la presente son puros en al menos alrededor del 80%, de forma usual al menos alrededor del 90%, y de preferencia, al menos alrededor del 95% de acuerdo con lo medido por intensidad de banda en un gel de tinción con plata.

La pureza o la homogeneidad de la proteína pueden ser indicadas por una cantidad de métodos conocidos en la técnica, como ser electroforesis en gel de poliacrilamida de una muestra de proteína, seguido de visualización luego de tinción. Para ciertos propósitos, será necesaria una alta resolución y la utilización de HPLC o medios similares para purificación.

Cuando los péptidos inmunogénicos presentan una longitud relativamente corta (es decir, menor que alrededor de 50 aminoácidos), con frecuencia son sintetizados mediante el uso de técnicas químicas de síntesis de péptidos estándar.

La síntesis de fase sólida donde el aminoácido de C–terminal de la secuencia está unido a un soporte insoluble seguido del agregado secuencial de los aminoácidos restantes en la secuencia es un método preferido para la síntesis química de los péptidos inmunogénicos descriptos en la presente. Las técnicas para síntesis de fase sólida resultan conocidas para los expertos en la técnica.

En forma alternativa, los péptidos inmunogénicos descriptos en la presente son sintetizados mediante el uso de metodología de ácido nucleico recombinante. En general, esto implica la creación de una secuencia de ácido nucleico que codifica el péptido, colocando el ácido nucleico en un casete de expresión bajo el control de un promotor en particular, que expresa el péptido en un huésped, aísla el péptido o polipéptido expresado y, de requerirse, renaturaliza el péptido. En la

literatura se encuentran técnicas suficientes para guiar al experto en la materia a través de dichos procedimientos.

Una vez expresados, los péptidos recombinantes pueden ser purificados de acuerdo con los procedimientos estándar, que incluyen precipitación con sulfato de amonio, columnas de afinidad, cromatografía en columna, electroforesis en gel y similares. Las composiciones sustancialmente puras con una homogeneidad de alrededor del 50% al 95% resultan de preferencia, y con una homogeneidad de entre 80% y 95% o más resultan de mayor preferencia para ser utilizadas como agentes terapéuticos.

El experto en la técnica reconocerá que, después de la síntesis química, la expresión biológica o la purificación, los péptidos inmunogénicos pueden poseer una conformación sustancialmente diferente de las conformaciones originales de los péptidos constituyentes. En este caso, con frecuencia es necesario desnaturalizar y reducir el péptido antiproliferativo y luego hacer que el péptido se repliegue en la conformación preferida. Los métodos para reducir y desnaturalizar proteínas e inducir el repliegue resultan conocidos para los expertos en la técnica.

La antigenicidad de la proteína purificada puede ser confirmada, por ejemplo, mediante la demostración de la reacción con suero inmune o bien, con antisueros producidos contra la proteína propiamente dicha.

Los términos "un" y "el" con sus respectivos femeninos de la forma utilizada en la presente son definidos de manera que signifiquen "uno o más" e incluyen los plurales a menos que el contexto resulte inapropiado.

Los términos "detectar" o "detectado" de la forma utilizada en la presente significan el uso de técnicas conocidas para detectar moléculas biológicas como ser métodos inmunoquímicos o histológicos y hacen referencia a la determinación cuantitativa o cualitativa de la presencia o concentración de la biomolécula en investigación.

El término "aislada" significa una molécula biológica libre de al menos algunos de los componentes en los que se presenta en forma natural.

Los términos "anticuerpo", "anticuerpos" o "partes funcionales de él", de la forma empleada en la presente, consisten en un término reconocido en la técnica y hacen referencia a moléculas o fragmentos activos de moléculas que se unen a antígenos conocidos, en particular a moléculas de inmunoglobulina y a porciones activas desde el punto de vista inmunológico de moléculas de inmunoglobulina, es decir, moléculas que contienen un sitio de unión que se une a un antígeno en forma inmunoespecífica. La inmunoglobulina de acuerdo con la invención puede ser de cualquier tipo (IgG, IgM, IgD, IgE, IgA e IgY) o clase (IgG1, IgG2, IgG3, IgG4, IgA1 y IgA2) o subclases de molécula de inmunoglobulina.

Dentro del alcance de la presente invención, el término "anticuerpos" pretende incluir anticuerpos monoclonales, policlonales, quiméricos, de cadena simple, biespecíficos, simionizados, anticuerpos humanos y humanizados, anticuerpos de camélido, diacuerpos, así como también partes funcionales o fragmentos activos de ellos. Entre los ejemplos de fragmentos activos de moléculas que se unen a antígenos conocidos se incluyen fragmentos Fab y $F(ab')_2$, incluyendo los productos de una biblioteca de expresión de inmunoglobulina Fab y fragmentos que unen epítopes de cualquiera de los anticuerpos y fragmentos arriba mencionados.

Estos fragmentos activos pueden derivar de un anticuerpo de la presente invención mediante una cantidad de técnicas. Por ejemplo, los anticuerpos monoclonales purificados pueden ser segmentados con una enzima, como ser la pepsina, y ser sometidos a filtración en gel por HPLC. La fracción apropiada que contiene fragmentos Fab puede luego ser recolectada y concentrada por filtración de membrana y similares. Para una descripción adicional de técnicas generales para el aislamiento de fragmentos activos de anticuerpos, referirse, por ejemplo, a

Khaw, B. A. et al. J. Nucl. Med. 23:1011–1019 (1982); Rousseaux et al. *Methods Enzymology*, 121:663–69, Academic Press, (1986).

La expresión "anticuerpo humanizado" hace referencia un tipo de anticuerpo modificado por ingeniería genética donde sus CDRs derivan de una inmunoglobulina dadora no humana, y las partes derivadas de inmunoglobulina restantes de la molécula derivan de una (o más) inmunoglobulina(s) humanas.

Un anticuerpo humanizado puede hacer referencia, además, a un anticuerpo con una región variable donde una o más de sus regiones estructurales cuentan con aminoácidos humanos o de primates. Además, los residuos del soporte estructural pueden ser modificados para conservar la afinidad de la unión. Los métodos para obtener "anticuerpos humanizados" resultan conocidos para los expertos en la técnica (referirse, por ejemplo, a Queen et al, Proc. Natl Acad Sci EE.UU, 86:10029–10032 (1989), Hodgson et al., *Bio/Technoloy*, 9:421 (1991)).

Asimismo, puede obtenerse un "anticuerpo humanizado" a través de un nuevo enfoque de modificación genética que permita la producción de anticuerpos policlonales tipo humano madurados por afinidad en animales grandes como ser, por ejemplo, conejos (http://www.rctech.com/bioventures/therapeutic.php).

La expresión "anticuerpo completamente humano" o anticuerpo "humano" hace referencia a un anticuerpo derivado de ratones transgénicos que llevan genes de anticuerpo humano o de células humanas. No obstante, para el sistema inmune humano, la diferencia entre anticuerpos "completamente humano", "humano" y "humanizados" puede ser insignificante o inexistente y como tal, las tres expresiones pueden ser de igual eficacia y seguridad.

La expresión "anticuerpo monoclonal" también es reconocida en la técnica y hace referencia a un anticuerpo que se produce en masa en el laboratorio a partir de un solo clon y que reconoce un solo antígeno. Los anticuerpos

monoclonales son producidos, típicamente, mediante la fusión de una célula B productora de anticuerpos, por lo general de corta vida, a una célula de rápido crecimiento, como ser una célula de cáncer (a veces mencionada como una célula "inmortal"). La célula híbrida resultante, o hibridoma, se multiplica con rapidez y crea un clon que produce grandes cantidades del anticuerpo.

El término "antígeno" hace referencia a una entidad o fragmento de él que puede inducir una repuesta inmune en un organismo, en particular de un animal, de forma más particular de un mamífero, incluso un ser humano. El término incluye inmunógenos y regiones responsables de la antigenicidad o de los determinantes antigénicos.

De la forma empleada en la presente, el término "soluble" significa disuelto en forma parcial o total en una solución acuosa.

Asimismo, de la forma empleada en la presente, el término "inmunogénico" hace referencia a sustancias que suscitan o mejoran la producción de anticuerpos, células T y otras células inmunes reactivas dirigidas contra un agente inmunogénico y contribuyen a una respuesta inmune en seres humanos o en animales.

Una respuesta inmune se presenta cuando un individuo produce suficientes anticuerpos, células T y otras células inmunes reactivas contra composiciones inmunogénicas administradas de la presente invención para moderar o mitigar el trastorno a ser tratado.

El término "hibridoma" es reconocido en la técnica y los expertos en la misma entienden que hace referencia a una célula producida por la fusión de una célula que produce anticuerpos y una célula inmortal, por ejemplo, una célula de mieloma múltiple. Esta célula híbrida es capaz de producir un suministro continuo de anticuerpos. Referirse a la definición de "anticuerpo monoclonal" anterior y a

los Ejemplos que aparecen más abajo para una descripción más detallada del método de fusión.

El término "portador", de la forma utilizada en la presente, significa una estructura a la que puede incorporarse un péptido antigénico o constructo supramolecular o puede estar asociado, lo cual presenta o expone péptidos antigénicos o parte del péptido al sistema inmune de un ser humano o de un animal. Cualquier partícula que pueda ser utilizada en forma adecuada en terapia animal o humana como ser, por ejemplo, una vesícula, una partícula o un cuerpo particulado puede ser empleada como portador dentro del contexto de la presente invención.

El término "portador" comprende, además, métodos de administración donde las composiciones de constructo antigénico supramolecular que comprenden el péptido antigénico pueden ser transportadas a sitios convenientes mediante mecanismos de administración. Un ejemplo de un sistema de administración como este utiliza metales coloidales como ser oro coloidal.

Entre las proteínas portadoras que pueden ser empleadas en las composiciones de constructo antigénico supramolecular de la presente invención se incluyen, pero sin que la enumeración sea taxativa, el péptido de unión de maltosa "MBP", albúmina de suero bovino "BSA", hemocianina de lapa "KLH", ovoalbúmina, flagelina, tiroglobulina, albúmina de suero de cualquier especie, gamaglobulina de cualquier especie, células singeneicas que transportan antígenos Ia y polímeros de aminoácidos D y/o L.

Además, la expresión "cantidad terapéuticamente efectiva" o "cantidad farmacéuticamente efectiva" hacen referencia a la cantidad de péptido de unión que, cuando es administrada a un ser humano o a un animal, es suficiente como para que de como resultado un efecto terapéutico en dicho ser humano o animal.

La cantidad efectiva es fácilmente determinada por los expertos en la técnica que sigan los procedimientos de rutina.

Las expresiones “pTau PHF”, “PHF” y "filamentos helicoidales pareados" son empleadas en la presente como sinónimas y hacen referencia a pares de filamentos de alrededor de 10 nm enrollados en hélices con una periodicidad de 160 nm visible en microscopio de electrones. El ancho varía entre 10 y 22 nm. Los PHF son estructuras predominantes en los ovillos neurofibrilares de la enfermedad de Alzheimer (EA) y en hilos de neurópilo. Los PHF también pueden verse en algunas, pero no en todas, neuritas distróficas asociadas con placas neuríticas. El componente principal de los PHF es una forma hiperfosforilada de proteína tau asociada a microtúbulos. Los PHF están compuestos por proteínas tau hiperfosforiladas antiparalelas unidas a disulfuro. Tau PHF puede ser separado de sus 20 residuos de aminoácidos de C–terminal. Los mecanismos subyacentes a la formación de PHF son inciertos pero la hiperfosforilación de tau puede desengancharla de los microtúbulos, lo que aumenta el grupo soluble de tau.

Dentro del alcance de la presente invención, se ha demostrado que la respuesta inducida por el anticuerpo a la composición antigénica de acuerdo con la invención es ampliamente independiente de la célula T. Se utilizó un modelo de ratón desnudo en esta cuestión y se vacunaron ratones desnudos y se midieron las respuestas del anticuerpo para evaluar la respuesta del anticuerpo específico de Aβ inducida por la composición antigénica de acuerdo con la invención en los ratones desnudos inmunizados. Los ratones desnudos llevan la mutación Foxn1nu y como consecuencia, presentan una función reducida de célula T debido a la falta de un timo adecuado.

La expresión "cantidad farmacéuticamente efectiva", de la forma empleada en la presente, hace referencia a una dosis del ingrediente activo en una

composición farmacéutica adecuada para la cura, o al menos para la detención parcial de los síntomas de la enfermedad, trastorno o estado a ser tratado o cualquier complicación asociada con ellos.

La presente invención provee péptidos de unión que reconocen y se unen a fosfoepítopes patológicos mayores de la proteína tau. En particular, la presente invención provee anticuerpos específicos contra fosfoepítopes lineales y conformacionales, simples y complejos, en proteína tau que son considerados responsables de la sinaptotoxicidad y neurotoxicidad en tauopatías, incluso en EA.

En consecuencia, la presente invención hace referencia en una forma de realización a un péptido de unión o una parte funcional de él, en particular a un anticuerpo, en particular a un anticuerpo monoclonal o una parte funcional de él, cuyo péptido de unión o anticuerpo reconoce y se une específicamente a un fosfoepítope en un mamífero, en particular en la proteína tau de un ser humano o en un fragmento de ella, en particular a un confórmero de proteína tau patológica, pero, en una forma de realización, no se une al epítope no fosforilado correspondiente y/o a epítopes no relacionados, donde dicho péptido de unión o anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 10 nM, en particular de al menos 8 nM, en particular de al menos 5 nM, en particular de al menos 2 nM, en particular de al menos 1 nM, en particular de al menos 500 pM, en particular de al menos 400 pM, en particular de al menos 300 pM, en particular de al menos 200 pM, en particular de al menos 100 pM, en particular de al menos 50 pM.

Proteína “tau soluble” tal como se usa en la presente se refiere a proteínas que consisten en ambos monómeros de proteína tau/péptido completamente solubilizados o de péptidos/proteínas tipo tau, o de péptidos/proteínas tau modificadas o truncadas o de otros derivados de monómeros de

péptidos/proteínas tau, y de oligómeros de proteína tau. “Tau soluble” excluye ovillos particularmente neurofibrilares (NFT).

Proteína “tau insoluble” tal como se usa en la presente se refiere a múltiples monómeros agregados de péptidos o proteínas tau o de péptidos/proteínas tipo tau, o de péptidos/proteínas tau modificadas o truncadas o de otros derivados de péptidos/proteínas tau que forman estructuras oligoméricas o poliméricas que son insolubles tanto *in vitro* en medio acuoso como *in vivo* en el organismo mamífero o humano, más en particular en el cerebro, pero en particular a múltiples monómeros agregados de tau o de péptidos/proteínas tau modificados o truncados o de sus derivados que son insolubles en el organismo mamífero o humano, más en particular en el cerebro, respectivamente. “Tal insoluble” incluye en particular ovillos neurofibrilares (NFT).

“Tau monomérico” o “monómero tau” tal como se usa en la presente se refiere a proteínas tau completamente solubilizadas sin complejos agregados en medio acuoso.

“Tau agregado”, “tau oligomérico” y “oligómero tau” se refieren a múltiples monómeros agregados de péptidos o proteínas tau o de péptidos/proteínas tipo tau, o de péptidos/proteínas tau modificados o truncados o de otros derivados de péptidos/proteínas tau que forman estructuras oligoméricas o poliméricas que son insolubles o solubles tanto en medio acuoso in vitro como in vivo en el organismo mamífero o humano, más en particular en el cerebro, pero en particular a múltiples monómeros agregados de tau o de péptidos/proteínas tau modificados o truncados o de sus derivados que son insolubles o solubles en el organismo mamífero o humano, más en particular en el cerebro, respectivamente.

En una forma de realización, la presente invención provee una composición farmacéutica que comprende un péptido de unión o una parte funcional de él, en particular un anticuerpo, en particular un anticuerpo monoclonal o una parte

funcional de él, o un polinucleótido que comprende una secuencia de ácido nucleico que codifica dicho péptido de unión o anticuerpo, de acuerdo con una cualquiera de las formas de realización descritas y reivindicadas en la presente, o una combinación de ellos, en una cantidad terapéuticamente efectiva junto con un portador farmacéuticamente aceptable.

Los portadores, diluyentes y/o excipientes farmacéuticos adecuados resultan conocidos en la técnica e incluyen, por ejemplo, soluciones salinas tamponadas con fosfato, agua, emulsiones tales como emulsiones de aceite/agua, diversos tipos de agentes humectantes, soluciones estériles, etc.

Los péptidos de unión de acuerdo con la invención que incluyen anticuerpos, particularmente anticuerpos monoclonales y sus fragmentos activos pueden ser preparados en una formulación fisiológicamente aceptable y pueden comprender un portador, diluyente y/o excipiente farmacéuticamente aceptable mediante el uso de técnicas conocidas. Por ejemplo, los péptidos de unión de acuerdo con la invención y de acuerdo con lo descripto en la presente que incluyen cualquier péptido de unión funcionalmente equivalente o partes funcionales de él, en particular los anticuerpos monoclonales de la invención que incluyen cualquier anticuerpo funcionalmente equivalente o partes funcionales de él, son combinados con un portador, diluyente y/o excipiente farmacéuticamente aceptable para formar una composición terapéutica. Los portadores, diluyentes y/o excipientes farmacéuticos adecuados resultan conocidos en la técnica e incluyen, por ejemplo, soluciones salinas tamponadas con fosfato, agua, emulsiones tales como emulsiones de aceite/agua, diversos tipos de agentes humectantes, soluciones estériles, etc.

La formulación de la composición farmacéutica de acuerdo con la invención puede ser lograda de acuerdo con la metodología estándar conocida por los expertos en la técnica.

Las composiciones de la presente invención pueden ser administradas a un sujeto en forma de sólido, líquido o aerosol en una dosis farmacéuticamente efectiva adecuada. Entre los ejemplos de composiciones sólidas se incluyen píldoras, cremas y unidades de dosificación implantables. Las píldoras pueden ser administradas en forma oral. Las cremas terapéuticas pueden ser administradas en forma tópica. Las unidades de dosificación implantables pueden ser administradas en forma local, por ejemplo, en el lugar del tumor, o pueden ser implantadas para la liberación sistemática de la composición terapéutica, por ejemplo, en forma subcutánea. Entre los ejemplos de composiciones líquidas se incluyen formulaciones adaptadas para la inyección intramuscular, subcutánea, intravenosa, intraarterial y formulaciones para la administración tópica e intraocular. Entre los ejemplos de formulaciones en aerosol se incluyen las formulaciones para inhalar para ser administradas a los pulmones.

Las composiciones pueden ser administradas por vías de administración estandares. En general, la composición puede ser administrada por vía tópica, oral, rectal, nasal, intradérmica, intraperitoneal o parenteral (por ejemplo, intravenosa, subcutánea o intramuscular).

Además, la composición puede ser incorporada en matrices de liberación constante tales como polímeros biodegradables, donde los polímeros son implantados en las proximidades de la zona donde se desea realizar la administración, por ejemplo, en el sitio de un tumor. El método incluye la administración de una dosis única, la administración de dosis repetidas a intervalos de tiempo previamente determinados y la administración constante durante un período de tiempo previamente determinado.

De la forma utilizada en la presente, una matriz de liberación constante es una matriz hecha de materiales, por lo general de polímeros, que son degradables a través de hidrólisis enzimática o de ácido/base o por disolución. Una vez

introducida en el cuerpo, la matriz reacciona ante las enzimas y los fluidos corporales. La matriz de liberación constante es seleccionada de manera conveniente mediante los materiales biocompatibles tales como liposomas, poliláctidos (ácido poliláctido), poliglicólido (polímero de ácido glicólico), co–glicólido poliláctido (copolímeros de ácido láctico y ácido glicólico), polianhídridos, poli(orto)ésteres, polipéptidos, ácido hialurónico, colágeno, sulfato de condroitina, ácidos carboxílicos, ácidos grasos, fosfolípidos, polisacáridos, ácidos nucleicos, ácidos de poliamino, aminoácidos tales como fenilalanina, tirosina, isoleucina, polinucleótidos, polivinilpolipropileno, polivinilpirrolidona y silicona. Una matriz biodegradable de preferencia es una matriz de uno de los siguientes: poliláctido, poliglicólido o co–glicólido poliláctido (copolímeros de ácido láctico y ácido glicólico).

Los expertos en la técnica saben que la dosificación de la composición dependerá de diversos factores tales como, por ejemplo, la afección que se esté tratando, la composición en particular que se emplee y otros factores clínicos tales como el peso, el tamaño, el sexo y la salud general del paciente, la superficie corporal, el compuesto particular o la composición particular a ser administradas, otros fármacos que sean administrados en ese momento y la vía de administración.

La composición de acuerdo con la invención puede ser administrada en combinación con otras composiciones que comprenden una sustancia o compuesto biológicamente activo como ser, por ejemplo, un compuesto conocido empleado en la medicación de tauopatías y/o de amiloidosis, un grupo de enfermedades y trastornos asociados con una proteína amiloide o tipo amiloide como ser la proteína β–amiloide involucrada en la enfermedad de Alzheimer.

La otra sustancia o compuesto biológicamente activo puede ejercer su efecto biológico mediante el mismo mecanismo o uno similar al de la vacuna

terapéutica de acuerdo con la invención o mediante un mecanismo de acción no relacionado o mediante una pluralidad de mecanismos de acción relacionados y/o no relacionados.

En general, el otro compuesto biológicamente activo puede incluir mejoradores de la transmisión de neutrones, fármacos psicoterapéuticos, inhibidores de acetilcolina esterasa, bloqueadores de canales de calcio, aminas biogénicas, tranquilizantes de benzodiacepina, síntesis de acetilcolina, mejoradores de almacenamiento o liberación, agonistas de receptores postsinápticos de acetilcolina, inhibidores de oxidasa A o B de monoamina, antagonistas de receptores de N–metil–D–aspartato glutamato, fármacos antiinflamatorios no esteroides, antioxidantes y antagonistas de receptores serotonérgicos.

En particular, el agente o compuesto biológicamente activo puede comprender al menos un compuesto seleccionado entre el grupo que consiste en compuestos contra estrés oxidativo, compuestos antiapoptóticos, quelantes de metal, inhibidores de reparación de ADN como ser pirencepina y metabolitos, ácido 3–amino–1–propanosulfónico (3APS), 1,3–propanodisulfonato (1,3PDS), activadores de secretasa, inhibidores de [beta]– y 7–secretasa, proteínas tau, neurotransmisores, rompedores de lámina 3, moléculas antiinflamatorias, "antipsicóticos atípicos" tales como, por ejemplo, clozapina, ziprasidona, risperidona, aripiprazol u olanzapina, o inhibidores de colinesterasa (ChEIs) como ser tacrina, rivastigmina, donepezil, y/o galantamina y otros fármacos y suplementos nutritivos tales como, por ejemplo, vitamina B12, cisteína, un precursor de acetilcolina, lecitina, colina, ginkgo biloba, acetil–L–carnitina, idebenona, propentofilina, o un derivado de xantina, junto con un péptido de unión de acuerdo con la invención que incluye anticuerpos, particularmente anticuerpos monoclonales y fragmentos activos de ellos, y en forma opcional, un portador

farmacéuticamente aceptable y/o un diluyente y/o un excipiente e instrucciones para el tratamiento de enfermedades.

En otra forma de realización, la composición de acuerdo con la invención puede comprender niacina o memantina junto con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, y en forma opcional, un portador y/o un diluyente y/o un excipiente farmacéuticamente aceptable.

En aun otra forma de realización de la invención, las composiciones provistas comprenden "antipsicóticos atípicos" tales como, por ejemplo, clozapina, ziprasidona, risperidona, aripiprazol u olanzapina para el tratamiento de síntomas psicóticos positivos y negativos que incluyen alucinaciones, delirios, trastornos en el pensamiento (manifestados por una incoherencia marcada, descarrilamiento, tangencialidad), y comportamiento desorganizado o fuera de lo común, así como también anhedonia, afecto aplanado, apatía e insociabilidad, junto con el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, y en forma opcional, un portador y/o un diluyente y/o un excipiente farmacéuticamente aceptable.

Otros compuestos que pueden ser empleados en forma adecuada en composiciones además del péptido de unión de acuerdo con la invención, son aquellos divulgados por ejemplo en el documento WO 2004/058258 (referirse en especial a las páginas 16 y 17) que incluyen dianas de fármacos terapéuticos (páginas 36 a 39), ácidos alcanosulfónicos y ácido alcanolsulfúrico (páginas 39 a 51), inhibidores de colinesterasa (páginas 51 a 56), antagonistas de receptores de NMDA (páginas 56 a 58), estrógenos (páginas 58 a 59), fármacos antiinflamatorios no esteroides (páginas 60 a 61), antioxidantes (páginas 61 a 62), agonistas de receptores activados por proliferadores de peroxisoma (PPAR) (páginas 63 a 67), agentes reductores de colesterol (páginas 68-75); inhibidores

de amiloides (páginas 75-77), inhibidores de formación de amiloide (páginas 77 a 78), quelantes de metal (páginas 78 a 79), antipsicóticos y antidepresivos (páginas 80 a 82), suplementos dietarios (páginas 83 a 89) y compuestos que aumentan la disponibilidad de sustancias biológicamente activas en el cerebro (referirse a las páginas 89 a 93), y profármacos (páginas 93 y 94), cuyo documento es incorporado a la presente a modo de referencia, pero en especial los compuestos mencionados en las páginas indicadas con anterioridad.

La materia farmacéuticamente activa proteinácea puede estar presente en cantidades comprendidas entre 1 ng y 10 mg por dosis. En general, el régimen de administración debe estar entre 0,1 [mu]g y 10 mg del anticuerpo de acuerdo con la invención, en particular entre 1,0 [mu]g y 1,0 mg, y en forma más particular entre 1,0 [mu]g y 100 [mu]g, y todos los números individuales que se encuentren dentro de estos rangos también son parte de la invención. Si la administración se produce a través de una infusión continua, una dosis más adecuada puede encontrarse entre unidades de 0,01 [mu]g y 10 mg por kilo de masa corporal por hora, y todos los números individuales que se encuentren dentro de estos rangos también son parte de la invención.

En general, la administración será parenteral, por ejemplo, intravenosa. Las preparaciones para la administración parenteral incluyen soluciones estériles acuosas o no acuosas, suspensiones y emulsiones. Entre los solventes no acuosos se incluyen, sin que la enumeración sea taxativa, propilenglicol, polietilenglicol, aceite vegetal como ser aceite de oliva y ésteres orgánicos inyectables tales como oleato de etilo. Los solventes acuosos pueden seleccionarse entre el grupo que consiste en agua, alcohol/soluciones acuosas, emulsiones o suspensiones que incluyen medios salinos o tamponados. Entre los vehículos parenterales se incluyen solución de cloruro de sodio, dextrosa de Ringer, cloruro de sodio y dextrosa, Ringer lactato o aceites fijos. Entre los

vehículos intravenosos se incluyen reaprovisionantes de fluidos y nutrientes, reaprovisionantes de electrolitos (como aquellos basados en dextrosa de Ringer) y otros. Los conservantes también pueden estar presentes tales como, por ejemplo, agentes antimicrobianos, antioxidantes, quelantes, gases inertes, etc.

La composición farmacéutica puede comprender, además, portadores proteináceos tales como, por ejemplo, albúmina de suero o inmunoglobulina, en particular de origen humano. Otros agentes biológicamente activos pueden estar presentes en la composición farmacéutica de la invención según su uso pretendido.

Cuando el objetivo de unión está ubicado en el cerebro, ciertas formas de realización de la invención contemplan el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, para atravesar la barrera hematoencefálica. Determinadas enfermedades neurodegenerativas están asociadas con un aumento de permeabilidad de la barrera hematoencefálica, de manera que el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales o su fragmento activo puedan ser introducidos con facilidad en el cerebro. Cuando la barrera hematoencefálica permanece intacta, existen diversos enfoques conocidos en la técnica para transportar moléculas a través de ella, que incluyen, pero sin que la enumeración sea taxativa, métodos físicos, métodos basados en lípidos y métodos basados en los canales y en los receptores.

Los métodos físicos para transportar el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales, o su fragmento activo, a través de la barrera hematoencefálica incluyen, pero sin que la enumeración sea taxativa, sortear la barrera hematoencefálica por completo o mediante la creación de aberturas en la barrera hematoencefálica. Los métodos para rodearla incluyen, pero sin que la enumeración sea taxativa, la inyección directa en el cerebro (referirse, por ejemplo, a Papanastassiou et

al, *Gene Therapy* 9: 398–406 (2002)) y al implante de un dispositivo de administración en el cerebro (referirse, por ejemplo, a Gill et al., *Nature Med.* 9: 589–595 (2003); y a Gliadel Wafers(TM), Guildford Pharmaceutical). Los método para crear aberturas en la barrera incluyen, sin que la enumeración sea taxativa, ultrasonido (referirse, por ejemplo, a la publicación de patente estadounidense N.º 2002/0038086), presión osmótica (por ejemplo, por administración de manitol hipertónico ((Neuwelt, E. A., *Implication of the Blood–Brain Barrier and its Manipulation*, Vols 1 & 2, Plenum Press, N. Y. (1989)), permeabilización, por ejemplo, por bradiquinina o permeabilizante A–7 (referirse, por ejemplo a las patentes estadounidenses N.º 5.112.596, 5.268.164, 5.506.206 y 5.686.416), y transfección de neuronas que unen la barrera hematoencefálica con vectores que contienen genes que codifican el péptido de unión o fragmento que une al antígeno (referirse, por ejemplo, a la publicación de la patente estadounidense N.º 2003/0083299).

Los métodos de transporte basados en lípidos del péptido de unión de acuerdo con la invención que incluyen anticuerpos, en particular anticuerpos monoclonales o uno de sus fragmentos activos a través de la barrera hematoencefálica incluyen, sin que la enumeración sea taxativa, el encapsulamiento del péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales o su fragmento activo en liposomas que son acoplados a fragmentos activos de ellos que se unen a receptores en el endotelio vascular de la barrera hematoencefálica (referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º 2002/0025313), y el revestimiento del péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales o su fragmento activo en partículas de lipoproteína de baja densidad (referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º

2004/0204354) o apolipoproteína E (referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º 2004/0131692).

Los métodos basados en canales y receptores para transportar el péptido de unión de acuerdo con la invención que incluyen anticuerpos, en particular anticuerpos monoclonales, o su fragmento activo, a través de la barrera hematoencefálica incluyen, pero sin que la enumeración sea taxativa, el uso de bloqueadores de glucocorticoide para aumentar la permeabilidad de la barrera hematoencefálica (referirse, por ejemplo, a las publicaciones de solicitudes de patentes estadounidenses N.º 2002/0065259, 2003/0162695 y 2005/0124533); la activación de canales de potasio (referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º 2005/0089473), la inhibición de transportadores de fármacos ABC ((referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º 2003/0073713); el revestimiento de anticuerpos con una transferrina y la modulación de actividad de uno o más receptores de transferrina (referirse, por ejemplo, a la publicación de solicitud de patente estadounidense N.º 2003/0129186) y la cationización de los anticuerpos (referirse, por ejemplo, a la patente estadounidense N.º 5.004.697).

Se pueden proporcionar únicas o repetidas administraciones de péptido de unión de acuerdo con la invención que incluyen anticuerpos, en particular anticuerpos monoclonales, o uno de sus fragmentos activos, o de una composición farmacéutica de acuerdo con la invención a un sujeto durante un período de tiempo prolongado. La duración de la administración puede ser de entre 1 semana y de hasta 12 meses o más. Durante este tiempo, el péptido de unión, anticuerpo o composición farmacéutica se puede administrar una vez por semana, una vez cada dos semanas, tres semanas, cuatro semanas, etc. o con una frecuencia mayor o menor según las necesidades del sujeto por tratar.

En otra forma de realización, la presente invención provee métodos y kits para la detección y el diagnóstico de enfermedades, trastornos o estados asociados con la proteína tau, que incluyen enfermedades o trastornos neurodegenerativos tales como tauopatías que comprenden un grupo heterogéneo de enfermedades y trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, panencefalitis esclerosante subaguda, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica. Las anomalías patológicas pueden ser producidas por la formación de lesiones neurofibrilares o pueden estar asociadas con ellas, donde la patología cerebral predominante es la tauopatía.

Además, la presente invención provee métodos y kits para diagnosticar una predisposición a enfermedades, trastornos o estados asociados con la proteína tau, que incluyen enfermedades o trastornos neurodegenerativos tales como tauopatías que comprenden un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran

coexistencia de patologías tau y amiloides, o para monitorear una enfermedad residual mínima en un paciente o para predecir la capacidad de respuesta de un paciente ante un tratamiento con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, o una composición de acuerdo con la invención y según se la describe en la presente. Estos métodos incluyen métodos inmunológicos conocidos comúnmente empleados para detectar o cuantificar sustancias en muestras biológicas o en un estado *in situ*.

El diagnóstico de una enfermedad o estado asociado con la proteína tau o de una predisposición a una enfermedad o estado asociado con la proteína tau en un sujeto que necesita de ello, en particular un mamífero, en forma más particular un ser humano, que incluye enfermedades o trastornos neurodegenerativos tales como tauopatías que comprenden un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides, puede lograrse mediante la detección de la unión inmunoespecífica de un péptido de unión de la invención, en particular de un anticuerpo, en particular de un anticuerpo monoclonal o un fragmento activo de él, con un epítope de la proteína tau en una muestra o in situ, que incluye poner la muestra o una parte del cuerpo específica o zona del cuerpo que se sospecha contiene la proteína tau en contacto con un anticuerpo que una un epítope de la proteína tau, lo que permite que el anticuerpo se una a la proteína Tau para formar un complejo inmunológico, detectando la formación del complejo inmunológico y la correlación de la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área del cuerpo específica, donde, en forma opcional, se compara la cantidad del complejo inmunológico con un valor control normal, donde un aumento en la cantidad del complejo inmunológico comparado con un valor control normal indica

que el sujeto sufre de una enfermedad o estado asociado a la proteína tau o se encuentra en riesgo de desarrollarlo.

El monitoreo de la enfermedad residual mínima en un sujeto, en particular un mamífero, en forma más particular un ser humano, después del tratamiento con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, o una composición de acuerdo con la invención pueden ser logrados mediante la detección de la unión inmunoespecífica de un péptido de unión de la invención, en particular de un anticuerpo, en particular de un anticuerpo monoclonal o un fragmento activo de él, con un epítope de la proteína tau en una muestra o in situ, que incluye poner la muestra o una parte del cuerpo específica o zona del cuerpo que se sospecha contiene la proteína tau en contacto con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, para unir un epítope de la proteína tau, lo que permite que el péptido de unión de acuerdo con la invención, que incluye anticuerpos, en particular anticuerpos monoclonales y fragmentos activos de él, se unan con la proteína tau para formar un complejo inmunológico, donde se detecta la formación del complejo inmunológico y se logra la correlación de la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área del cuerpo específica, donde, en forma opcional, se compara la cantidad de dicho complejo inmunológico con un valor control normal, donde un aumento en la cantidad de dicho complejo inmunológico comparado con un valor control normal indica que el sujeto aún puede sufrir de una enfermedad residual mínima.

La predicción de la capacidad de respuesta de un sujeto, en particular un mamífero, en forma más particular un ser humano, a un tratamiento con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, o una composición

de acuerdo con la invención puede lograrse mediante la detección de la unión inmunoespecífica de un péptido de unión, en particular de un anticuerpo monoclonal o un fragmento activo de él, con un epítope de la proteína tau en una muestra o *in situ*, que incluye poner la muestra o una parte del cuerpo específica o zona del cuerpo que se sospecha contiene la proteína tau en contacto con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, que une un epítope de la proteína tau, lo que permite que el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales o sus fragmentos activos, se una con la proteína tau para formar un complejo inmunológico, donde se detecta la formación del complejo inmunológico y se logra la correlación de la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área del cuerpo específica, donde, en forma opcional, se compara la cantidad de dicho complejo inmunológico antes y después del inicio del tratamiento, donde una disminución en la cantidad de dicho complejo inmunológico indica que dicho paciente tiene una gran probabilidad de responder al tratamiento.

Las muestras biológicas que pueden emplearse en el diagnóstico de una enfermedad o estado asociado con la proteína tau, para diagnosticar una predisposición a una enfermedad o estado asociado co la proteína tau, que incluye enfermedades o trastornos neurodegenerativos tales como tauopatías que comprenden un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides, o para monitorear una enfermedad residual mínima en un paciente o para predecir la capacidad de respuesta de un paciente ante un tratamiento con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos

activos, o una composición de acuerdo con la invención y según se la describe en la presente, por ejemplo, fluidos tales como suero, plasma, saliva, secreciones gástricas, moco, fluido cerebroespinal, fluido linfático y similares o muestras de tejido o células obtenidas de un organismo como ser tejido neural, cerebral, cardíaco o vascular. Para determinar la presencia o ausencia de la proteína tau en una muestra, puede emplearse cualquier inmunoensayo conocido para los expertos en la técnica como ser, por ejemplo, ensayos que utilicen métodos de detección indirecta con reactivos secundarios para su detección, ensayos de aglutinación e inmunoprecipitación y ELISA. Una descripción detallada de estos ensayos se encuentra, por ejemplo, en Harlow y Lane, *Antibodies: A Laboratory Manual* (*Cold Spring Harbor Laboratory*, Nueva York 1988 555–612, WO96/13590 de Maertens y Stuyver, Zrein et al. (1998) y WO96/29605.

Para un diagnóstico *in situ*, el péptido de unión de acuerdo con la invención incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, de la invención o cualquier parte activa y funcional de él puede ser administrado al organismo a ser diagnosticado a través de métodos conocidos en la técnica como ser, por ejemplo, inyección intravenosa, intranasal, intraperitoneal, intracerebral, intraarterial de modo que pueda ocurrir una unión específica entre un anticuerpo de acuerdo con la invención con una región eptitópica en la proteína amiloide. El complejo péptido de unión /antígeno puede ser detectado en forma conveniente a través de una etiqueta unida al péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales, o un fragmento funcional de él, o cualquier otro método de detección conocido en la técnica.

Los inmunoensayos empleados en aplicaciones de diagnóstico o en aplicaciones para diagnosticar una predisposición a una enfermedad o estado asociado con la proteína tau, que incluyen enfermedades o trastornos

neurodegenerativos tales como tauopatías que comprenden un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides, o para monitorear una enfermedad residual mínima en un paciente o para predecir la capacidad de respuesta de un paciente ante un tratamiento con un péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, o una composición de acuerdo con la invención y según se la describe en la presente, típicamente se basan en antígenos etiquetados, péptidos de unión o reactivos secundarios para su detección. Estas proteínas o reactivos pueden ser etiquetados con compuestos que en general son conocidos para los expertos en la técnica, que incluyen enzimas, radioisótopos y sustancias fluorescentes, luminiscentes y cromogénicas que incluyen, pero sin que la enumeración sea taxativa, partículas coloreadas tales como cuentas de látex y oro coloidales. De estos, puede usarse el etiquetado radioactivo para casi todos los tipos de ensayos y con la mayoría de las variantes. Las etiquetas conjugadas por enzimas son en particular útiles cuando debe evitarse radioactividad o cuando se necesitan resultados rápidos. Aunque los fluorocromos requieren de un equipo costoso para su uso, proveen un método muy sensible para su detección. Los péptidos de unión útiles en estos ensayos son aquellos divulgados y reivindicados en la presente que incluyen anticuerpos, en particular anticuerpos monoclonales, anticuerpos policlonales y anticuerpos policlonales purificados por afinidad.

En forma alternativa, el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, puede ser etiquetado en forma indirecta mediante la reacción con sustancias etiquetadas que cuentan con una afinidad para la inmunoglobulina, como ser los anticuerpos secundarios o de proteína A o G. El péptido de unión de

acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, puede ser conjugado con una segunda sustancia y detectado con una tercera sustancia etiquetada que presenta una afinidad para la segunda sustancia conjugada al anticuerpo. Por ejemplo, el péptido de unión de acuerdo con la invención que incluye anticuerpos, en particular anticuerpos monoclonales y sus fragmentos activos, puede ser conjugado con biotina y el conjugado de biotina/péptido de unión detectado mediante avidina etiquetada o estreptavidina. De forma similar, el péptido de unión puede ser conjugado con un hapteno y el conjugado de hapteno/péptido de unión detectado mediante el uso de péptido de unión anti–hapteno etiquetado.

Los expertos en la técnica conocerán estas y otras etiquetas adecuadas que pueden ser empleadas de acuerdo con la presente invención. La unión de estas etiquetas a péptidos de unión o sus fragmentos puede ser lograda usando técnicas convencionales conocidas comúnmente por los expertos en la técnica. Las técnicas típicas son descriptas por Kennedy, J. H., et al., 1976 (*Clin. Chim. Acta* 70:1–31), y Schurs, A. H. W. M., et al. 1977 (*Clin. Chim Acta* 57:1–40). Las técnicas de acoplamiento mencionadas en estos últimos casos consisten en el método de glutaraldehído, el método de periodato, el método de dimaleimida y otros, y todos se incorporan a la presente a modo de referencia.

Los inmunoensayos actuales utilizan un método de anticuerpo doble para detectar la presencia de un analito, donde el anticuerpo es etiquetado en forma indirecta por su reactividad con un segundo anticuerpo que ha sido etiquetado con una etiqueta detectable. El segundo anticuerpo es, de preferencia, uno que se una con anticuerpos del animal del que deriva el anticuerpo monoclonal. En otras palabras, si el anticuerpo monoclonal es un anticuerpo de ratón, entonces el segundo anticuerpo etiquetado será un anticuerpo anti–ratón. Para el anticuerpo a ser usado en el ensayo descripto en la presente, esta etiqueta es, de preferencia,

una cuenta revestida con el anticuerpo, en particular, una cuenta magnética. Para el anticuerpo a ser usado en el inmunoensayo descripto en la presente, la etiqueta es, de preferencia, una molécula detectable como ser una sustancia radioactiva, fluorescente o electroquimiluminiscente.

Un sistema de anticuerpo doble alternativo, al que con frecuencia se hace referencia como sistemas de formato rápido porque están adaptados a determinaciones rápidas de la presencia de un analito, también puede ser empleado dentro del alcance de la presente invención. El sistema requiere de una alta afinidad entre el anticuerpo y el analito. De acuerdo con una forma de realización de la presente invención, la presencia de la proteína amiloide es determinada mediante el uso de un par de anticuerpos, cada uno específico para la proteína amiloide. Uno de dichos pares de anticuerpos es mencionado en la presente como un "anticuerpo detector" y el otro de dicho par de anticuerpos es mencionado en la presente como un "anticuerpo de captura". El anticuerpo monoclonal de la presente invención puede ser empleado como un anticuerpo de captura o bien, como un anticuerpo detector. El anticuerpo monoclonal de la presente invención también puede ser empleado como un anticuerpo de captura y detector, juntos en un único ensayo. Una forma de realización de la presente invención utiliza, de esta forma, el método tipo sándwich de anticuerpo doble para detectar la proteína amiloide en una muestra de fluido biológico. En este método, el analito (proteína amiloide) se encuentra entre el anticuerpo detector y el anticuerpo de captura, donde el anticuerpo de captura es inmovilizado en forma irreversible sobre un soporte sólido. El anticuerpo detector contendría una etiqueta detectable, con el fin de identificar la presencia del sándwich de anticuerpo–analito y así, la presencia del analito.

Entre las sustancias en fase sólida ilustrativas se encuentran, sin que la enumeración sea taxativa, placas de microtitulación, tubos de ensayo de

poliestireno, cuentas magnéticas, plásticas o de vidrio y platinas que son conocidas en el campo de los radioinmunoensayos e inmunoensayos de enzima. Los métodos para acoplar anticuerpos a fases sólidas también son conocidos para los expertos en la técnica. Últimamente, se ha empleado una cantidad de materiales porosos tales como nylon, nitrocelulosa, acetato de celulosa, fibras de vidrio y otros polímeros porosos como soportes sólidos.

La presente invención también hace referencia a un kit de diagnóstico para detectar la proteína tau en una muestra biológica que comprende una composición de acuerdo con lo definido con anterioridad. Además, la presente invención se refiere al kit de diagnóstico que se acaba de mencionar que, además de una composición de acuerdo con lo definido con anterioridad, también comprende un reactivo de detección de acuerdo con lo definido con anterioridad. La expresión "kit de diagnóstico" hace referencia, en general, a un kit de diagnóstico cualquiera conocido en la técnica. En forma más específica, esta última expresión hace referencia a un kit de diagnóstico de acuerdo con lo descripto en Zrein et al. (1998).

Otro objetivo de la presente invención es el de provee nuevas inmunosondas y kits de ensayo para la detección y diagnóstico de enfermedades y estados asociados con la proteína tau, que comprende péptidos de unión de acuerdo con la presente invención. Para las inmunosondas, los péptidos de unión están unidos en forma directa o indirecta a una molécula informante adecuada, por ejemplo, una enzima o un radionúclido. El kit de prueba incluye un recipiente que contiene uno o más péptidos de unión de acuerdo con la presente invención e instrucciones para el uso de los péptidos de unión con el fin de unirse a un antígeno de tau para formar un complejo inmunológico y detectar la formación del complejo inmunológico de manera que la presencia o ausencia del complejo inmunológico se correlacione con la presencia o ausencia de la proteína tau.

EJEMPLOS

Ejemplo 1: Generación y detección selectiva de hibridomas y anticuerpos

El objetivo de este estudio fue el de generar y detectar en forma selectiva mAbs anti–tau (anticuerpos monoclonales). Se generaron hibridomas por fusión en un bazo de ratón inmunizado con vacuna de tau con una línea celular de mieloma. Se evaluaron los hibridomas para comprobar su capacidad de reacción con relación a la proteína tau de longitud completa fosforilada y no fosforilada, así como también los péptidos antigénicos de tau fosforilados y no fosforilados empleados en la preparación de la vacuna. Asimismo, se llevó a cabo una detección selectiva de hibridoma para comprobar la capacidad de reacción del sobrenadante de hibridomas respecto de los ovillos de tau mediante el uso de inmunoquímica en cortes de cerebro de ratón transgénico tau.

1.1 Métodos

1.1.1 Fusión

Se usó un ratón C57BL/6 de tipo salvaje vacunado con ACI–33 (Tau5–20 [pY18] para la producción de hibridoma. El ratón recibió un refuerzo con la vacuna ACI–33 el día 0 y nuevamente en el día 4 y se llevó a cabo la fusión el día 7. Se fundieron $173x10^6$ (ACI–33) esplenocitos a partir del ratón inmunizado con células de mieloma SP2–O–Ag14 a una razón de 5 esplenocitos por cada célula de mieloma.

Se usó un ratón C57BL/6 de tipo salvaje vacunado con ACI–35 (Tau5–408 [PS396, pS404] para la producción de hibridoma. El ratón recibió un refuerzo con la vacuna ACI–35 el día 0 y nuevamente en el día 4 y se llevó a cabo la fusión el día (sic) se fundieron (sic) 6×10^7 (ACI–35) esplenocitos a partir del ratón inmunizado con células de mieloma 2×10^7 SP2–O–Ag14 a una razón de 3 esplenocitos por cada célula de mieloma.

Se usó un ratón C57BL/6 de tipo salvaje vacunado con ACI–36 (Tau401–418 [pS404/S409]) para la producción de hibridoma. El ratón recibió un refuerzo con la vacuna ACI–36 el día 0 y nuevamente en el día 4 y se llevó a cabo la fusión el día 7. Se fundieron $84x10^6$ esplenocitos a partir del ratón inmunizado con células de mieloma SP2–O–Ag14 a una razón de 5 esplenocitos por cada célula de mieloma.

Se usó un ratón C57BL/6 de tipo salvaje vacunado con ACI–41 (combinación de Tau206–221 [pT212/pS214] y Tau196–211 [pS202/pT205]) para la producción de hibridoma. El ratón recibió un refuerzo con la vacuna ACI–41 el día 0 y nuevamente en el día 4 y se llevó a cabo la fusión el día 8. Se fundieron 162x106 esplenocitos a partir del ratón inmunizado con células de mieloma SP2–O–Ag14 a una razón de 5 esplenocitos por cada célula de mieloma.

Las cuatro fusiones dieron como resultado 8 x 96 placas de cavidades y los clones fueron denominados de acuerdo con la placa (del 1 al 8), luego con la hilera (de A a G) y por último, con la columna (de 1 a 12).

1.1.2 Método de detección selectiva de clones

En primer lugar, se evaluaron las 8 x 96 placas de cavidades dos veces para comprobar la expresión de IgG. Luego, se transfirieron los clones de expresión positiva en 24 placas de cavidades y se testearon los sobrenadantes celulares (= clones) de células en desarrollo en una prueba ELISA tau y una detección selectiva TAUPIR de inmunohistoquímica. Los sobrenadantes positivos en ELISA y/o TAUPIR fueron transferidos a T25 matraces y los clones fueron nuevamente sometidos a detección selectiva para comprobar la expresión de IgG en la prueba ELISA aTau y la detección selectiva TAUPIR.

1.1.3 Detección selectiva de IgG

Se revistieron placas de ELISA con 50 ul/cavidad de anticuerpo IgG antirratón (CER Groupe, Maloie, Bélgica) en un tampón de revestimiento durante

16 horas a una temperatura de 4 ºC. Después de lavar las placas con PBS/Tween se aplicaron 100 ul/cavidad de una solución bloqueante durante 1 hora a temperatura ambiente. Se incubaron 50 ul de sobrenadante de hibridoma sin diluir durante 1 hora a temperatura ambiente. Después del paso del lavado, se aplicó una combinación de IgG1, IgG2a, IgG2b e IgG3 antirratón conjugado con peroxidasa de rábano picante (HRP) (Ab Serotec, Raleigh, NC, EE. UU.) en las placas durante 1 hora a temperatura ambiente. Después de un lavado final, se llevó a cabo la detección con TMB (3–3',5,5'–tetrametilbencidina), el sustrato de fosfatasa para HRP y se leyeron las placas a 405 nm mediante el uso de un lector de placas ELISA. Los resultados son expresados como densidad óptica (DO).

1.1.4 Detección selectiva de ELISA de hibridomas tau

Se llevó a cabo una prueba ELISA de hibridomas en péptido pTau (ACI–33, T1.5: Tau5–20 [pY18]; ACI–35, T3.5: Tau393–408[pS396/pS404]; ACI–36, T4.5: Tau401–418 [pS404/S409]; ACI–41, T8.5: Tau206–221 [pT212/pS214] y T9.5: Tau196–211 [pS202/pT205] PolyPeptide Laboratories, Hillerød, Dinamarca), correspondientes al péptido tau (ACI–33, T1.6: Tau5–20; ACI–36, T4.6: Tau401–4; ACI–41, T8.6: Tau206–221 y T9.6: Tau196–211, PolyPeptide Laboratories, Hillerød, Dinamarca), proteína tau de longitud completa fosforilada (441aa) (*pTau protein*, Vandebroek et al., 2005) y proteína tau de longitud completa (441aa) (*Tau protein*, SignalChem, Richmond, Canadá). Por último, se usó albúmina de suero bovino (BSA) como control negativo.

Se revistieron placas con 10 µg/ml del péptido tau correspondiente y 1 µg/ml de la proteína tau correspondiente durante la noche a 4 ºC. Después de lavar cada cavidad con PBS al 0,05% Tween 20 y de bloquear con BSA al 1% en PBS al 0,05% de Tween 20, se agregaron el sobrenadante de hibridoma sin diluir o control negativo de medio a las placas y se las incubó a 37 ºC durante 2 horas. Después del lavado, se incubaron las placas con un anticuerpo total de IgG

antirratón conjugado con fosfatasa (AP) (Jackson Laboratories, Baltimore, PA, EE. UU.) durante 2 horas a 37 ºC. Después del lavado, se incubaron las placas con pNPP (paranitrofenilfosfato), el sustrato de fosfatasa para AP y se leyeron las placas a 405 nm mediante el uso de un lector de placas ELISA. Los resultados son expresados como densidad óptica (DO).

1.1.5 Detección selectiva de hibridomas IHC: unión de anticuerpos anti–tau con ovillos en secciones de cerebro de ratones transgénicos (TAUPIR)

Los experimentos TAUPIR fueron realizados de acuerdo con el protocolo del Ejemplo 3.1.2.

1.1.6 Detección selectiva de IgG de matraces T25

Se revistieron placas de ELISA con 5 ug/ml de anticuerpo de fragmento específico IgG F(ab')2 antirratón (Jackson Laboratories, Baltimore, PA, EE. UU.) en un tampón de revestimiento de carbonato–bicarbonato con un pH de 9,6 (Sigma, Buchs, Suiza) durante la noche a 4 ºC. Después de lavar las placas, se incubaron el sobrenadante de hibridoma sin diluir, el anticuerpo IgG de control positivo (6E10 a 1ug/ml: Covance, Emeryville, CA, EE. UU.) o de control negativo (medio de cultivo solo) durante 1 hora a temperatura ambiente. Después del paso del lavado, se incubó el anticuerpo de fragmento específico Fcγ de IgG antirratón de cabra conjugado con AP secundario (subclases 1+2a+2b+3) (Jackson Laboratories, Baltimore, PA, EE. UU.) en placas durante 2 horas a 37 ºC. Después del lavado final, se llevó a cabo la detección con pNPP (paranitrofenilfosfato), el sustrato de fosfatasa para AP y se leyeron las placas a 405 nm mediante el uso de un lector de placas ELISA. Los resultados son expresados como densidad óptica (DO).

1.2 Resultados

1.2.1 Hibridomas ACI–33

Se sometieron a detección selectiva los sobrenadantes celulares de las 8 x 96 placas de cavidades resultantes de la fusión para la producción de IgG. En las 768 placas (8 x 96 placas), 277 placas testeadas resultaron positivas respecto de la expresión de IgG y fueron transferidas a 24 placas de cavidades. En las 24 placas de cavidades, se hizo desarrollar 79 clones y se analizó el sobrenadante a partir de esas células. Los clones positivos fueron transferidos además en matraces T25 y los sobrenadantes fueron sometidos a detección selectiva para la producción de IgG, ELISA y TAUPIR (Tabla 2).

El clon 6C10 fue el único positivo en las 3 detecciones selectivas y fue seleccionado por subclonación.

1.2.2 Hibridomas ACI–36

Se sometieron a detección selectiva los sobrenadantes celulares de las 8 x 96 placas de cavidades resultantes de la fusión para la producción de IgG. En las 768 placas (8 x 96 placas), 333 placas testeadas resultaron positivas respecto de la expresión de IgG y fueron transferidas a 24 placas de cavidades. En las 24 placas de cavidades, se hizo desarrollar 75 clones y se analizó el sobrenadante a partir de esas células. Los clones positivos fueron transferidos además en matraces T25 y los sobrenadantes fueron sometidos a detección selectiva para la producción de IgG, ELISA y TAUPIR (Tabla 3).

Con el fin de seleccionar clones para los siguientes pasos, se llevó a cabo una clasificación de todos los sobrenadantes positivos para detecciones selectivas de IgG/ELISA/TAUPIR sobre la base de los resultados de ELISA y TAUPIR. La clasificación de los resultados de ELISA y TAUPIR fue llevada a cabo de acuerdo con lo explicado en la sección de métodos. La tinción TAUPIR fue casi idéntica para los cinco clones primeros y esto correspondió a los resultados de ELISA. Se descartó 4C12 dado que fue encontrado en la misma placa que 4C1, lo que aumentó la probabilidad de que los 2 clones fueran los mismos (reconociendo

el mismo epítope). Los mejores 4 clones seleccionados fueron 3A8, 2B6, 4C1 y 6H1. Los otros 6 clones (4C12, 2G1, 2F9, 7D6, 3B9, 4E12) fueron conservados como soporte.

Se llevó a cabo una clasificación de los 10 clones que mostraron positividad en la prueba ELISA y TAUPIR para seleccionar los mejores (Tabla 4). Los resaltados en gris son los mejores 5 clones.

1.2.3 Hibridomas ACI–41

Se sometieron a detección selectiva los sobrenadantes celulares de las 8 x 96 placas de cavidades resultantes de la fusión para la producción de IgG. En las 768 placas (8 x 96 placas), 215 placas testeadas resultaron positivas respecto de la expresión de IgG y fueron transferidas a 24 placas de cavidades. En las 24 placas de cavidades, se hizo desarrollar 81 clones y se analizó el sobrenadante a partir de esas células. Los clones positivos fueron transferidos además en matraces T25 y los sobrenadantes fueron sometidos a detección selectiva para la producción de IgG, ELISA y TAUPIR (Tabla 5).

Los clones 5D10 y 7C2 fueron los únicos positivos en las 3 detecciones selectivas y fueron seleccionados por subclonación. El clon 5D10 une solamente el péptido T8.5 mientras que el clon 7C2 une los dos péptidos de la vacuna ACI–41 (T8.5 y T9.5) (referirse a la Figura 10 en la solicitud de patente de PCT (PCT/EP2010/054418).

El subclón 5D10A4 que se origina a partir de 5D10 era específico para el péptido p–tau.

1.3. Conclusión

Los anticuerpos generados han mostrado una alta especificidad a los péptidos pTau con solo una unión marginal con los péptidos no fosforilados.

A partir de 4 fusiones (ACI–33, ACI–36, ACI–35 y ACI–41), se depositó un total de 16 clones en DSMZ (Tabla 1) y fueron seleccionados para una subclonación adicional.

Los clones madre positivos mencionados con anterioridad fueron cultivados en forma adicional en 96 placas de cavidades, luego en 24 placas de cavidades y por último, en matraces T25. En cada etapa, los sobrenadantes de los clones de hibridoma fueron sometidos a detección selectiva por ELISA, TAUPIR y transferencia Western.

Ejemplo 2: Clonación de regiones variables de cadena ligera y pesada de anticuerpos

Los genes de región variable pesada y ligera de anticuerpos a partir de células de hibridoma son clonados y las secuencias de ADN y la ubicación de las regiones que determinan complementariedad (CDRs) son determinadas así como también las características de unión de los anticuerpos.

Se preparó ARN total a partir de 3 x 10^6 células de hibridoma (1 vial) mediante el uso del *Qiagen RNeasy mini kit* (Cat No: 74104). Se eluyó ARN en 50 mL de agua y se lo verificó en un gel de agarosa al 1,2%.

Se prepararon cADNs V_H y V_K mediante el uso de transcriptasa inversa con cebadores de región constante IgG y kappa. Se ampliaron las primeras cadenas de cADNs mediante por PCR mediante el uso de un gran conjunto de cebadores de secuencia de señal. Los ADNs amplificados fueron purificados con gel y clonados en el vector pGem® T Easy (Promega). Los clones V_H y V_K obtenidos fueron evaluados para comprobar los insertos del tamaño esperado. La secuencia de ADN de clones seleccionados fue determinada en ambas direcciones por secuenciación de ADN automatizada. Las ubicaciones de las regiones que determinan complementariedad (CDRs) en las secuencias fueron determinadas con referencia a otras secuencias de anticuerpo (Kabat EA *et al.*, 1991).

Ejemplo 3: Estudios de unión I

El objetivo fue medir la unión de fosfo–tau (pTau) de los anticuerpos generados a partir de hibridomas subclonados derivados de ratones inmunizados con las vacunas liposomales tau. Para evaluar esto, se utilizó un ensayo de inmunoabsorción unida a enzimas (ELISA) para medir la unión de los anticuerpos purificados tanto en la proteína tau de longitud completa fosforilada y no fosforilada, así como también los péptidos antigénicos de tau fosforilados y no fosforilados empleados en la preparación de la vacuna liposomal.

La detección selectiva fue completada a través de otros dos métodos. Se realizó inmunohistoquímica (IHC) en secciones de cerebro de una animal transgénico tau (TAUPIR) mediante el uso de un anticuerpo anti–tau como el anticuerpo primario. En forma adicional, se llevó a cabo una transferencia Western (WB) en homogenatos de proteína de cerebro de ratones transgénicos tau, mediante el uso de un anticuerpo anti–tau como anticuerpo de transferencia.

3.1 Métodos

3.1.1 Ensayo de unión fosfo–tau

Se generaron los anticuerpos anti–fosfo tau (isotipo IgG3 de ratón) de ratones vacunados con tau liposomal. Las vacunas liposomales eran preparaciones fosforiladas de un péptido fosfo–tau (p–tau). Los subclones de hibridoma que producen los anticuerpos anti–tau fueron seleccionados mediante la limitación de la dilución de los clones madre. Se realizó isotipificación para indicar la presencia de un clon de un solo isotipo. Los anticuerpos fueron producidos en frascos rotativos, purificados por cromatografía de afinidad, sometidos a filtración estéril a 0,22 µm, y cuantificados. Para evaluar la unión del anticuerpo de tau y p–tau, se usó un ensayo ELISA. En resumen, se revistieron 96 placas de cavidades Nunc MaxiSorp (Nunc, Roskilde, Dinamarca) con 1 µg/mL de proteína tau de longitud completa (441 aa) (SignalChem, Richmond, Canadá) o

proteína tau fosforilada de longitud completa (441 aa) (Vandebroek et al., 2005). En forma adicional, se revistieron placas con 10 µg/mL de péptido derivado de tau. Para realizar una prueba de capacidad de reacción cruzada en secuencias de tau y p–tau que no fueron usadas en la preparación de la vacuna, se revistieron placas con 10 µg/mL de los siguientes péptidos: Tau5–20 (fosforilado y no fosforilado en Y18), Tau393–408 (fosforilado y no fosforilado en S396 y S404), Tau401–418 (fosforilado y no fosforilado en S404 y S409), Tau206–221 (fosforilado y no fosforilado en T212 y S214) y Tau196–211 (fosforilado y no fosforilado en S202 y T205). El revestimiento fue realizado durante la noche en solución salina tamponada con fosfato (PBS) a 4 ºC. Se lavaron las placas con cuidado con 0,05% de Tween 20/PBS y luego fueron bloqueadas con 1% de albúmina de suero bovino (BSA) en 0,05% de Tween 20/PBS durante 1 hora a 37 ºC. Luego, se agregó el anticuerpo que estaba siendo sometido a prueba en una serie de dilución de 8 a 16 dos veces entre 20 y 0 µg/mL, y se lo dejo incubar durante 2 horas a 37 ºC. Luego, se lavaron las placas de acuerdo con lo descripto con anterioridad y se agregó anticuerpo secundario IgG antirratón conjugado con AP (Jackson ImmunoResearch Laboratories, Suffolk, Inglaterra) en una dilución de 1/6000 en 0,05% de Tween 20/PBS durante 2 horas a 37 ºC. Después del lavado, se incubaron las placas con una solución de fosfatasa de hexahidrato disódico de p–nitrofenilfosfato (pNPP; Sigma–Aldrich, Buchs, Suiza) y se leyeron las placas a 405 nm después de 2 a 16 horas de incubación mediante el uso de un lector de placas ELISA. Los resultados son expresados como densidad óptica (DO).

3.1.2 Unión de anticuerpos anti–tau con ovillos tau en secciones de cerebro de animales transgénicos tau (TAUPIR)

Se usaron cortes de cerebro de ratones transgénicos biGT doble transgénico adultos (de más de 18 meses de edad) (ratones transgénicos GSK–

3–beta cruzados con ratones TPLH, que contienen la isoforma más prolongada (441aa) de tau humana con la mutación P301L). En forma adicional, también se usaron secciones de ratones nuligénicos tau (TKO, de 6 meses de edad). Las secciones del cerebro fueron lavadas durante 5 minutos en PBS y luego se las incubó durante 15 minutos a temperatura ambiente en 1,5% de H_2O_2 en PBS:MeOH (1:1) para bloquear la actividad de peroxidasa endógena. Después de lavar las secciones 3 veces en PBST (PBS/0.1% TritonX100), fueron incubadas durante 30 minutos a temperatura ambiente en una solución de bloqueo de PBST + 10% de FCS (suero de ternero fetal). La incubación con el anticuerpo anti–tau que se sometió a prueba fue realizada durante la noche a 4 ºC en diluciones indicadas en PBST/10% FCS. Luego, se lavaron las secciones 3 veces en PBST antes de la incubación con anticuerpo secundario antirratón de cabra conjugado con HRP (adquirido en Dako, Glostrup, Dinamarca) en PBST/10% FCS durante 1 hora a temperatura ambiente. Antes de la detección, se lavaron las secciones 3 veces con PBST y se la incubó en 50 mM Tris/HCl con un pH de 7,6 durante 5 minutos. La detección se llevó a cabo mediante la incubación de las secciones durante 3 minutos en diaminobencidina (DAB: 1 comprimido en 10 ml de 50 mM Tris.HCl + 3 ul H_2O_2 30%; MP Biomedicals, Solon, OH, EE. UU.). La reacción fue detenida mediante el lavado de las secciones 3 veces en PBST. Luego, se transfirieron las secciones a placas silanizadas y secadas con aire sobre una placa calentada a 50 ºC durante 2 horas. El contraste de color fue realizado mediante el uso de incubación con hematoxilina Mayers (Fluka Chemie, Buchs, Suiza) durante 1 minuto, seguido de un paso de lavado durante 4 minutos bajo agua corriente. Se deshidrataron las secciones haciéndolas pasar por un baño al 50%, 70%, 90% y 100% de etanol (dos veces en este último caso), y luego dos veces por xilol durante 1 minuto. Por último, las secciones fueron colocadas sobre DePex (BDH Chemicals Ltd, Poole, Inglaterra) bajo una placa de vidrio.

Adicionalmente, se usaron sobrenadantes de hibridoma en dilución 1/10 (todos los anticuerpos derivados de ACI–35 mostrados en la Tabla 1) para secar membranas con proteínas de homogenados de cerebro separados por SDS–PAGE de ratones transgénicos tau, ratones de tipo salvaje o ratones knock–out tau.

3.1.3 Unión de anticuerpo anti–tau con ovillos tau en secciones de cerebro de pacientes con AD y tauopatías (TAUPIR)

El ensayo de inmunorreacción del anticuerpo anti–tau ACI–36–3A8–Ab1 a p–tau en el cerebro humano fue realizado por TAUPIR.

Las secciones de parafina del cerebro fueron desparafinizadas haciéndolas pasar por xilol 2 veces durante 5 minutos y 2 veces durante 1 minuto en 100% EtOH, seguido del lavado de 1 minuto en 90%, 70% y 50% de EtOH y agua destilada, seguido de 2 lavados de 5 minutos en PBS.

Para recuperar el antígeno, se trataron las secciones con calentamiento durante 10 minutos en solución 0,01 M de ácido cítrico en agua (con un pH de 6,0) y enfriamiento durante 20 minutos. Las secciones fueron incubadas durante 15 minutos a temperatura ambiente en 1,5% de H_2O_2 en PBS:MeOH (1:1) para bloquear la actividad de la peroxidasa endógena. Después de lavar las secciones 3 veces en PBST (PBS/0,05%Tween–20), estas fueron incubadas durante 30 minutos en PBST + suero de ternero fetal al 10% (FCS) como solución de bloqueo. La incubación con el anticuerpo anti–ptau primario ACI–36–3A8–Ab1 (410 ng/mL en tampón de bloqueo) fue realizada durante la noche a 4 ºC. Luego, se lavaron las secciones 3 veces en PBST antes de la incubación con anticuerpo secundario antirratón de cabra conjugado con HRP (Dako, Glostrup, Dinamarca) diluido en una proporción de 1 a 500 en PBST/10% FCS durante 1 hora a temperatura ambiente. Antes de la detección, se lavaron las secciones 3 veces con PBS y se las incubó en 50 mM Tris/HCl con un pH de 7,6 durante 5 minutos.

La detección fue realizada mediante la incubación de las secciones durante 3 minutos en diaminobencidina (DAB: 1 comprimido en 10 mL de 50 mM Tris–HCl + 3 µL H_2O_2 30%; MP Biomedicals, Solon, OH, EE. UU.). La reacción fue detenida mediante el lavado de las secciones 3 veces en PBS. El contraste de color fue realizado mediante el uso de incubación con hematoxilina Mayers (Fluka Chemie, Buchs, Suiza) durante 1 minuto, seguido de un paso de lavado durante 4 minutos bajo agua corriente. Se deshidrataron las secciones haciéndolas pasar por un baño al 50%, 70%, 90% y 100% de etanol (dos veces en este último caso), y luego dos veces por xilol durante 1 minuto.

Por último, las secciones fueron colocadas sobre DePex (BDH Chemicals Ltd, Poole, Inglaterra) bajo una placa de vidrio. Las secciones con tinción fueron examinadas por microscopía por luz blanca e imágenes digitales tomadas con una cámara 3CCD (Leica, Wetzlar, Alemania). Se tomaron las imágenes y se las analizó mediante el uso de un software dedicado (IM500, Leica). Las imágenes son mostradas con una aplicación de 20 x 1,6.

3.1.4 Transferencia Western (WB)

La unión del anticuerpo sometido a prueba a p–tau en el extracto de cerebro del animal transgénico fue realizada por WB. La homogenización del cerebro de ratones TKO, biGT, TPLH y FVB fue realizada en el tampón siguiente: 25 mM Tris/HCl pH7,6, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 30 mM NaF, 0,2 mM Na_3VO_4, 1 nM ácido ocádico, 1 mM fenilmetilsulfonilfluoruro (PMSF), 5 mM Na4P2O7, 1 comprimido cóctel de inhibidor de proteasa completo (CPIC) por cada 12 ml en total. Para obtener un homogenato de cerebro total, se homogenizó el cerebro en hielo en 1 vol/peso del hemisferio (ml/gr) con una mano de mortero de teflón/tubo de vidrio tipo alfarero accionado a motor a 700 rpm. Los homogenatos de cerebro totales fueron diluidos a la mitad en un tampón de muestra (125 mM Tris/HCl pH 6,8, 4% (p/v) sulfato de dodecilo de sodio (SDS),

20% glicerol, 0,01% azul bromofenol y 5% beta–mercapto–etanol), luego llevado con rapidez a 95 °C. Las muestras fueron mantenidas durante 5 minutos, diluidas a un cuarto en tampón de muestra, llevadas nuevamente a 95 ºC y luego enfriadas y agitadas a 14.000 rpm durante 5 minutos para aclarar todo resto que no hubiera sido solubilizado. Se recolectaron los sobrenadantes y se los cargó en gel de SDS–PAGE. La transferencia en membrana nitrocelulosa (Hybond–ECL) fue realizada en un tampón de transferencia (25 mM Tris pH 8,6, 190 mM glicina, 20% metanol). Las membranas fueron transformadas en la solución de bloqueo (0,1% de Tween de TBS (50 mM Tris.HCl, pH7,6, 150 mM NaCl y 5% de leche en polvo seca) antes de incubarlas durante la noche a 4 ºC con el anticuerpo de la prueba diluido en la solución de bloqueo. La incubación con anticuerpo secundario de antirratón de cabra conjugado con HRP (Dako, Glostrup, Dinamarca) diluido en una proporción de 1 a 10.000 en solución de bloqueo fue llevada a cabo a temperatura ambiente durante 1 hora. La detección se llevó a cabo mediante el uso de reactivos de detección por transferencia Western de ECl de GE Healthcare.

3.2 Resultados

3.2.1 Ensayo ELISA y TAUPIR usando secciones de cerebro en ratones tau transgénicos tau positivos en ovillo

Se midió la unión de anticuerpos en comparación con el péptido tau fosforilado usado como inmunógeno, y con la proteína humana tau de longitud completa, fosforilada. Se trata de isoforma más prolongada de proteína humana tau que consiste en 441 aminoácidos. El péptido no fosforilado correspondiente y la proteína humana tau de longitud completa también fueron incluidos. De acuerdo con lo indicado en la Tabla 6, los anticuerpos demostraron una alta capacidad de unión con el péptido tau fosforilado, con una unión limitada o sin unión alguna a la proteína humana tau de longitud completa fosforilada. No se

observó ninguna unión con el péptido tau no fosforilado correspondiente o con la proteína humana tau de longitud completa no fosforilada. Esto demuestra una alta capacidad de unión de anticuerpos anti–tau con los péptidos tau humana fosforilados.

Para evaluar la unión no específica respecto de otras secuencias tau fosforiladas y no fosforiladas, el anticuerpo fue sometido a una prueba para verificar la unión con cinco péptidos tau no fosfo y fosfo, uno de los cuales fue empleado como la secuencia de péptido de antígeno. No se observó ningún péptido tau fosfo o no fosfo con capacidad de reacción cruzada, salvo los péptidos usados en la vacuna, incluso a altas concentraciones de péptido.

La unión de anticuerpos anti–tau con p–tau en cerebros de ratones transgénicos tau fue evaluada por tinción de TAUPIR (Figura 1) y por WB (Figura 2). Los anticuerpos que demuestran unión con ovillos tau y cabezas de neurópilos están presentes en la corteza y el hipocampo de los cerebros de ratones transgénicos tau (biGT). Las diluciones de anticuerpo usadas para TAUPIR variaban de 0,05 a 0,0033 ug/mL. También se usaron anticuerpos anti–tau como un anticuerpo primario en una WB mediante el uso de homogenatos de cerebro totales de ratones TKO, biGT, TPHL y FVB de tipo salvaje y se separaron por SDS–PAGE. Se usaron dos anticuerpos anti–p–tau comerciales como controles, MC1 y Tau5. Todos los anticuerpos anti–tau unidos a pTau están presentes en los cerebros de ratones transgénicos tau.

Blotting

En membranas que contenían homogenatos de proteína separados por SDS–PAGE de ratones transgénicos tau, ratones de tipo salvaje y ratones knock–out tau, todos anticuerpos ACI–35 (revelado en la Tabla 1) se unieron a bandas de proteína que tenían idéntico patrón de migración de 46 kDa que Tau y pTau (datos no mostrados).

3.2.2 Estudio de TAUPIR en secciones de cerebro de pacientes con EA y tauopatías

La capacidad del anticuerpo ACI–36–3A8–Ab1 para unirse con agregados de tau alojados en secciones de cerebro humano de sujetos con tauopatías diagnosticadas, incluso EA, FAD, AGD, FTDP–17, CBD y PSP, fue examinada por inmunohistoquímica TAUPIR (Fgura 2). El anticuerpo anti–ptau ACI–36–3A8–Ab1 unido a p–tau que contiene ovillos neurofibrilares (NFTs), hilos de neurópilo en secciones de cerebro humano y otras formas de acumulaciones de p–tau presentes en neuronas y en tipos de célula glial. En forma más específica, NFTs con tinción prominente de ACI–36–3A8–Ab1, hilos de neurópilo y neuritos distróficos que rodean las placas amiloides en cerebros con EA, que resultó de fácil observación en los sujetos diagnosticados con EA y FAD. En secciones de cerebro de AGD, tanto NFTs con tinción de ACI–36–3A8–Ab1 y los hilos de neurópilo con múltiples gránulos/granos argirofílicos claramente visibles (Figura 2). La tinción de las secciones de cerebro de PSP con ACI–36–3A8–Ab1 mostraron NFTs, hilos de neurópilo y neuritos distróficos. En forma adicional, las inclusiones tipo cuerpo de Pick y los astrositos positivos p–tau en penacho fueron advertidos con claridad al ser una característica abundante en PSP, donde la tinción de p–tau se extiende a través de toda la célula, incluso en procesos distales. En FTDP–17, el patrón de tinción también ilustró la heterogeneidad conocida de la enfermedad, no solo con NFTs detectados sino también con neuronas "aglobadas" acromáticas. El anticuerpo ACI–36–3A8–Ab1 también coloreó neuronas acromáticas hinchadas que eras ligeramente tau positivas, característica principal de CBD. Otra característica patológica prominente de CBD, es decir las inclusiones oligodendrogliales, denominadas cuerpos en espiral, también fueron bien detectadas por el anticuerpo ACI–36–3A8–Ab1. No se

detectó ninguna tinción en un sujeto control AT8 negativo mientras que se identificó una tinción débil en un sujeto control AT8 positivo.

El uso de TAUPIR en secciones de cerebro humano de sujetos a los que previamente se les ha diagnosticado diferentes formas de tauopatía, el anticuerpo anti–ptau ACI–36–3A8–Ab1 demostró una buena unión con varias características patológicas ricas en p–tau presentes en los cerebros de estos sujetos.

Ejemplo 4: Estudios de unión II

El objetivo del estudio fue el de determinar la afinidad de unión entre los anticuerpos anti–tau y el péptido fosfo–tau mediante el uso de resonancia de plasmón superficial (SPR). El péptido fosfo–tau corresponde a la secuencia de péptido usada en la preparación de la vacuna para generar el anticuerpo anti–tau. Para estudiar esta interacción, se inmovilizaron fosfopéptidos en la superficie de un chip sensor y se monitoreó la unión en tiempo real utilizando SPR al hacer pasar el anticuerpo sobre el chip.

4.1 Métodos

4.1.1 Ensayo de unión por SPR

Todos los experimentos de SPR fueron llevados a cabo en un instrumento Biacore X (GE Healthcare). Los reactivos para la inmovilización (EDC, NHS y etanolamina), el chip sensor CM5 (carboximetildextrano) así como también el HBS–EP del tampón de corrida fueron adquiridos de GE Healthcare. Se solubilizaron péptidos fosfo–tau en PBS/tampón de acetato sódico (10 mM, con un pH de 5,0) en una relación de 1:1 vol/vol para dar una concentración de péptido final de 250 µg/ml. Esta solución de péptido fue luego acoplada a través de una célula de flujo (fc) 2 de un chip sensor CM5 que fue previamente activado mediante EDC/NHS. Después del acoplamiento, se pasó etanolamina sobre la superficie, lo que dio un nivel de inmovilización final de 218 RUs. Se sometieron a ensayo cinco concentraciones de los anticuerpos anti–tau mediante diluciones en

serie a través de un tampón de corrida. Se produjeron inyecciones comenzando por la concentración más baja y se pasaron por la fc 1 y 2 a una velocidad baja de 30 µL/min durante 180 segundos. La célula de flujo 1 fue no derivatizada y las respuestas fueron tomadas de fc 2 para corregir el ruido del instrumento y los cambios de refracción de masa. Una vez finalizada la inyección, se lavaron las superficies de inmediato con tampón de corrida durante 300 segundos. Para eliminar el anticuerpo unido restante del chip, se llevó a cabo una regeneración de superficie mediante la inyección de un pulso (típicamente de 3 µl) de NaoOH 8 mM en NaCl 1M con contenido de agua. Se realizó un análisis cinético mediante el uso de algoritmos para integración numérica y análisis global a través de BIAevaluation 3.0. Los sensogramas obtenidos para las inyecciones de anticuerpo en diferentes concentraciones superpuestos y las líneas de base ajustadas en cero. Para el ajuste de la curva, se adaptaron todos los datos en forma simultánea a un modelo homogéneo 1:1 (Langmuir).

De modo alternativo, se inmovilizó el péptido T3 biotinilado inmovilizado (T3.30) en un chip de estreptavidina Biacore SA (GE Healthcare) usando un instrumento Biacore X. Los anticuerpos se diluyeron en tampón de corrida de HBS–EP (GE Healthcare) y se inyectaron a 50 ul/min durante 120 s seguido de 100 s de disociación. La regeneración superficial se realizó usando un pulso (1–3 ul) de 16 mM de NaOH. El filtrado se realizó usando BIAevaluación y asumiendo una interacción de unión de Langmuir de 1:1.

Péptidos utilizados

T1.5	H–K(Ac)K(Ac)–RQEFEVMEDHAGTY[PO3H2]GL–K(Ac)K(Ac)–NH2	lot AW11309D
T4.5	H–K(Ac)K(Ac)–GDTS[PO3H2]PRHLS[PO3H2]NVSSTGSID–	lot CF09168

	K(Ac)K(Ac)–NH2	
T3.30	Biotin–ligador LC– GVYKS[PO3H2]PVVSGDTS[PO3H2]PRHL–NH2	lot MI89P9– P12–2

4.2 Resultados

La unión de los anticuerpos anti–tau con el péptido tau fosforilado fue monitoreada en tiempo real mediante el uso de SPR. Podrían emplearse análisis de fases de asociación y disociación de unión de anticuerpos para determinar la constante de velocidad de asociación (k_a), la constante de velocidad de disociación (k_d) así como también la constante de disociación K_D. El anticuerpo ACI–33–6C10–Ab1 se une específicamente con un péptido T1.5 sobre la superficie de carboximetildextrano no derivatizada en el rango de 3.7 → 367 nM del anticuerpo. Los análisis cinéticos de los sensogramas revelaron una constante de velocidad de asociación de 9,46 x 10^5 $M^{-1}s^{-1}$ y la constante de velocidad de disociación de 3,27 x 10^{-3} s^{-1} (Tabla 7). Por lo tanto, la constante de disociación K_D fue determinada en 3,46 nM, lo que muestra que el anticuerpo reconoce el fosfopéptido T1.5 con una muy alta afinidad. Todos los anticuerpos evaluados mostraron una alta afinidad respecto de sus fosfopéptidos respectivos usados para la inmunización y la generación de hibridomas, pero también demostraron una pequeña afinidad con los no fosfopéptidos.

Ejemplo 5: Mapeo de epítope de anticuerpos anti–p–tau

5.1 Métodos

El mapeo de epítope de anticuerpos monoclonales de ratón anti fosfo tau fue realizado por ELISA mediante el uso de diferentes librerías de fosfo o no fosfo péptidos. Las secuencias de aminoácidos de librerías de péptidos usadas son mostradas en la Tabla 8. Cada librería consistía en péptidos biotinilados cortos que abarcan secuencias fosfo y no fosfo presentes en la vacuna de péptido. Las librerías de péptidos fueron adquiridas en ANAWA Trading S.A. El mapeo de

epítope fue realizado de acuerdo con las instrucciones del fabricante (Mimotopes). En resumen, las placas revestidas con estreptavidina (NUNC) fueron bloqueadas con BSA al 1% en solución salina tamponada con fosfato (PBS) durante la noche a 4 ºC. Después del lavado con PBS– Tween 20 al 0,05%, las placas fueron revestidas durante 1 hora a temperatura ambiente con los diversos péptidos de cada librería, diluidos en BSA al 0,1%, azida de sodio al 0,1% en PBS hasta llegar a una concentración final de 10 µM. Después del lavado, se incubaron las placas durante 1 hora a temperatura ambiente con el anticuerpo a ser sometido a ensayo diluido en 40 ng/ml en BSA al 2%, y azida de sodio al 0,1% en PBS. Se lavaron las placas en anticuerpo secundario IgG antirratón conjugado con AP ((Jackson ImmunoResearch Laboratories, Suffolk, Inglaterra) en una dilución de 1/6.000 durante 1 hora a temperatura ambiente. Después del lavado final, se llevaron las placas incubadas con una solución sustrato de fosfatasa de hexahidrato de p–nitrofenilfosfato disódico (pNPP, Sigma–Aldrich, Buchs, Suiza) y se leyeron las placas a 405 nm transcurridas 2 horas de la incubación mediante el uso de un lector de placas ELISA. La unión fue considerada positiva si la densidad óptica (DO) era de al menos 2 veces mayor que la DO base.

5.2 Resultados

Como resultado de los experimentos de mapeo de epítope, los epítopes podrían ser identificados a través de la inclusión del residuo de aminoácidos fosforilados requeridos (referirse a la Tabla 9) al que los anticuerpos divulgados en la presente se unen específicamente.

- Tau aa 15–20, con un requerimiento de pY18 (6C10F9C12A11; 6C10E5E9C12)
- Tau aa 405–412, con un requerimiento de pS409 (6H1A11C11; 6H1G6E6)
- Tau aa 405–411, con un requerimiento de pS409 (2B6A10C11; 2B6G7A12; 3A8A12G7; 3A8E12H8)

- Tau aa 208–218, con un requerimiento de pT212 y pS214 (7C2(1)F10C10D3)
- Tau aa 393–401, con un requerimiento de pS396 (A4–2A1–18; A4–2A1–40)
- Tau aa 396–401, con un requerimiento de pS396 (A4–4A6–18)
- Tau aa 394–400, con un requerimiento de pS396 (A6–1D2–12)
- Tau aa 402–406, con un requerimiento de pS404 (A6–2G5–08)
- Tau aa 393–400, con un requerimiento de p396 (A6–2G5–30; A6–2G5–41)

Ejemplo 6: Inmunización pasiva de unas semana de ratones transgénicos tau

6.1. Métodos

Para todos los estudios *in vivo*, se usaron ratones transgénicos tau a los que se les administró el tratamiento de anticuerpos de acuerdo con lo mostrado en la Tabla que aparece a continuación.

Ratones transgénicos y anticuerpos usados para estudios *in vivo*

N.º de estudio	Modelo transgénico tau	Edad del ratón al inicio del estudio (en meses)	Duración del estudio (en semanas)	Anticuerpos administrados	Dosis (mg/kg)	Cantidad de administraciones i.p.	Lectura
1	TMHT (h*Tau*$^{V337M/R406W}$)	6.3	1	ACI–36–2B6–Ab1	0*, 3 o 10	2	MSD, IHC, WB
				ACI–36–3A8–Ab1	0 o 3		
2	TMHT	4.2	4	ACI–36–	0,1 o 3	4	MSD,

	(h*Tau*$^{V337M/R406W}$)			2B6–Ab1 o ACI–36–3A8–Ab1			IHC, WB, MWM
3	TMHT (h*Tau*$^{V337M/R406W}$)	3.0	12	ACI–36–2B6–Ab1 o ACI–36–3A8–Ab1	0,1 o 3	13	MSD, IHC, WB, MWM
4	biGT (hTau^{P301L} x hGSK3β)	4.5	12	ACI–36–2B6–Ab1 o ACI–36–3A8–Ab1	0,1 o 3	13	WB

*control del vehículo para todos los estudios; en forma intraperitoneal (i.p.)

6.1.1. Ratones y los tratamientos

Se usaron ratones Tg macho y hembra de 6,3 meses de edad (±3 días) de sobreexpresión de la isoforma TAU441 de tau humana de longitud completa, que soportan mutaciones de cambio de sentido V337M y R406W bajo el control de activador de murina Thy–1 (ratones TMHT), para el estudio N.º 1 (referirse a la Tabla que se encuentra más abajo). Los ratones fueron sometidos a eutanasia 1 día después de la última administración para determinar la patología tau en el cerebro.

6.1.2 Identificación y alojamiento del animal

En el transcurso de proceso para la genotipificación, a los animales se los numeró en forma consecutiva a través de la clásica marca en la oreja. Se volvió a

determinar el genotipo de todos los animales antes del inicio del estudio. Los ratones fueron mantenidos de acuerdo con los procedimientos operativos estándar de JSW sobre la base de estándares internacionales. Se colocó a los animales en jaulas individuales ventiladas con una base para roedores estandarizada de Rettenmaier®. Se mantuvo la temperatura a alrededor de 24 ºC y la humedad relativa fue mantenida entre 40% y 70%. Los animales fueron alojados según un ciclo de luz constante (12 horas de luz/oscuridad). Los animales disponían *ad libitum* de agua corriente normal y alimento para roedores estándar, seco, pelletizado (Altromin®). Se comprobaban los signos clínicos en forma regular a cada uno de los animales y se tomaba nota en una planilla individual de cada animal.

6.1.3. Sangrados in vivo

Transcurridos siete días desde la primera inmunización, se provocaban sangrados *in vivo* por muestreo mandibular de la vena/arteria facial. Las muestras de sangre son una combinación de sangre venosa y arterial. Para obtener el plasma, se reunía sangre en tubos de heparina y se los centrifugaba (1000 x g, 10 minutos, temperatura ambiente). Se congelaba el plasma en dos alícuotas hasta su uso.

6.1.4. Análisis cuantitativo inmunohistoquímico (IHC)

Se analizaron los hemisferios de todos los cerebros criocongelados. Se cortaron en forma longitudinal 15 criosecciones por nivel (5 niveles en total), cada una de 10µm de espesor (Leica CM 3050S). Se eligieron los niveles cerebrales de acuerdo con el atlas morfológico sobre el cerebro del ratón de Paxinos y Frankin (2da edición). El corte de los cinco niveles comenzó con un corte aleatorio y el posterior muestreo continuo de manera uniforme y sistemática, siempre manteniendo 15 cortes por nivel en series y descartando 150 µm entre los niveles.

Para determinar la patología tau en el hipocampo y las amígdalas, se sometieron a tinción 5 cortes (1 de cada nivel) por región cerebral y animal mediante el uso de anticuerpos AT180 (N.º MN1040, Thermo Scientific) y HT7 (N.º MN1000, Thermo Scientific). Se visualizaron los anticuerpos primarios por el anticuerpo secundario acoplado a Cy–3 (Jackson Laboratories) y con posterioridad, se evaluó el área inmunoactiva mediante el uso de software Image Pro Plus (versión 6.2).

Se midieron objetos inmunoactivos sobre una restricción de tamaño (30 μm^2 en las amígdalas, 7 μm^2 en el hipocampo) y sobre un umbral de intensidad dinámico. Se registró en forma automática el área total y la intensidad total de los objetos y el umbral individual. De usarse, se definió el umbral dinámico como la "intensidad media dentro del AOI más la cantidad de veces del factor de la desviación estándar de intensidades de píxeles dentro del AOI". En cualquiera de los casos, los valores debían exceder un umbral mínimo establecido. Los niveles exactos del umbral son dados en la tabla siguiente.

Umbrales	Valor mínimo	Factor dinámico
Amígdala AT180	25	2
Hipocampo AT180	28	–
Amígdala HT7	35	2
Hipocampo HT7	25	0.5

El tamaño de la región fue medido por delineación manual del hipocampo y de la amígdala. Se normalizaron los datos del área HT7 y AT180 IR al tamaño de las regiones.

Los datos relacionados con todos los IHC con n > 4 siguieron una distribución gaussiana de acuerdo con la prueba de normalidad de Kolmogorov Smirnov y son

representados como media + SEM. Para el grupo vehículo que consiste en cuatro animales solamente, por lo que resultan demasiado pocos para una prueba de normalidad, se dio por supuesta la distribución gaussiana. Se calcularon diferencias de grupo mediante ANOVA paramétrica de un sentido seguida de una prueba *post hoc* de Newman Keuls, calculada con software GraphPadPrism. El nivel de error alfa fue establecido en 0,05.

Se determinó la patología tau del cerebro en el hipocampo y en la amígdala mediante un análisis cuantitativo inmunohistoquímico (IHC) a través del uso de anticuerpos AT180 (anti–ptau) y HT7 (anti–tau). Además, los efectos del tratamiento sobre tau y p–tau soluble en la corteza y en el hipocampo fueron medidos en la fracción homogénea soluble mediante el uso de tecnología doble MesoScale Discovery (MSD), que sondea p–tau y tau total.

Ninguno de los anticuerpo usado para el ensayo de IHC o el de MSD cuentan con un epítope que se superponga con los dos anticuerpos del tratamiento usados en este estudio.

6.1.5. Generación de la fracción para el análisis cuantitativo del nivel de proteína tau soluble en las fracciones cerebrales solubles de ratones Tg

Los ratones tratados de acuerdo con el método 6.1.1 fueron sometidos a eutanasia 1 día después de la segunda administración para determinar la patología tau en el cerebro. En resumen, se homogeneizaron muestras de corteza solubles de un hemisferio cerebral en 100 a 200 µL de tampón de extracción en frío (25 mM Tris–HCl pH=7,4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM β–glicerofosfato, 30 mM NaF, 2 mM Na_3VO_4, cóctel inhibidor de proteasa y fosfatasa). Se centrifugaron los homogenatos (74.200 x g durante 15 minutos a una temperatura de 4 ºC) y se usaron los sobrenadantes para el análisis de tau soluble. Se determinó la concentración de proteína total en las fracciones solubles

de muestras de corteza mediante un ensayo de cuantificación de proteína BCA (Thermo Fisher Scientific, Rockford, IL, EE. UU.).

6.1.6. Análisis de la presencia de p–tau por transferencia Western

Para investigar la inmunorreactividad de los cerebros de ratones a los que se les administró ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2, se usaron dos anticuerpos que, según los informes, unen epítopes de PHF p–tau (Greenberg et al., 1992; Reig et al., 1995; Hoffmann et al., 1997) en ensayos de transferencia Western (WB).

Se diluyeron fracciones solubles de la corteza mediante el agregado de un volumen igual de tampón de muestra A (125 mM Tris/HCl pH 6,8, 4% (p/v) sulfato de dodecilo de sodio (SDS), 20% glicerol, 0,01% azul bromofenol y 5% beta–mercapto–etanol), y luego se llevaron las muestras a una temperatura de 95 °C durante 10 minutos.

Se cargaron 30 µg de muestra en un gel Bis–Tris de 4–12% (Invitrogen, Basel, Suiza) y se corrió el tampón de SDS MOPS (Invitrogen). Las proteínas fueron transferidas a una membrana de PVDF de 0,45 µm en un tampón de transferencia (25 mM Tris pH 8,6, 190 mM glicina, 20% metanol). Para verificar la transferencia de proteína, se sometieron a tinción las membranas con Ponceau S durante 5 minutos, se las lavó y bloqueó durante 1 hora en tampón de bloqueo (BSA al 5% en TBS [50 mM Tris–HCl, pH 7,6, 150 mM NaCl]). Se transfirieron las membranas durante la noche a 4 ºC con los anticuerpos primarios en tampón de bloqueo y Tween al 0,1%. Los dos anticuerpos primarios de PHF específico p–tau usados para las WBs fueron: anti–pS396 (PHF–13 epítope; AbCam, Cambridge, Reino Unido; usado en 3 µg/mL), específico de Ser396 (pS396) fosforilado de p–tau de murina humana (Hoffmann et al., 1997), y AD2 (PHF–1 epítope; BioRad, Reinach, Suiza; usado en 0,4 µg/mL), específico para murina pS396 y humana y Ser404 fosforilado (pS404; Reig et al., 1995). Para WBs de tau totales, Tau5 (0,5 µg/mL),

se usó un anticuerpo que liga tau humana y murina (BD Biosciences, Allschwil, Suiza). Después de la incubación con el anticuerpo primario, se lavaron las membranas con Tween al 0,1% en TBS, y se las incubó con los anticuerpos secundarios: antirratón de cabra–IEDye 800 o anticonejo de cabra IRDye 680 (ambos de Li–Cor Biosciences, NE, EE. UU.) ambos diluidos en 1:15.000 en BB y Tween al 0,1%. Luego, se incubaron las membranas durante 1 hora a temperatura ambiente protegidas de la luz, se las lavó durante 15 minutos 3 veces con Tween al 0,1% en TBS, y durante 5 minutos 2 veces con TBS, y se cuantificaron las bandas mediante el uso del sistema por imágenes infrarrojas cercanas Odyssey de Li–Cor (Li–Cor). Las bandas fueron normalizadas a la expresión de beta–actina (AbCam; que se usó a 0,4 µg/mL). Para verificar la identificación de las bandas transgénicas humanas en comparación con las tau endógenas de ratón, se investigaron las transferencias con un anticuerpo específico para tau total humana (Tau13, AbCam; que no se muestran). En forma adicional, se investigaron las membranas con un anticuerpo primario antirratón, para verificar que los anticuerpos del tratamiento, ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2, no estuvieran presentes en las muestras de las pruebas desnaturalizadas en una cantidad suficiente como para que interfieran en la unión de anti–pS396 o AD2. No se detectaron anticuerpos del tratamiento intactos o desnaturalizados (cuyos resultados no se muestran) en las muestras usadas para este estudio.

6.1.7. Análisis estadístico

Se analizaron los datos mediante el uso de estadísticas de suma de rangos de Kruskal–Wallis, y de ser significativo a un nivel de $P < 0,05$, se usó la prueba *post hoc* de Dunn que compara todos los grupos (GraphPad Prism, GraphPad Software, CA, EE. UU.). Los resultados son presentados como puntos de datos

individuales que muestran una media ± SEM. Se consideraron las diferencias con P < 0,05 como significativas desde el punto de vista estadístico.

6.2 Resultados

6.2.1. Patología tau cerebral por análisis cuantitativo inmunohistoquímico (IHC)

Dos inyecciones i.p. de ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2 no mostraron ningún efecto colateral importante durante el período del estudio. La tinción para pT231 y pS235 mediante el uso de AT180 por IHC mostró una mayor área inmunorreactiva (IR) en la amígdala después del tratamiento con ACI–36–3A8–Ab2. Los ratones tratados con 3 mg/kg de ACI–36–2B6–Ab2 presentaban un área IR de AT 180 significativamente menor en el hipocampo.

El tratamiento con ACI–36–3A8–AB2 aumentó p–tau de IR con AT 180 en comparación con el grupo PBS en la amígdala. En el hipocampo, el tratamiento con ACI–36–2B6–AB2 redujo la p–tau. AT 180 etiqueta específicamente p–tau. La frecuencia de células IR de AT 180 fue reducida en los ratones tratados con ACI–36–2B6–AB2. Este efecto fue mayor en el grupo de dosis baja (3 mg/kg) (ACI–36–2B6–AB2 LD). El patrón de tinción somal no difiere entre los grupos.

A mayores dosis de 10 mg/kg, se observó una tendencia no significativa para menos IR de AT 180 en el hipocampo tanto para ACI–36–2B6–Ab2 como para ACI–36–3A8–Ab2, cuando se lo comparó con los ratones tratados con el vehículo control. En forma cualitativa, los animales tratados con ACI–36–2B6–Ab2 mostraron una cantidad menor de neuronas hipocampales con un etiquetado de AT180 muy intenso.

6.2.2. Reducción del nivel de tau total en la fracción cerebral posterior a la inmunización pasiva

El efecto de los tratamientos en p–tau y tau en la fracción cerebral que contiene proteínas solubles fue medido mediante el uso de un ensayo doble de MSD. Los niveles de TAU soluble total en la corteza se vieron muy reducidos en

los ratones tratados con ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2 (p<0.01; Figura 3, panel superior). Los niveles de Piau soluble también se vieron muy reducidos (p < 0,05; Figura 3, panel inferior), donde la dosis de 3 mg/kg de ACI–36–2B6–Ab2 demuestra la gran reducción (p < 0.01). La relación entre Piau y TAU total permaneció sin cambios. Los niveles de TAU soluble y pTAU no cambiaron en las muestras del hipocampo (que no se muestran aquí).

6.2.3. Efectos de la administración de ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2 en la presencia de epítopes fosfo–tau que se encuentran en filamentos helicoidales pareados (PHFs)

En un sentido estructural, los ovillos neurofibrilares (NFTs) consiste en filamentos helicoidales pareados (PHFs) compuestos por la proteína tau asociada a microtúbulos, que se encuentra principalmente en un estado hiperfosforilado (Alonso et al., 1997). El objetivo de este estudio fue el de usar anticuerpos que reconocen PHF de pTau para investigar y cuantificar estos epítopes de PHF de pTau en los cerebros de ratones transgénicos Tau, después de la administración de ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2.

Para medir los efectos de dos administraciones de ACI–36–2B6–Ab2 o ACI–36–3A8–Ab2 en la cantidad de fosfo epítopes de PHF Tau bien documentada, se investigaron fracciones solubles de corteza cerebral de ratones tratados Tg Tau con AD2 (epítope PHF–1, pS396/pS404) y anticuerpo anti–pS396 (epítope PHF–13, pS396) mediante el uso de WBs. Se cuantificó la inmunorreactividad mediante el uso de un sistema por imágenes infrarrojas. Los efectos del tratamiento de ACI–36–3A8–AB2 y ACI–36–2B6–AB2 en la inmunorreactividad de PHF de AD2 en la corteza de ratones Tg Tau fueron determinados a través de AD2 que estudia pS396 y pS404, dos residuos fosfo de PHF de Tau documentados con anterioridad (Greenberg et al., 1992; Reig et al., 1995).

Se cuantificaron las bandas, lo que indica pTau humana y de ratón fosforilada en S396 y S404 mediante el uso del anticuerpo AD2 (PHF–1), a través del uso de un sistema por imágenes infrarrojas de Li–Cor. Se determinaron los valore para cada uno de los ratones así como también la media ± SEM.

Se observó una tendencia no significativa para una reducción en la inmunorreactividad de pTau de AD–2 positivo para la banda de pTau humana transgénica. No obstante, se observó una reducción significativa en la cantidad de pTau de AD2 positiva de ratón en los ratones tratados con 3 mg/kg de ACI–36–2B6–Ab2 y una tendencia no significativa cuando se los trató con 10 mg/kg de ACI–36–2B6–Ab2 o ACI–36–3A8–Ab2.

Cuando se usó un anticuerpo diferente que reconoce específicamente pTau pS396 para realización una tinción (Hoffmann et al., 1997), se observó un efecto incluso mayor. Los ratones tratados con 3 mg/kg de ACI–36–2B6–Ab2 presentaban pTau endógena de ratón y humana de pS396 positiva menos significativa, con una tendencia hacia la reducción cuando son tratados con 10 mg/kg de ACI–36–2B6–Ab2 o ACI–36–3A8–Ab2. Para evaluar los efectos en Tau total de ratón y humana, que incluye tanto pTau total y fosforilada, se verificaron las transferencias con el anticuerpo Tau5. En comparación con el vehículo control, no se moduló Tau total por ACI–36–2B6–Ab2 o ACI–36–3A8–Ab2 administrado en 10 mg/kg, sin embargo, se observó una tendencia para Tau total reducida para ACI–36–2B6–Ab2 administrado de ratón en 3 mg/kg.

6.2.4 Resumen

Dos administraciones periféricas de Tau Tg de ratón con el anticuerpo ACI–36–3A8–Ab2 anti–pTAU redujeron en forma significativa TAU soluble y pTAU soluble en la corteza cerebral. Dos administraciones periféricas de Tau Tg de ratón con el anticuerpo ACI–36–2B6–Ab2 anti–pTAU redujeron en forma significativa TAU soluble y pTAU soluble en la corteza cerebral. En forma

adicional, en el hipocampo, el ACI–36–2B6–AB2 redujo en forma significativa la inmunorreactivida de pTAU. Estos resultados demuestran la capacidad de inmunización anti–pTAU pasiva mediante el uso de anticuerpos ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2 para reducir la tauopatía.

Dos administraciones periféricas de ACI–36–2B6–Ab2 en 3 mg/kg a ratones Tg Tau redujeron la presencia de epítopes de PHF pTau en la corteza de acuerdo con lo medido por transferencia Western. A una dosis mayor de 10 mg/kg, tanto ACI–36–2B6–Ab2 como ACI–36–3A8–Ab2 demostraron una tendencia reducida a la inmunorreactividad de epítope de PHF de pTau. Estos resultados muestran que los anticuerpos ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2 pueden ser usados en forma adecuada en inmunoterapia pasiva contra tauopatías tales como la enfermedad de Alzheimer.

Ejemplo 7: Tratamiento de 1 mes en ratones que sobreexpresan Tau humana

7.1 Métodos

7.1.1 Ratones y los tratamientos

Se usaron ratones transgénicos tau a los que se les administró el tratamiento de anticuerpos de acuerdo con lo mostrado en la Tabla en el Método 6.1. (Estudio N.º 2.)

7.1.2. Prueba de comportamiento: tarea de laberinto de agua de Morris (MWM)

Después de la última administración, se llevó a cabo la tarea de laberinto de agua (MWM) para evaluar el rendimiento de la memoria espacial en ratones tratados de acuerdo con el 6.1.1. La prueba de MWM fue llevada a cabo con todos animales enjaulados en 4 semanas después del inicio. La MWM consiste en un recipiente circular blanco con un diámetro de 100 cm, lleno de agua corriente a una temperatura de 21±2 °C. El recipiente está virtualmente dividido en cuatro sectores. Se coloca una plataforma transparente (de 8 cm de diámetro) de alrededor de 0,5 cm por debajo del nivel del agua. Durante todas las sesiones de

la prueba, la plataforma es ubicada en el cuadrante sudoeste del recipiente. Cada ratón debe realizar tres intentos en cada uno de los cuatro días consecutivos. Un solo intento duró un máximo de un minuto. Durante ese tiempo, el ratón tuvo la oportunidad de encontrar el objetivo diáfano oculto. Después de cada intento, se dejó descansar a los ratones sobre la plataforma durante 10–15 segundos para que se orienten en los alrededores. Al menos una hora después del último intento en el día 4, los ratones debieron completar una prueba de sondeo (PT). Durante la PT, se retiró la plataforma del recipiente y la persona a cargo del experimento registró la cantidad de veces que cada ratón cruzó sobre la posición objetivo anterior junto con la permanencia en este cuadrante. Para la cuantificación de la latencia de escape (el tiempo [en segundos] que necesitó el ratón para encontrar la plataforma oculta y por lo tanto, escapar del agua), del camino (la longitud de la trayectoria [en metros] para alcanzar el objetivo), de los cruces de la zona objetivo y de la permanencia en el cuadrante objetivo en la PT, se usó un sistema de seguimiento computarizado (Biobserve Software). Todos los animales debían realizar una prueba visual después de la PT en el último día para eliminar la influencia de capacidades visuales insuficientes en los resultados de comportamiento.

7.1.3. Determinación de la patología tau cerebral por análisis cuantitativo inmunohistoquímico (IHC)

Los ratones fueron sometidos a eutanasia 1 día después de la prueba de MWM (1 semana después de la última administración) para determinar la patología Tau en el cerebro. Se determinó la patología Tau del cerebro en el hipocampo y en la amígdala mediante un análisis cuantitativo inmunohistoquímico (IHC) a través del uso de anticuerpos AT180 (anti–pTau, pT231/pS235) y HT7 (anti–Tau específico de humano). Además, los efectos del tratamiento sobre Tau y pTau soluble en la corteza y en el hipocampo fueron medidos en la fracción

homogénea mediante el uso de tecnología doble MesoScale Discovery (MSD), que sondea pTau (pT231) y Tau total. Ninguno de los anticuerpos usados para el ensayo de IHC o el de MSD cuentan con un epítope que se superponga con el anticuerpo del tratamiento usado en este estudio.

7.1.4. Preparación de la muestra para el análisis de Tau soluble en la corteza y en el hipocampo

Los ratones fueron sometidos a eutanasia durante la recolección de tejido, una semana después de la última administración del tratamiento. Se homogeneizaron la corteza y el hipocampo en 100 a 200 µL de tampón de extracción en frío 1 (25 mM Tris–HCl pH=7,4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM ß–glicerofosfato, 30 mM NaF, 2 mM Na_3VO_4, cóctel de inhibidor de proteasa y fosfatasa). Se centrifugaron los homogenatos (74.200 x g durante 15 minutos a una temperatura de 4 ºC) y se usaron los sobrenadantes para el análisis de Tau soluble en la corteza y en el hipocampo (Figura 4). Se volvieron al suspender los gránulos en 100–200 µL de tampón de extracción 2 (10 mM Tris HCl pH=7,4, 800 mM NaCl, 300 mM sacarosa, 1 mM de EGTA, cócteles de inhibidores de fosfatasa y proteasa) y los transfirió a un tubo de 1,5 mL. Se centrifugaron las soluciones (4.000 g durante 20 minutos a una temperatura de 4 ºC) y se transfirieron los sobrenadantes a tubos de ultracentrifugación. Luego, se agregó sarcosilo (una solución acuosa al 30%) a la concentración final de 1% y se la incubó durante 1,5 horas a temperatura ambiente. Después de la centrifugación (74.200 g durante 30 minutos a una temperatura de 4 ºC) se descartaron los sobrenadantes y se volvieron a suspender los gránulos en 100 µL de tampón 3 (50 mM Tris–HCl, pH=7,4). Se usaron los gránulos vueltos a suspender como Tau insoluble en sarcosilo (SinT) en la corteza y en el hipocampo. Se determinó la concentración de proteína total en las muestras de fracciones solubles y SinT

mediante un ensayo de cuantificación de proteína BCA (Thermo Fisher Scientific, Rockford, IL, EE. UU.).

7.1.5. Transferencia Western para Tau y PHF pTau

Para investigar el efecto de la administración de ACI–36–2B6–Ab1 en presencia de PHF pTau en la corteza cerebral y en el hipocampo, se informó que dos anticuerpos unen epítopes de PHF pTau (Greenberg et al., 1992; Reig et al., 1995; Hoffmann et al., 1997) en ensayos de transferencia Western (WB). Las fracciones solubles y SinT de la corteza y del hipocampo fueron diluidas mediante el agregado de un volumen igual del tampón de muestra A (125 mM Tris/HCl pH 6,8, 4% (p/v) sulfato de dodecilo de sodio (SDS), 20% glicerol, 0,01% azul bromofenol y 5% beta–mercapto–etanol), y luego se llevaron las muestras a una temperatura de 95 °C durante 10 minutos. Se cargaron 30 µg de muestra en un gel Bis–Tris de 4–12% (Invitrogen, Basel, Suiza) y se corrió el tampón de SDS MOPS (Invitrogen). Las proteínas fueron transferidas a una membrana de PVDF de 0,45 µm en un tampón de transferencia (25 mM Tris pH 8,6, 190 mM glicina, 20% metanol). Para verificar la transferencia de proteína, se sometieron a tinción las membranas con Ponceau S durante 5 minutos, luego se las lavó y bloqueó durante 1 hora en tampón de bloqueo (BSA al 5% en TBS [50 mM Tris–HCl, pH 7,6, 150 mM NaCl]). Se transfirieron las membranas durante la noche a 4 ºC con los anticuerpos primarios en tampón de bloqueo y Tween al 0,1%.

Los dos anticuerpos primarios de PHF específico p–tau usados para las WBs fueron: anti–pS396 (PHF–13 epítope; AbCam, Cambridge, Reino Unido; usado en 3 µg/mL), específico de Ser396 (pS396) fosforilado de p–tau de murina humana (Hoffmann et al., 1997), y AD2 (PHF–1 epítope; BioRad, Reinach, Suiza; usado en 0,4 µg/mL), específico para murina pS396 y humana y Ser404 fosforilado (pS404; Reig et al., 1995). Para investigar el efecto de la administración de ACI–36–2B6–Ab1 en presencia de PHF pTau en la corteza

cerebral y en el hipocampo, se informó que dos anticuerpos unen epítopes de PHF pTau en ensayos de transferencia Western (WB). Para WBs de Tau totales, Tau5, se usó un anticuerpo que liga Tau humana y murina (BD Biosciences, Allschwil, Suiza) en 0,5 µg/mL. Se transfirieron todas las membranas en forma adicional para que se normalizara la beta–actina (AbCam; usada a 0,4 µg/mL) para la carga de proteína.

Después de la incubación con el anticuerpo primario, se lavaron las membranas con Tween al 0,1% en TBS, y se las incubó con los anticuerpos secundarios: antirratón de cabra–IRDye 800 o anticonejo de cabra IRDye 680 (ambos de Li–Cor Biosciences, NE, EE. UU.) ambos diluidos en 1:15.000 en BB y Tween al 0,1%. Luego, se incubaron las membranas durante 1 hora a temperatura ambiente protegidas de la luz, se las lavó durante 15 minutos 3 veces con Tween al 0,1% en TBS, y durante 5 minutos 2 veces con TBS, y se cuantificaron las bandas mediante el uso del sistema por imágenes infrarrojas cercanas Odyssey de Li–Cor (Li–Cor). Las bandas de interés fueron normalizadas en expresión de beta–actina. Para verificar la identificación de las bandas transgénicas humanas en comparación con las Tau endógenas de ratón, se investigaron las transferencias con un anticuerpo específico para Tau total humana sin la reacción cruzada con murina (Tau13, AbCam; que no se muestran). En forma adicional, se investigaron las membranas con un anticuerpo primario antirratón, para verificar que los anticuerpos del tratamiento no estuvieran presentes en las muestras de las pruebas desnaturalizadas en una cantidad suficiente como para que interfieran en la unión de los anticuerpos de transferencia primarios. No se detectaron anticuerpos del tratamiento intactos o desnaturalizados (cuyos resultados no se muestran) en las muestras usadas para este estudio. Los valores son expresados como inmunorreactividad (IR) corregida por beta–actina.

7.1.6. Análisis estadístico

Se analizaron los datos mediante el uso de ANOVA de un sentido seguido de una prueba *post hoc* de comparación múltiple de Dunnett (GraphPad Prism, GraphPad Software, CA, EE.UU.) que compara cada tratamiento con ratones tratados con Tg control. Los resultados son presentados como puntos de datos individuales que muestran una media ± SEM. Se consideraron las diferencias con $p < 0,05$ como significativas desde el punto de vista estadístico. No se incluyeron los valores simples que fueron identificados como valores atípicos significativos ($p < 0,5$) por test t de Student de desvío extremo.

7.2 Resultados

7.2.1. Prueba de comportamiento: tarea de laberinto de agua de Morris (MWM) después de la inmunización pasiva

Las cuatro inyecciones i.p. de ACI–36–2B6–Ab1 administradas en forma semanal a 3 mg/kg o 1 mg/kg durante un período de cuatro semanas no mostraron ningún efecto adverso grave.

Durante la última semana del tratamiento, se evaluó el aprendizaje de navegación espacial y la memoria de los animales. Los animales debían completar un entrenamiento de 4 días con 3 intentos por día seguido de una prueba de sondeo y una prueba visual. Se evaluó la latencia de escape (el tiempo [en segundos] que necesitó el ratón para encontrar la plataforma oculta y por lo tanto, escapar del agua), el camino (la longitud de la trayectoria [en metros] para alcanzar el objetivo), la velocidad de nado (coeficiente calculado del camino y la latencia de escape), y la cantidad de cruces objetivo y la permanencia en el cuadrante objetivo.

Los animales Tg (el grupo A) así como también los animales de control nTg (F) tratados con el vehículo mostraron las curvas de aprendizaje esperadas cuando se evaluó la latencia de escape y la longitud de la trayectoria del nado

para alcanzar la plataforma durante los cuatro días de prueba. Los animales de control Tg (A) contaban con una deficiencia significativa en el aprendizaje de acuerdo con lo mostrado por las curvas de aprendizaje más planas en latencias de escape y las trayectorias del nado en comparación con animales de control nTg (F). Las latencias de escape y las trayectorias del nado fueron significativamente más largas (ANOVA de dos días) en los días 3 y 4 del entrenamiento ($p < 0,001$; prueba posterior de Bonferroni). El tratamiento con ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1, de dosis alta o baja (grupos B y C y D y E, respectivamente) no condujeron a una mejora significativa de las capacidades de aprendizaje espacial en comparación con los animales control Tg (grupo A) y demostraron curvas de aprendizaje similares. Al ajustar el rendimiento del día 1 de cada grupo en 100% y todos los demás días como porcentaje del día 1, puede observarse una mejora para los ratones tratados con ACI–36–3A8–Ab1 (ambas dosis). El efecto alcanzó una importancia estadística para la longitud de la trayectoria del nado en el día 3 ($p < 0,01$ en el grupo D y $p < 0,05$ en el grupo E) y en el día 4 ($p < 0,05$ en el grupo D).

Para los ratones tratados con ACI–36–2B6–Ab1 (ambas dosis), puede observarse una ligera mejoría en la longitud de la trayectoria del nado, aunque sin importancia estadística.

No se detectaron diferencias entre grupos de tratamiento en términos de velocidad de nado en los cuatro días de entrenamiento.

Los resultados de la prueba de MWM demostraron tendencias hacia un aprendizaje espacial mejorado para los ratones tratados con ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1.

7.2.2. Patología Tau cerebral por análisis cuantitativo inmunohistoquímico (IHC)

El anticuerpo AT180 produce una tinción en pTAU humana y endógena (doblemente fosforilado en Thr231 y Ser235).

Se determinó IR de AT180 en la amígdala y en el hipocampo después de inmunización con ACI–36–2B6–AB1 y ACI–36–3A8–AB1. Se midió el porcentaje del área de IR de AT180 en la amígdala y en el hipocampo.

La cantidad de PTAU intrasomal en los controles de nTg fue significativamente menor en comparación con los grupos Tg ($p < 0,001$). En la amígdala, se observó una tendencia a aumentar pTAU somal para el tratamiento de ACI–36–2B6–Ab1 de 3 mg/kg. Por el contrario, ambas dosis de ACI–36–2B6–Ab1 tendieron a reducir pTAU en comparación con los animales tratados con el vehículo en el hipocampo. Las intensidades de tinción media fueron comparables en todos los grupos transgénicos.

El tratamiento ACI–36–3A8–Ab1 con no alteró pTAU somal en el hipocampo y en la amígdala y los niveles de pTAU neuronales en la amígdala y en el hipocampo no difirieron significativamente entre grupos transgénicos tratados. La suma de las intensidades de tinción y la media fueron comparables en todos los grupos transgénicos.

Dado que el anticuerpo HT7 es específico para TAU humana, solo se midió una pequeña señal en controles nTg, que deriva de la autofluorescencia de los puntos de lipofuscina que superan los siete píxeles de tamaño. El tratamiento de ACI–36–2B6–Ab1 no alteró el área de IR positiva de HT7 somal en el hipocampo en comparación con el vehículo control (PBS). En la amígdala, los ratones que recibieron la dosis menos de ACI–36–2B6–Ab1 tendieron a presentar mayores niveles de TAU humana total (prueba T: $p = 0,0954$) en términos del área de IR. Asimismo, este aumento fue visible en términos cualitativos como un aumento del área de tinción y la intensidad de tinción en los somas neuronales individuales. No se observaron diferencias inducidas por el tratamiento que fueran significativas desde el punto de vista estadístico en las neuronas del hipocampo.

El tratamiento de ACI–36–3A8–Ab1 no alteró en forma significativa el área de IR positiva de HT7 somal en la amígdala y el hipocampo en comparación con el vehículo control (PBS). La suma de las intensidades de tinción y la media fueron comparables en todos los grupos transgénicos (los datos no se muestran).

La patología Tau cerebral no mostró ningún cambio en los niveles de Tau total o pTau en la fracción soluble cerebral, no obstante, la inmunotinción de secciones cerebrales demostró una reducción en pTau del hipocampo en ratones tratados con ACI–36–2B6–Ab1.

7.2.3. Efectos de la administración de anticuerpo anti–Tau en epítopes fosfo–Tau presentes en filamentos helicoidales pareados (PHFs)

La enfermedad de Alzheimer (EA) se caracteriza desde el punto de vista neuropatológico, por ovillos neurofibrilares (NFTs, Braak, Braak & Bohl, 1993). En un sentido estructural, los NFTs consisten en filamentos helicoidales pareados (PHFs) compuestos por la proteína Tau asociada a microtúbulos, que se encuentra principalmente en un estado hiperfosforilado (Alonso et al., 1997). El objetivo de este estudio fue el de reducir estos epítopes de PHF de pTAu en los cerebros de ratones transgénicos Tau a través de cuatro administraciones del anticuerpo anti–pTau ACI–36–2B6–Ab2 y ACI–36–3A8–Ab2.

Para medir los efectos de cuatro administraciones de ACI–36–2B6–Ab2 en la cantidad de fosfo epítopes de PHF Tau bien documentada, se investigaron fracciones SinT y solubles de corteza cerebral y de hipocampo de ratones tratados Tg Tau con AD2 (epítope PHF–1, pS396/pS404) y anticuerpo anti–pS396 (epítope PHF–13, pS396) mediante el uso de WBs. Como marcadores de PHFs de Tau, la presencia de pS396/pS404 han sido previamente documentada (Greenberg et al., 1992; Reig et al., 1995) y en forma más específica, el sitio pS396 (Hoffmann et al., 1997). En los ratones Tg Tau, Tau es expresada como Tau de murina endógena y como el transgén de Tau humana, con una diferencia

en peso molecular que puede ser claramente identificada en WBs cuando un anticuerpo de transferencia se une a Tau desde ambas especies y Tau desde dos transcripciones diferentes es expresada en cantidades suficientes. Por lo tanto, cuando es posible, las bandas de Tau transgénica humana y de ratón endógena son identificadas para cada anticuerpo de transcripción y son cuantificadas por separado. Para verificar los patrones de migración para estas bandas de Tau en nuestros ensayos de WB, se usó un anticuerpo anti–Tau que se une a Tau total, pero es específico humano (Tau13), y por lo tanto, solo muestra el transgén humano en cerebros Tg Tau para muestras de control Tg Tau para verificar patrones de migración para Tau de ratón y de humano en los ratones transgénicos de Tau. En forma adicional, se normalizaron todas las bandas cuantificadas con beta–actina.

La presencia de epítopes de PHF, investigadas en la fracción soluble de la corteza cerebral, fue reducida en los ratones tratados con ACI–36–2B6–Ab1 y in ACI–36–3A8–Ab1. Esto resultó significativo tanto para las bandas de ratón como para las humanas mediante el uso del anticuerpo pS396 (PHF–13) para las WBs (Figuras 5A y B).

Cuando se usó el anticuerpo AD2 (PHF–1, pS396/pS404) para WBs de extractos de ratones tratados con ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1, se observo una significativa reducción (Figura 5C).

Debe destacarse que aunque los dos anticuerpos de PHF específico que son usados para estas WBs tienen epítopes similares y una buena especificidad respecto de sus objetivos fosforilados, el anticuerpo pS396 (PHF–13) parece tener una mejor relación entre señal y ruido y fue el mejor anticuerpo general para estas WBs.

El efecto objetivo directo del anticuerpo del tratamiento fue investigado en la corteza mediante el uso de ACI–36–2B6–Ab1 y ACI–36–2B6–Ab1,

respectivamente, como el anticuerpo de transferencia. Este anticuerpo anti–pTau se une con el epítope fosfo–Tau como el anticuerpo del tratamiento usado en este estudio. Se han probado las transferencias con anterioridad con un solo anticuerpo IgG antirratón secundario.

Se cuantificó la transferencia mediante el uso de un sistema por imágenes infrarrojas. Se determinaron los valore para cada uno de los ratones así como también la media ± SEM.

No se detectó ninguna señal sobre la base, lo que verifica la falta de efectos de bloqueo o de interferencia por parte del anticuerpo del tratamiento en estas muestras (cuyos datos no se muestran).

En ratones tratados con ACI–36–2B6–Ab1, se observó una tendencia hacia una señal reducida al nivel de ratones nTg tratados control, lo que indica un efecto objetivo directo (Figura 5G). En ratones tratados con ACI–36–3A8–Ab1, no se observaron efectos significativos del tratamiento (Figura 5G).

El significativo efecto del tratamiento de ACI–36–2B6–Ab1 en Tau total en la corteza soluble de ratones Tg de Tau se observó a través del uso de un anticuerpo Tau5 para transferencia que une la Tau de ratón endógena y la Tau humana transgénica (Figuras 5H y 5I). Se observó una reducción significativa en Tau total para Tau de ratón engódena y para Tau humana transgénica en la fracción soluble de la corteza cerebral.

Las bandas, que indican la Tau total de ratón A) y humana B) (Tau5), fueron cuantificadas mediante el uso de un sistema por imágenes infrarrojas. Se determinaron los valores para cada uno de los ratones así como también la media ± SEM.

La presencia de epítopes de PHF en la fracción insoluble en detergente fue llevada a cabo por la preparación de fracciones de cerebro (SinT) insolubles en sarcosilo.

Se cuantificó la transferencia mediante el uso de un sistema por imágenes infrarrojas. Se determinaron los valore para cada uno de los ratones así como también la media ± SEM.

En esta fracción, estuvo presente mucha menos Tau cuando se la compara con la fracción Tau soluble tanto en la corteza como en el hipocampo. Esto puede deberse a la edad de los ratones Tg de Tau usado en este estudio, que a los 4 meses no pueden haber acumulado una cantidad significativa de Tau agregada e insoluble en el hipotálamo y en la corteza . Por lo tanto, cuando se investigan epítopes de PHF en la fracción SinT, solo el anticuerpo AD2 (PHF–1, pS396/pS404) proveyó una señal que resultó suficiente para la cuantificación confiable de las bandas.

Los ratones tratados con 1 mg/kg de ACI–36–2B6–Ab1 y con 1 mg/kg de ACI–36–3A8–Ab1, respectivamente, presentaron una reducción significativa en el epítope de PHF–1 en bandas que representan la Tau de ratón endógena (Figura 5C). Las señales observadas para la banda humana transgénica no fueron lo suficientemente intensas como para ser cuantificadas en forma confiable.

También se investigó el hipocampo, mediante el uso de los mismos anticuerpos y fracciones que para la corteza. Se detectaron señales más bajas para todos los anticuerpos de transferencia en fracciones del hipocampo en comparación con la corteza.

Se determinaron los efectos del tratamiento con ACI–36–2B6–AB1 y ACI–36–3A8–AB1 en la inmunorreactividad de pS396 (PHF–13) en el hipocampo soluble de ratones Tg de Tau. Las bandas, que indican los epítopes de pTau de ratón A) y humana B) pS396 (PHF–13) fueron cuantificadas mediante el uso de un sistema por imágenes infrarrojas. Se determinaron los valores para cada uno de los ratones así como también la media ± SEM.

El tratamiento con ACI–36–2B6–Ab1 no alteró en forma significativa la presencia del epítope pS396 (PHF–13) en la fracción de hipocampo soluble de Tau de ratón, con una pequeña tendencia a una reducción en la banda de transgén humano.

El tratamiento con ACI–36–3A8–Ab1 mostró tendencias a una reducción en presencia del epítope pS396 (PHF–13) en la fracción de hipocampo soluble de Tau de ratón y la banda de transgén humano.

De forma similar a lo que se observó para WBs de pS396 (PHF–13), se detectó una tendencia para una señal reducida en ambos extractos de ratones tratados con ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1 para Tau total en la fracción de hipocampo soluble de Tau humana.

De forma similar a las muestras SinT de la corteza, la fracción SinT del hipocampo presentaba muy bajos niveles de pTau. Los ratones tratados con ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1, respectivamente, no presentaron ningún cambio en el epítope de PHF–1 (pS396/pS404) en bandas que representan la Tau de ratón endógena. Las señales observadas para la banda humana transgénica no fueron lo suficientemente intensas como para ser cuantificadas en forma confiable.

7.2.4 Resumen

El estudio indica que la inmunización pasiva mediante el uso de cuatro administraciones de los anticuerpos anti–pTau de fosfosita específica ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1 mejora el aprendizaje espacial y reduce la patología pTau cerebral.

Cuatro administraciones periféricas de anticuerpo anti–pTau ACI–36–2B6–Ab1 en 1 y 3 mg/kg a ratones Tg Tau redujeron la presencia de epítopes de PHF pTau en la corteza de acuerdo con lo medido por transferencia Western. Se observó una tendencia a la reducción en el hipocampo. De forma similar, también

se observó una reducción en la Tau total. Se observó una reducción significativa en la inmunorreactividad de PHF–1 de pTau en la fracción de corteza soluble, y también se observó una tendencia que indicó efectos objetivo directos del tratamiento con el anticuerpo. Estos resultados proveen un mayor sustento para los anticuerpos ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1 en inmunoterapia pasiva contra tauopatías tales como la enfermedad de Alzheimer.

Ejemplo 8: Tratamiento de 3 meses en ratones que sobreexpresan Tau humana

8.1 Métodos

8.1.1 Ratones y los tratamientos

Se usaron ratones transgénicos tau a los que se les administró el tratamiento de anticuerpos de acuerdo con lo mostrado en la Tabla en el Método 6.1. (estudio N.º 3) y se asignaron ratones en cuatro grupos de tratamiento diferentes de acuerdo con lo descripto en la tabla a continuación.

Grupo	Cepa de ratón	Genotipo	Edad al comienzo	Género	N	Tratamiento		
A	TMHT	Tg	3 meses (±2 semanas)	mixto	15+1	PBS (control)	i.p. 10 µl/g b.w.	por semana
B	TMHT	Tg	3 meses (±2 semanas)	mixto	15+1	ACI–36–2B6–Ab1 (1 mg/kg)	i.p. 10 µl/g b.w.	por semana
C	TMHT	Tg	3 meses (±2 semanas)	mixto	15+1	ACI–36–2B6–Ab1 (3	i.p. 10 µl/g b.w.	por semana

						mg/kg)		
F	TMHT	nTg	3 meses (±2 semanas)	mixto	15+1	PBS (control)	i.p. 10 µl/g b.w.	por semana

Se trataron en total 45 ratones Tg más 3 reservas asignados a los grupos de tratamiento A a C y 15 ratones nTg más 1 reserva (grupo F) en el día 0, 7, 14, 21, 28, 35, 42, 56, 63, 70, 77 y 84 por inyección i.p. de anticuerpo anti–pTAU o PBS (vehículo control), ACI–36–2B6–Ab1 o ACI–36–3A8–Ab1. Se dividieron en forma aleatoria los animales en 5 grupos de inicio diferentes (escalas) que comprenden animales de todos los grupos tratados. La cantidad de animales en una escala fue limitada para asegurar la misma edad y un manejo uniforme. Después de la duodécima administración, se llevó a cabo una tarea de laberinto de agua (MWM) para evaluar el rendimiento de la memoria espacial. Después de la MWM, se les administró a los ratones el artículo de prueba una vez más (inyección décimo tercera) antes de ser sometidos a eutanasia 24 horas más tarde de haber determinado la patología Tau. Se determinó la patología tau del cerebro en el hipocampo y en la amígdala mediante un análisis cuantitativo inmunohistoquímico (IHC) a través del uso del anticuerpo AT180 (anti–pTau, pT231/pS235). Además, los efectos del tratamiento sobre Tau y pTau soluble e insoluble en sarcosilo en la corteza y en el hipocampo fueron medidos mediante el uso de tecnología MesoScale Discovery (MSD), que sondea pTau (pT231 y pS396) y Tau total.

8.1.2. Prueba de comportamiento: tarea de laberinto de agua de Morris (MWM)

Este experimento fue llevado a cabo de acuerdo con el protocolo descripto en el Ejemplo 7.1.2. En la duodécima semana, se evaluó la navegación espacial con la prueba de laberinto de agua de Morris (MWM) para comprobar el aprendizaje y la memoria.

8.1.3. Biología molecular

Se cuantificó Tau total y Tau fosforilado en Thr 231 y en pS396 en homogenatos de cerebro de animales Tg mediante el uso de un ensayo inmunoabsorbente de MesoScale Discovery (MSD).

8.1.4. Determinación de la patología tau cerebral por análisis cuantitativo inmunohistoquímico (IHC)

Este experimento fue llevado a cabo de acuerdo con el protocolo descripto en el Ejemplo 7.1.3. Se determinó la patología TAU mediante la inmunorreactividad de AT180 en el hipocampo y en la amígdala de 8 animales por grupo.

8.1.5. Efectos de la administración de anticuerpo anti–Tau durante tres meses en epítopes fosfo–Tau presentes en filamentos helicoidales pareados (PHFs)

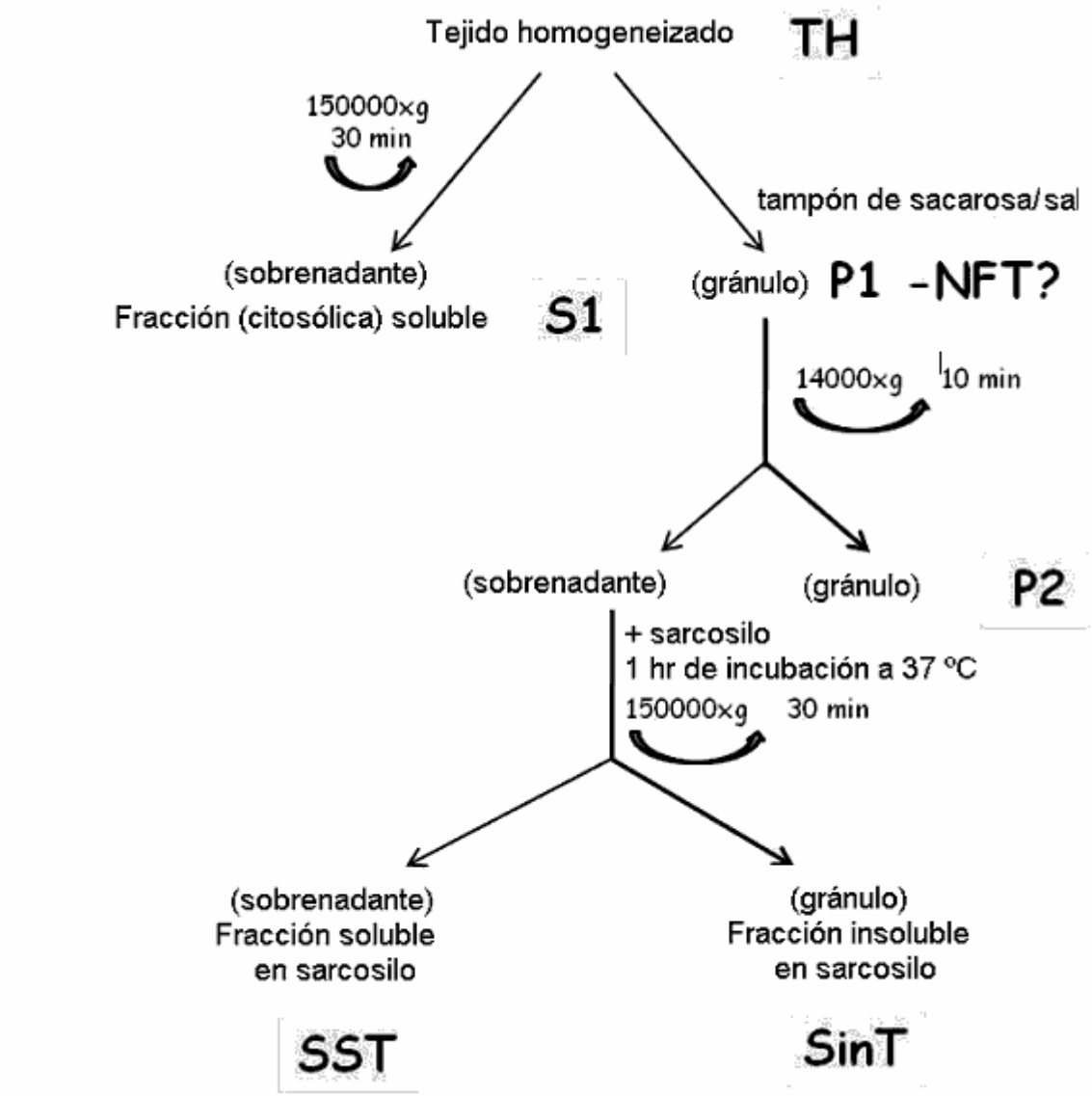
Este experimento fue llevado a cabo de acuerdo con los protocolos descriptos en 7.1.4., 7.1.5. y 7.1.6. Para medir los efectos de cuatro administraciones de ACI–36–2B6–Ab1 y ACI–36–3A8–Ab1 en la cantidad de fosfo epítopes de PHF Tau bien documentada, se investigaron fracciones SinT y solubles de corteza cerebral y de hipocampo de ratones tratados Tg Tau con AD2 (epítope PHF–1, pS396/pS404), anticuerpo anti–pS396 (epítope PHF–13, pS396) y AT180 (pT231/pS235) mediante el uso de WBs.

8.1.6. Efectos de la administración de anticuerpo anti–Tau durante tres meses en epítopes fosfo–Tau mediante el uso de ratones bigénicos Tau biGT

El estudio N.° 4 fue llevado a cabo mediante el uso de ratones Tau bigénicos según lo muestra el Método 6.1. Se prepararon muestras de corteza cerebral de acuerdo con lo mostrado en la figura siguiente, a través del uso del homogenato total (TH) o la fracción soluble (S1) para transferencia Western. Se investigaron las membranas mediante el uso de los siguientes anticuerpos de transferencia para pTau o Tau total:

- HT–7 (26ng/ml), específica de Tau humana total
- PHF–13 (pS396) con una dilución de 1/7500
- AT180 (pT231) en 2,47ug/ml
- AT8 (pS202) en 3ug/ml
- pS404 con una dilución de 1:5000
- pS400 con una dilución de 1:5000

Se normalizaron todas las cuantificaciones con beta–actina.



8.2 Resultados para el anticuerpo ACI–36–2B6–Ab1

Las trece inyecciones i.p. de ACI–36–2B6–Ab1 administradas en forma semanal a 1 ó 3 mg/kg durante un período de doce semanas de estudio no mostraron ningún efecto adverso grave.

8.2.1. Resultados de comportamiento – Laberinto de agua de Morris

Los resultados de la prueba de MWM demostraron fuertes tendencias hacia un aprendizaje espacial mejorado para los ratones tratados con ACI–36–2B6–Ab1 (Figura 9).

Durante la última semana del tratamiento, se evaluó el aprendizaje de navegación espacial y la memoria de los animales. Los animales debían completar un entrenamiento de 4 días con 3 intentos por día seguido de una prueba de sondeo y una prueba visual. Se evaluó la latencia de escape (el tiempo [en segundos] que necesitó el ratón para encontrar la plataforma oculta y por lo tanto, escapar del agua), el camino (la longitud de la trayectoria [en metros] para alcanzar el objetivo), la velocidad de nado (coeficiente calculado del camino y la latencia de escape), y la cantidad de cruces objetivo y la permanencia en el cuadrante objetivo. Los animales control Tg (el grupo A) y los animales de control nTg (grupo F) tratados con el vehículo mostraron las curvas de aprendizaje esperadas en términos de latencia de escape y longitud de la trayectoria del nado para alcanzar la plataforma durante los cuatro días de prueba. Los animales de control Tg (A) mostraron una deficiencia significativa en la capacidad de aprendizaje reflejada en las curvas de aprendizaje más planas de latencias de escape y las trayectorias del nado en comparación con animales de control nTg (grupo F). Las latencias de escape y las trayectorias del nado fueron significativamente diferentes (ANOVA de dos

vías) en los días 3 ($p < 0,001$, longitud; prueba posterior de Bonferroni) y 4 del entrenamiento ($p< 0,01$; prueba posterior de Bonferroni). El tratamiento con ACI–36–2B6–Ab1, de dosis alta o baja (B y C) no mejoró en forma significativa la capacidad de aprendizaje espacial en comparación con los animales control Tg (A). Al ajustar el rendimiento de cada grupo en 100% el día 1 de entrenamiento y todos los demás días como porcentaje del día 1, puede observarse una leve mejora para los ratones tratados con ACI–36–2B6–Ab1 (ambas dosis) en longitud de trayectoria del nado, aunque sin importancia desde el punto de vista estadístico. No se detectaron diferencias de tratamiento entre grupos cuando se calculó la velocidad de nado en los cuatro días de entrenamiento. En la prueba de sondeo (PT), se registró la permanencia en el cuadrante objetivo (cuadrante sudoeste) así como también los cruces de zona objetivo. Los controles nTg (grupo F) ocuparon más tiempo en el cuadrante objetivo y cruzaron la zona objetivo con mayor frecuencia respecto de los controles Tg (grupo A) pero sin importancia sustancial.

El tratamiento con ninguna dosis, ya sea alta o baja, condujo a una mejora de las capacidades de aprendizaje espacial en comparación con los ratones de control Tg de acuerdo con lo evaluado en la PT.

8.2.2. Biología molecular

8.2.2.1. Tau en fracción soluble de homogenatos de la corteza

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en la fracción soluble de homogenatos de la corteza de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), B (Tg, ACI–36–2B6–Ab1 1 mg/kg) y C (Tg, ACI–36–2B6–Ab1 3 mg/kg). Un tratamiento con ACI–36–2B6–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos de la corteza soluble. No obstante, se observó un ligero aumento (sin importancia) de la TAU total media, p231TAU y p396TAU ante el tratamiento con ACI–36–

2B6–Ab1. No se vio afectada la fosforilación de TAU evaluada como las relaciones entre pTAU y TAU total.

8.2.2.2. Tau en fracción insoluble de sarcosilo de homogenatos de la corteza

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en las fracciones insolubles de sarcosilo de los homogenatos de la corteza de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), B (Tg, ACI–36–2B6–Ab1 1 mg/kg) y C (Tg, ACI–36–2B6–Ab1 3 mg/kg). Un tratamiento con ACI–36–2B6–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos de la corteza insoluble en sarcosilo. Se observó un ligero aumento (sin importancia) de la TAU total media y p231TAU ante el tratamiento con ACI–36–2B6–Ab1. No se vio afectada la fosforilación de TAU evaluada como las relaciones entre pTAU y TAU total.

8.2.2.3. Tau en fracción soluble de homogenatos del hipocampo

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en la fracción soluble de homogenatos del hipocampo de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), B (Tg, ACI–36–2B6–AB1 1 mg/kg) y C (Tg, ACI–36–2B6–Ab1 3 mg/kg). Un tratamiento con ACI–36–2B6–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos del hipocampo soluble. Se observó una ligera reducción (sin importancia) de la fosforilación de TAU evaluada como las relaciones entre pTAU y TAU total ante el tratamiento con ACI–36–2B6–Ab1.

8.2.2.4. Tau en fracción insoluble de sarcosilo de homogenatos del hipocampo

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en las fracciones insolubles de sarcosilo de los homogenatos del hipocampo de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), B (Tg, ACI–36–2B6–AB1 1 mg/kg) y C (Tg, ACI–36–2B6–Ab1 3 mg/kg). Un tratamiento con ACI–36–2B6–Ab1 no afectó en forma significativa TAU total y

pTAU en los homogenatos del hipocampo insoluble en sarcosilo. Un tratamiento con 3 mg/kg aumentó la TAU total media, p231TAU así como también p396TAU, aunque sin alcanzar una importancia significativa, mientras se observó una ligera reducción de la TAU total media, p231TAU así como también de p396TAU ante un tratamiento de 1 mg/kg. La fosforilación de TAU evaluada como las relaciones entre pTAU y TAU total fue ligeramente aumentada ante el tratamiento con 3 mg/kg de ACI–36–2B6–Ab1.

8.2.2.5. Western Blots para coteza soluble

El tratamiento con ACI–36–2B6–Ab1 en función de la dosis redujo la presencia tanto de epítopes tau pS396/pS404 (Figura 5D) como de pT181 (Figuras 5E y 5F) en la fracción soluble de la corteza cerebral con un efecto significativo en la dosis de 3 mg/kg.

8.2.2.6 TAU en ratones bigénicos Tau biGT

Los ratones biGT tratados con ACI–36–2B6–Ab1 durante 3 meses presentaron una TAU total significativamente reducida en la fracción soluble de corteza cerebral (Figura 6A y 6B). Se observó una reducción significativa para epítopes pTau pT231/AT180 (Figuras 6C y 6D), pS202/AT8 (Figura 6E) y pS396 (Figuras 6F y 6G). También se observó una reducción significativa en el homogenato total (TH) para epítope pTAU pS400 (Figuras 6H y 6I) y pS404 (Figuras 6L y 6M). Además, también se observo una reducción significativa en la fracción soluble para epítopes pTau pS400 (Figuras 6J y 6K) y una tendencia a reducirse para el epítope pTau pS404 (Figuras 6N y 6O)

8.2.3. Histología

8.2.3.1. Morfometría – Determinación de áreas de la región

Las áreas de la región medida del hipocampo y de la amígdala no difieren en forma significativa en todos los cerebros investigados, lo que excluye los efectos negativos en el tejido durante la disección e IHC o tinción

(por ejemplo, la contracción inequívoca, el seccionamiento diferente) y hasta un cierto punto, la atrofia inducida por el tratamiento. Cada una de las secciones puede desviarse de la media del grupo e individual por ejemplo, por el pliegue de tejido o pérdida de partes de la sección durante la ejecución del protocolo de etiquetado. Por lo tanto, el área inmunorreactiva total [en µm2] de cualquier etiquetado fue normalizada en el área de las regiones individuales de las secciones [en mm2] mediante el cálculo del porcentaje del área etiquetada dentro del área de la región [área etiquetada/(área de la región * 10.000)].

8.2.3.2. Resultados de AT180 IH

El anticuerpo AT180 detecta la pTAU humana y endógena (doblemente fosforilada en Thr231 y Ser235). La cantidad de pTAU intrasomal en los controles de nTg fue significativamente menor en comparación con los grupos Tg ($p < 0,01$ así como también $p < 0,001$). En la amígdala, la dosis mayor de ACI–36–2B6–Ab1 (3 mg/kg – grupo C) redujo significativamente la pTAU somal en comparación con animales tratados con el vehículo (Figura 7 izquierda). La dosis menor (1 mg/kg – grupo C) mostró la misma tendencia pero no alcanzó importancia. El mismo efecto resultó detectable en el hipocampo donde ACI–36–2B6–Ab1 redujo pTAU de dosis dependiente, significativa para la dosis mayor y tendenciosamente para la menor (Figura 7 derecha). Asimismo, esta reducción fue visible en términos cualitativos como una reducción del área de tinción y la intensidad de tinción en los somas neuronales individuales. Los resultados de la suma de intensidades de tinción normalizadas en el tamaño de AOI de IR de AT180 medida en los somas neuronales fueron comparables con el porcentaje de área de IR de AT180 medidos, incluso una dependencia a la dosis significativa del efecto mayor de la dosis mayor en la amígdala. En el hipocampo, las comparaciones *post hoc* no alcanzaron un nivel significativo.

8.2.4 Resumen

La patología Tau cerebral de forma en que fue medida por MSD no mostró un cambio significativo, no obstante, la inmunotinción de secciones del cerebro demostró una dependencia a la dosis con hasta un 60% de reducción en la inmunotinción AT180 (pT231/pS235) en somas neuronales.

El estudio indica que la inmunización pasiva mediante el uso de trece administraciones de un anticuerpo anti–pTau de fosfosita específica ACI–36–2B6–Ab1 puede mejorar el aprendizaje espacial y reducir la patología pTau cerebral.

8.3 Resultados del anticuerpo ACI–36–3A8–Ab1

Las trece inyecciones i.p. de ACI–36–3A8–Ab1 administradas en forma semanal a 1 o 3 mg/kg durante un período de doce semanas de estudio no mostraron ningún efecto adverso grave.

8.3.1. Resultados de comportamiento – Laberinto de agua de Morris

Los resultados de la prueba de MWM demostraron un efecto significativo de un aprendizaje espacial mejorado para los ratones tratados con ACI–36–3A8–Ab1 en 3 mg/kg (Figura 10).

Los animales control Tg (el grupo A) y los animales de control nTg (grupo F) tratados con el vehículo mostraron las curvas de aprendizaje esperadas en términos de latencia de escape y longitud de la trayectoria del nado para alcanzar la plataforma durante los cuatro días de prueba. Los animales de control Tg (A) mostraron una deficiencia significativa en la capacidad de aprendizaje reflejada en las curvas de aprendizaje más planas de latencias de escape y las trayectorias del nado en comparación con animales de control nTg (grupo F). Las latencias de escape y las trayectorias del nado fueron significativamente diferentes (ANOVA de dos vías) en los días 3 ($p < 0,001$, longitud; prueba posterior de Bonferroni) y 4 del entrenamiento ($p< 0,01$; prueba posterior de Bonferroni). El tratamiento con ACI–36–3A8–Ab1, de dosis

alta o baja (D y E) no mejoró en forma significativa la capacidad de aprendizaje espacial en comparación con los animales control Tg (A) cuando se analizaron en valores absolutos. Al ajustar el rendimiento de cada grupo en 100% en el día 1 de entrenamiento y todos los demás días como porcentaje del día 1, puede observarse una mejora en la capacidad de aprendizaje y memoria ante un tratamiento de ACI–36–3A8–Ab1 (ambas dosis). Los animales tratados con la dosis baja de ACI–36–3A8–Ab1 (grupo D) rindieron solo un poco mejor en la MWM si se los compara con los controles Tg (grupo A). El efecto de un tratamiento semanal con 3 mg/kg ACI–36–3A8–Ab1 (grupo E) fue mucho más pronunciado hasta casi restablecer el rendimiento de animales nTg. Comparado con los controles Tg (A), el efecto de 3 mg/kg ACI–36–3A8–Ab1 resultó significativo desde el punto de vista estadístico para la longitud de la trayectoria del nado en el día 3 y en el día 4 ($p < 0,05$). No se detectaron diferencias de tratamiento entre grupos cuando se calculó la velocidad de nado en los cuatro días de entrenamiento.

En la prueba de sondeo (PT), se registró la permanencia en el cuadrante objetivo (cuadrante sudoeste) así como también los cruces de zona objetivo. Los controles nTg (grupo F) ocuparon más tiempo en el cuadrante objetivo y cruzaron la zona objetivo con mayor frecuencia respecto de los controles Tg (grupo A) pero sin importancia sustancial. El tratamiento con ninguna dosis, ya sea alta o baja, condujo a una mejora significativa desde el punto de vista estadístico en comparación con los ratones de control Tg de acuerdo con lo evaluado en la PT. No obstante, los animales tratados con ACI–36–3A8–Ab1 presentaron más cruces de zona objetivo (aunque sin importancia estadística) en comparación con el control Tg, que se encuentra de acuerdo con el resultado de la longitud de nado durante los 4 días de entrenamiento.

8.3.2. Biología molecular

8.3.2.1. Tau en fracción soluble de homogenatos de la corteza

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en la fracción soluble de homogenatos de la corteza de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), y D (Tg, ACI–36–3A8–Ab1 1 mg/kg) y de animales n = 15 a partir del grupo E (Tg, ACI–36–3A8–Ab1, 3 mg/kg). Un tratamiento con ACI–36–3A8–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos de la corteza soluble. No obstante, se observó un ligero aumento (sin importancia) de la TAU total media, p231TAU y p396TAU ante el tratamiento con ACI–36–3A8–Ab1. La fosforilación de TAU en 231 evaluada como las relaciones entre p231TAU y TAU total fue ligeramente reducida ante el tratamiento con 3 mg/kg de ACI–36–3A8–AB1.

8.3.2.2. Tau en fracción insoluble de sarcosilo de homogenatos de la corteza

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en la fracción insoluble en sarcosilo de homogenatos de la corteza de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), y D (Tg, ACI–36–3A8–Ab1 1 mg/kg) y de animales n = 15 a partir del grupo E (Tg, ACI–36–3A8–Ab1, 3 mg/kg). Un tratamiento con ACI–36–3A8–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos de la corteza insoluble en sarcosilo. Se observó una ligera reducción (sin importancia) de la TAU total media, p231TAU y p396TAU ante el tratamiento con ACI–36–3A8–Ab1. Además, las relaciones entre p231TAU y TAU total de animales tratados con 1 mg/kg ACI–36–3A8–Ab1 mostraron una variabilidad ligeramente menor en comparación con los animales tratados con el vehículo (pero sin importancia en la prueba F: p = 0,184) sin cambiar las relaciones entre p231TAU media y TAU total de los dos grupos. Para grupos tratados con 1 mg/kg y 3 mg/kg ACI–36–3A8–Ab1, se observó un ligero aumento en la fosforilación de p396TAU.

8.3.2.3. Tau en fracción soluble de homogenatos del hipocampo

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en la fracción soluble de homogenatos del hipocampo de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), y D (Tg, ACI–36–3A8–Ab1 1 mg/kg) y de animales n = 15 a partir del grupo E (Tg, ACI–36–3A8–Ab1, 3 mg/kg). Los niveles de TAU y pTAU en las fracciones de hipocampo soluble de IRN6301 (grupo D) eran valores atípicos y los excluyó. Un tratamiento con ACI–36–3A8–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos de hipocampo soluble. Se observó una ligera reducción (sin importancia) de la fosforilación de TAU en 231 evaluada como las relaciones entre pTAU y TAU total ante el tratamiento con ACI–36–3A8–Ab1.

8.3.2.4. Tau en fracción insoluble de sarcosilo de homogenatos del hipocampo

Se evaluaron TAU total, p231TAU, p396TAU y las relaciones entre pTAU y TAU total en las fracciones insolubles de sarcosilo de los homogenatos del hipocampo de animales n = 16 a partir del grupo A (grupo vehículo Tg; PBS), B (Tg, ACI–36–3A8–AB1 1 mg/kg) y C (Tg, ACI–36–3A8–Ab1 3 mg/kg). Un tratamiento con ACI–36–3A8–Ab1 no afectó en forma significativa TAU total y pTAU en los homogenatos del hipocampo insoluble en sarcosilo. Se observó un ligero aumento (sin importancia) de la TAU total media, p231TAU y p396TAU ante el tratamiento con ACI–36–3A8–Ab1. La fosforilación de TAU evaluada como la relación entre p231TAU y TAU total no fue afectada y el tratamiento con 1mg/kg de ACI–36–3A8–Ab1 ligeramente redujo la relación entre p396TAU y TAU total.

8.3.2.5. Western Blots para corteza soluble

Una significativa reducción de epítope pTau pS396/pS404 en la corteza cerebral soluble en ratones tratados con 1 ó 3 mg/kg de ACI–36–3A8–Ab1 (Figura 5D). La presencia de epítope pTau pT181 humano/transgénico se redujo en la fracción de la corteza soluble, con un significativo efecto en

ratones tratados con 1 mg/kg y la tendencia en ratones tratados con 3 mg/kg (Figura 5E). Se observó una tendencia de una reducción en la cantidad de pTau pT181 endógeno (Figura 5F).

8.3.2.6TAU en ratones bigénicos Tau biGT

Los ratones biGT tratados con ACI–36–3A8–Ab1 durante 3 meses presentaron una TAU total significativamente reducida en la fracción soluble de corteza cerebral (Figura 6A y 6B). Se observó una reducción significativa para epítopes pTau pT231/AT180 (Figuras 6C y 6D), pS202/AT8 (Figura 6E) y pS396 (Figuras 6F y 6G). También se observó una reducción significativa en el homogenato total (TH) para epítope pTAU pS400 (Figuras 6H y 6I) y pS404 (Figuras 6L y 6M). Además, también se observo una reducción significativa en la fracción soluble para epítopes pTau pS400 (Figuras 6J y 6K) y una tendencia a reducirse para el epítope pTau pS404 (Figuras 6N y 6O)

8.3.3. Histología

8.2.3.1. Morfometría – Determinación de áreas de la región

Referirse al Ejemplo 8.2.3.1.

8.3.3.2. Resultados de AT180 IH

El anticuerpo AT180 detecta la pTAU humana y endógena (doblemente fosforilada en Thr231 y Ser235). La cantidad de PTAU intrasomal en los controles de nTg fue significativamente menor en comparación con los grupos Tg ($p < 0{,}001$). En la amígdala, ambas dosis de ACI–36–3A8–Ab1 [(1 mg/kg – grupo D) y 3 mg/kg (grupo E)] redujeron significativamente la pTAU somal en comparación con animales tratados con el vehículo (Figura 8 izquierda). Un efecto similar pudo detectarse en el hipocampo donde la dosis menor de ACI–36–3A8–Ab1 redujo pTAU; no obstante, en este caso, la dosis mayor fue menos efectiva y solo llevó a una reducción tendenciosa (Figura 8 derecha). Asimismo, esta reducción fue visible en términos cualitativos como

una reducción del área de tinción y la intensidad de tinción en los somas neuronales individuales. Los resultados de la suma normalizada de intensidades de IR de AT180 medida en los somas neuronales fueron comparables en la amígdala con un porcentaje de área de IR de AT180 medida pero alcanzó importancia solo para la dosis mayor. En el hipocampo, el resultado fue totalmente comparable con el porcentaje del área de IR.

8.3.4 Resumen

La patología Tau cerebral de forma en que fue medida por MSD no mostró un cambio significativo, no obstante, la inmunotinción de secciones del cerebro demostró una dependencia a la dosis con hasta un 40% de reducción en la inmunotinción AT180 (pT231/pS235) en somas neuronales.

El estudio indica que la inmunización pasiva mediante el uso de trece administraciones de un anticuerpo anti–pTau de fosfosita específica ACI–36–3A8–Ab1 mejora el aprendizaje espacial y reducir la patología pTau cerebral.

Depósitos:

Se depositaron las siguientes líneas celulares de hibridoma a nombre de AC Immune SA, PSE–EPFL Building B, 1015 Lausanne, Suiza y de Katholieke Universiteit Leuven, Minderbroedersstraat 8a – Box 5105, B–3000 Leuven ante el “Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) en Braunschweig, Inhoffenstrasse 7 B, 38124 Braunschweig, en virtud de las disposiciones del Tratado de Budapest:

Nombre del hibridoma	**Número de depósito**	**Fecha del depósito**
6C10F9C12A11	DSM ACC3079	25 de agosto de 2010
6C10E5E9C12	DSM ACC3081	25 de agosto de 2010

6H1A11C11	DSM ACC3080	25 de agosto de 2010
6H1G6E6	DSM ACC3088	25 de agosto de 2010
2B6A10C11	DSM ACC3084	25 de agosto de 2010
2B6G7A12	DSM ACC3087	25 de agosto de 2010
3A8A12G7	DSM ACC3086	25 de agosto de 2010
3A8E12H8	DSM ACC3085	25 de agosto de 2010
7C2(1)F10C10D3	DSM ACC3082	25 de agosto de 2010
7C2(2)B9F11D5	DSM ACC3083	25 de agosto de 2010
A4–4A6–48	DSM ACC3136	30 de agosto de 2011
A6–2G5–30	DSM ACC3137	30 de agosto de 2011
A6–2G5–41	DSM ACC3138	30 de agosto de 2011
A4–2A1–18	DSM ACC3139	30 de agosto de 2011
A4–2A1–40	DSM ACC3140	30 de agosto de 2011
A6–1D2–12	DSM ACC3141	6 de septiembre de 2011

Tabla 1. Secuencia Tau, vacuna y descripción del anticuerpo

Descripción	**Vacuna**	**Secuencia*, longitud (n), número de ID de secuencia**	**Hibridomas**	**Anticuerpos**
T1: Tau 5–20 [pY18]	ACI–33	RQEFEVMEDHAGTY(p)GL (n = 16) (SEQ ID NO: 59)	6C10F9C12A11	ACI–33–6C10–Ab1
			6C10E5E9C12	ACI–33–6C10–Ab2
T8: Tau 206–221 [pT212, pS214] T9: Secuencia 9: Tau 196–211 [pS202, pT205]	ACI–41	PGSRSRT(p)PS(p)LPTPPTR (n = 16) (SEQ ID NO: 60) GYSSPGS(p)PGT(p)PGSRSR (n = 16) (SEQ ID NO: 61)	6H1A11C11	ACI–36–6H1–Ab1
			6H1G6E6	ACI–36–6H1–Ab2
			2B6A10C11	ACI–36–2B6–Ab1
			2B6G7A12	ACI–36–2B6–Ab2
			3A8A12G7	ACI–36–3A8–Ab1
			3A8E12H8	ACI–36–3A8–Ab2
T4: Tau 401–418 [pS404, pS409]	ACI–36	GDTS(p)PRHLS(p)NVSSTGSID (n = 18) (SEQ ID NO: 63)	7C2(1)F10C10D3	ACI–41–7C2–Ab1
			7C2(2)B9F11D5	ACI–41–7C2–Ab1
T3: Tau 393–408 [pS396, pS404]	ACI–35	VYKS(p)PVVSGDTS(p)PRHL (n = 16) (SEQ ID NO: 62)	A4–4A6–48	ACI–35–4A6–Ab2
			A6–2G5–30	ACI–35–2G5–Ab2

			A6–2G5–41	ACI–35–2G5–Ab3
			A4–2A1–18	ACI–35–2A1–Ab1
			A4–2A1–40	ACI–35–2A1–Ab2
			A6–1D2–12	ACI–35–1D2–Ab1
T5: Secuencia de control: Tau 379–408 [pS396, pS404]	ACI–37	RENAKAKTDHGAEIVYKS(p)PVVSGDTS(p)PRHL (n = 30) (SEQ ID NO: 58)		
T8: Tau 206–221 [pT212, pS214]	ACI–39	PGSRSRT(p)PS(p)LPTPPTR (n = 16) (SEQ ID NO: 60)		
T9 : Tau 196–211 [pS202, pT205]	ACI–40	GYSSPGS(p)PGT(p)PGSRSR (n = 16) (SEQ ID NO: 61)		
T2: Tau 200–216 [pS202+ pT205 & pT212+pS214]	ACI–34	PGS(p)PGT(p)PGSRSRT(p)PS(p)LP (n = 17) (SEQ ID NO: 64)		
T10: Tau 407–418 [pS409]	ACI–42	HLS(p)NVSSTGSID (n = 12) (SEQ ID NO: 65)		
T11: Tau 399–408 [pS404]	ACI–43	VSGDTS(p)PRHL (n = 10) (SEQ ID NO: 66)		

*En base a la isoforma más larga de Tau humana (Tau441). p indica residuo fosforilado.

Tabla 2. Resultados del control de hibridoma ACI–33

Control con placa de 24 cavidades		*Control con recipientes T25*		
Positivo en ELISA	**Positivo en TAUPIR**	**Positivo en control de IgG**	**Positivo en ELISA**	**Positivo en TAUPIR**
1A7		1A7		
	1A11			
	1C11	1C11		
2C9		2C9		
3C3		3C3	3C3	
3C5		3C5		
3E8		3E8		
3G10	3G10	3G10	3G10	
6C10	6C10	6C10	6C10	6C10
6F3		6F3		
6F8		6F8		

Tabla 3. Resultados del control de hibridoma ACI–36

Control con placa de 24 cavidades		*Control con recipientes T25*		
Positivo en ELISA	**Positivo en TAUPIR**	**Positivo en control de IgG**	**Positivo en ELISA**	**Positivo en TAUPIR**
2B6	2B6	2B6	2B6	2B6
2F9	2F9	2F9	2F9	2F9
2G1		2G1	2G1	2G1

3A8	3A8	3A8	3A8	3A8
3B9		3B9	3B9	3B9
3F11	3F11	3F11		3F11
	4A3			4A3
4C1		4C1	4C1	4C1
4C12		4C12	4C12	4C12
4E12		4E12	4E12	4E12
5E10		5E10	5E10	
5F5		5F5	5F5	
7D6	7D6	7D6	7D6	7D6
6H1		6H1	6H1	6H1

Tabla 4: Clasificación para clones positivos en ELISA y TAUPIR de ACI–36

clasif. para ELISA	clasif. para TAUPIR
3A8	6H1
2B6	4C1
4C1	3A8
6H1	4C12
4C12	2B6
2G1	2F9
2F9	3B9
7D6	2G1
3B9	7D6
4E12	4E12

Tabla 5. Resultados del control de hibridoma ACI–41

Control con placa de 24 cavidades		*Control con recipientes T25*		
Positivo en ELISA	**Positivo en TAUPIR**	**Positivo en ELISA**	**Positivo en TAUPIR**	**Positivo en ELISA**
	3D11	3D11		3D11

4H6		4H6		4H6
5D10	5D10	5D10	5D10	5D10
5E6	5E6			
5F10		5F10		
6B7		6B7	6B7	
7C2	7C2	7C2	7C2	7C2
	8G8			8G8
	8H8	8H8		8H8

Tabla 6. Control de hibridomas para unirse con el blanco

Hibridomas	**Anticuerpos**	**ELISA**				**TAUPIR**	**Western Blot**
		Péptido Tau p	*Péptido Tau*	*pTau de longitud total*	*Tau de longitud total*		
6C10F9C12A11	ACI–33–6C10–Ab1	**+**	**–**	**+/–**	**–**	**+**	**–**
6C10E5E9C12	ACI–33–6C10–Ab2	**+**	**–**	**+/–**	**–**	**+**	**–**
6H1A11C11	ACI–36–6H1–Ab1	**+**	**–**	**+**	**–**	**+**	**+**
6H1G6E6	ACI–36–6H1–Ab2	**+**	**–**	**+**	**–**	**+**	**+**

2B6A10C11	ACI–36–2B6–Ab1	+	–	+	–	+	+
2B6G7A12	ACI–36–2B6–Ab2	+	–	+	–	+	+
3A8A12G7	ACI–36–3A8–Ab1	+	–	+	–	+	+
3A8E12H8	ACI–36–3A8–Ab2	+	–	+	–/+	+	+
7C2(1)F10C10D3	ACI–41–7C2–Ab1	+	–	+	–	+	–
7C2(2)B9F11D5	ACI–41–7C2–Ab2	+	–	+	–	+	–
A4–2A1–18	ACI–35–2A1–Ab1	+	–	+	–		
A4–2A1–40	ACI–35–2A1–Ab2	+	–	+	–		
A4–4A6–18	ACI–35–4A6–Ab1	+	–	–	+		
A4–4A6–48	ACI–35–4A6–Ab2						
A6–1D2–12	ACI–35–1D2–Ab1	+	–	+	–		
A6–2G5–08	ACI–35–2G5–Ab1	+	–	–	–		
A6–2G5–30	ACI–35–2G5–Ab2	+	–	+	–		
A6–2G5–41	ACI–35–	+	–	+	–		

	2G5–Ab3						

Tabla 7. Afinidad de unión de anticuerpos anti–tau

Hibridomas	**Anticuerpos**	**Constante de velocidad de asociación (k_d) (1/Ms)**	**Constante de velocidad de disociación (k_a) (1/s)**	**Constante de disociación (K_D) (nM)**
6C10F9C12A 11	ACI–33–6C10–Ab1	9.46×10^{5}	3.27×10^{-3}	3.46
6H1A11C11	ACI–36–6H1–Ab1	3.53×10^{4}	6.80×10^{-5}	1.93
6H1G6E6	ACI–36–6H1–Ab2	9.99×10^{4}	9.58×10^{-5}	0.96
2B6A10C11	ACI–36–2B6–Ab1	6.90×10^{5}	1.63×10^{-4}	0.24
2B6G7A12	ACI–36–2B6–Ab2	9.11×10^{5}	1.11×10^{-4}	0.12
3A8A12G7	ACI–36–3A8–Ab1	1.01×10^{6}	1.09×10^{-4}	0.11
3A8E12H8	ACI–36–3A8–Ab2	8.43×10^{5}	1.43×10^{-4}	0.17
A4–4A6–18	ACI–35–4A6–Ab1	2.00×10^{5}	3.10×10^{-3}	16
A6–1D2–12	ACI–35–1D2–Ab1	1.60×10^{3}	9.30×10^{-6}	≤ 6
A6–2G5–08	ACI–35–2G5–	4.80×10^{5}	5.30×10^{-3}	10

	Ab1			

Tabla 8. Librerías de péptidos usadas para mapeo de epítope

Librería de péptidos para T1																
N.º de aminoácidos Tau(441)		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Aminoácido		R	Q	E	F	E	V	M	E	D	H	A	G	T	Y (P)	G
	Péptido N.º															
Fosfopéptidos	T1.18											A	G	T	Y(P)	G
	T1.17										H	A	G	T	Y(P)	G
	T1.16									D	H	A	G	T	Y(P)	G
	T1.15								E	D	H	A	G	T	Y(P)	G
	T1.14							M	E	D	H	A	G	T	Y(P)	G
	T1.13						V	M	E	D	H	A	G	T	Y(P)	G
	T1.12					E	V	M	E	D	H	A	G	T	Y(P)	G
	T1.11				F	E	V	M	E	D	H	A	G	T	Y(P)	G
	T1.10			E	F	E	V	M	E	D	H	A	G	T	Y(P)	G
	T1.9		Q	E	F	E	V	M	E	D	H	A	G	T	Y(P)	G
	T1.7	R	Q	E	F	E	V	M	E	D	H	A	G	T	Y(P)	G
Aminoácido		R	Q	E	F	E	V	M	E	D	H	A	G	T	Y	G
	Péptido N.º															
No fosfopéptidos	T1.28											A	G	T	Y	G
	T1.27										H	A	G	T	Y	G
	T1.26									D	H	A	G	T	Y	G
	T1.25								E	D	H	A	G	T	Y	G
	T1.24							M	E	D	H	A	G	T	Y	G
	T1.23						V	M	E	D	H	A	G	T	Y	G
	T1.22					E	V	M	E	D	H	A	G	T	Y	G
	T1.21				F	E	V	M	E	D	H	A	G	T	Y	G
	T1.20			E	F	E	V	M	E	D	H	A	G	T	Y	G
	T1.19		Q	E	F	E	V	M	E	D	H	A	G	T	Y	G
	T1.8	R	Q	E	F	E	V	M	E	D	H	A	G	T	Y	G

Tabla 8. Continuación

Librería de péptidos para T4

N.º aminoácido Tau(441)		401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	4
Aminoácido		G	D	T	S(p)	P	R	H	L	S(p)	N	V	S	S	T	G	S	
	Péptido N.º																	
Fosfopéptidos	**T3.17**	G	D	T	S(p)	P	R	H	L									
	T4.11		D	T	S(p)	P	R	H	L	S(p)								
	T4.12			T	S(p)	P	R	H	L	S(p)	N							
	T4.13				S(p)	P	R	H	L	S(p)	N	V						
	T4.14					P	R	H	L	S(p)	N	V	S					
	T4.15						R	H	L	S(p)	N	V	S	S				
	T4.16							H	L	S(p)	N	V	S	S	T			
	T4.17								L	S(p)	N	V	S	S	T	G		
	T4.18									S(p)	N	V	S	S	T	G	S	
	T4.19										N	V	S	S	T	G	S	
	T4.20											V	S	S	T	G	S	
Aminoácido		G	D	T	S	P	R	H	L	S	N	V	S	S	T	G	S	
	Péptido N.º																	
No fosfopéptidos	**T3.26**	G	D	T	S	P	R	H	L									
	T4.21		D	T	S	P	R	H	L	S								
	T4.22			T	S	P	R	H	L	S	N							
	T4.23				S	P	R	H	L	S	N	V						
	T4.24					P	R	H	L	S	N	V	S					
	T4.25						R	H	L	S	N	V	S	S				
	T4.26							H	L	S	N	V	S	S	T			
	T4.27								L	S	N	V	S	S	T	G		
	T4.28									S	N	V	S	S	T	G	S	
	T4.19										N	V	S	S	T	G	S	
	T4.20											V	S	S	T	G	S	

Tabla 8. Continuación

Librería de péptidos para T8

N.º de aminoácido Tau(441)		206	207	208	209	210	211	212	213	214	215	216	217	218	219
Aminoácido		P	G	S	R	S	R	T(p)	P	S(p)	L	P	T	P	P
	Péptido N.º														
Fosfopéptidos	**T8.7**	P	G	S	R	S	R	T(p)	P						
	T8.8		G	S	R	S	R	T(p)	P	S(p)					
	T8.9			S	R	S	R	T(p)	P	S(p)	L				
	T8.10				R	S	R	T(p)	P	S(p)	L	P			
	T8.11					S	R	T(p)	P	S(p)	L	P	T		
	T8.12						R	T(p)	P	S(p)	L	P	T	P	
	T8.13							T(p)	P	S(p)	L	P	T	P	P
	T8.14								P	S(p)	L	P	T	P	P
	T8.15									S(p)	L	P	T	P	P
Aminoácido		P	G	S	R	S	R	T	P	S	L	P	T	P	P
	Péptido N.º														
No fosfopéptidos	**T8.16**	P	G	S	R	S	R	T	P						
	T8.17		G	S	R	S	R	T	P	S					
	T8.18			S	R	S	R	T	P	S	L				
	T8.19				R	S	R	T	P	S	L	P			
	T8.20					S	R	T	P	S	L	P	T		
	T8.21						R	T	P	S	L	P	T	P	
	T8.22							T	P	S	L	P	T	P	P
	T8.23								P	S	L	P	T	P	P
	T8.24									S	L	P	T	P	P

Tabla 8. Continuación

Librería de péptidos para T9														
N.º de aminoácidos Tau(441)		196	197	198	199	200	201	202	203	204	205	206	207	208	209
Aminoácido		G	Y	S	S	P	G	S(p)	P	G	T(p)	P	G	S	R
	Péptido N.º														
Fosfopéptidos	T9.7	G	Y	S	S	P	G	S(p)	P						
	T9.8		Y	S	S	P	G	S(p)	P	G					
	T9.9			S	S	P	G	S(p)	P	G	T(p)				
	T9.10				S	P	G	S(p)	P	G	T(p)	P			
	T9.11					P	G	S(p)	P	G	T(p)	P	G		
	T9.12						G	S(p)	P	G	T(p)	P	G	S	
	T9.13							S(p)	P	G	T(p)	P	G	S	R
	T9.14								P	G	T(p)	P	G	S	R
	T9.15									G	T(p)	P	G	S	R
Aminoácido		G	Y	S	S	P	G	S	P	G	T	P	G	S	R
	Péptido N.º														
No fosfopéptidos	T9.16	G	Y	S	S	P	G	S	P						
	T9.17		Y	S	S	P	G	S	P	G					
	T9.18			S	S	P	G	S	P	G	T				
	T9.19				S	P	G	S	P	G	T	P			
	T9.20					P	G	S	P	G	T	P	G		
	T9.21						G	S	P	G	T	P	G	S	
	T9.22							S	P	G	T	P	G	S	R
	T9.23								P	G	T	P	G	S	R
	T9.24									G	T	P	G	S	R

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Tabla 8. Continuación

Librería de péptidos para T3															
N.º de aminoácidos Tau(441)		393	394	395	396	397	398	399	400	401	402	403	404	405	406	407
Aminoácidos		V	Y	K	S(p)	P	V	V	S	G	D	T	S(p)	P	R	H
	Péptido N.º															
Fosfopéptidos	T3.9	V	Y	K	S(p)	P	V	V	S							
	T3.10		Y	K	S(p)	P	V	V	S	G						
	T3.11			K	S(p)	P	V	V	S	G	D					
	T3.12				S(p)	P	V	V	S	G	D	T				
	T3.13					P	V	V	S	G	D	T	S(p)			
	T3.14						V	V	S	G	D	T	S(p)	P		
	T3.15							V	S	G	D	T	S(p)	P	R	
	T3.16								S	G	D	T	S(p)	P	R	H
	T3.17									G	D	T	S(p)	P	R	H
Aminoácido		V	Y	K	S	P	V	V	S	G	D	T	S	P	R	H
	Péptido N.º															
No fosfopéptidos	T3.18	V	Y	K	S	P	V	V	S							
	T3.19		Y	K	S	P	V	V	S	G						
	T3.20			K	S	P	V	V	S	G	D					
	T3.21				S	P	V	V	S	G	D	T				
	T3.22					P	V	V	S	G	D	T	S			
	T3.23						V	V	S	G	D	T	S	P		
	T3.24							V	S	G	D	T	S	P	R	
	T3.25								S	G	D	T	S	P	R	H
	T3.26									G	D	T	S	P	R	H

Tabla 9. Aminoácidos Tau y fosfo–residuos requeridos para la unión de anticuerpos.

Vacuna	Hibridoma	Epítope*
ACI–33	6C10F9C12A11	Tau aa 15–20, con requerimiento por pY18
ACI–33	6C10E5E9C12	Tau aa 15–20, con requerimiento por pY18
ACI–36	6H1A11C11	Tau aa 405–412, con requerimiento por pS409
ACI–36	6H1G6E6	Tau aa 405–412, con requerimiento por pS409
ACI–36	2B6A10C11	Tau aa 405–411, con requerimiento por pS409
ACI–36	2B6G7A12	Tau aa 405–411, con requerimiento por pS409
ACI–36	3A8A12G7	Tau aa 405–411, con requerimiento por pS409
ACI–36	3A8E12H8	Tau aa 405–411, con requerimiento por pS409
ACI–41	7C2(1)F10C10D3	Tau aa 208–218, con requerimiento por pT212 y pS214
ACI–35	A4–2A1–18	Tau aa 393–401, con requerimiento por pS396
ACI–35	A4–2A1–40	Tau aa 393–401, con requerimiento por pS396
ACI–35	A4–4A6–18	Tau aa 396–401, con requerimiento por pS396

ACI–35	A6–1D2–12	Tau aa 394–400, con requerimiento por pS396
ACI–35	A6–2G5–08	Tau aa 402–406, con requerimiento por pS404
ACI–35	A6–2G5–30	Tau aa 393–400, con requerimiento por pS396
ACI–35	A6–2G5–41	Tau aa 393–400, con requerimiento por pS396

*En base a la isoforma más larga de Tau humana (Tau441)

Tabla 10. Secuencia de aminoácidos de las regiones variables de cadena pesada y de cadena liviana (VH y VK) y CDRs

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**	**VH CDR1**	**VH CDR2**	**VH CDR3**	**VK CDR1**	**VK CDR2**	**VK CDR3**
ACI–36	3A8A12G7*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49/ y SEQ ID NO: 51)	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNRGGTTYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCASYYAVGYWGQGTTLTVSS (SEQ ID NO: 1)	DIVMTQSPSSLAMSVGQKVTMSCKSSQSVFNSGNQKNSLAWYQQKPGQSPKLLVYFASTRESGVPDRFIGSGSGTDFSLTISSVQAEDLADYFCQEHYTTPPTFGTGTKLELK (SEQ ID NO: 6)	GYTFTDYYMN (SEQ ID NO: 12)	DINPNRGGTTYNQKFKG (SEQ ID NO: 13)	YYAVGY (SEQ ID NO: 14)	KSSQSVFNSGNQKNSLA (SEQ ID NO: 21)	FASTRES (SEQ ID NO: 22)	QEHYTTPPT (SEQ ID NO: 23)
ACI–36	3A8A12G7*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_G (SEQ ID NO: 50 y SEQ ID	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNRGGTTYNQKFK	DVVMTQTPLSLPVSLGDQASISCRSSQRLVHSHGKTYLHWYLQKPGQSPKLLIYKVSNRFSGVPD	GYTFTDYYMN (SEQ ID NO: 12)	DINPNRGGTTYNQKFKG (SEQ ID	YYAVGY (SEQ ID NO: 14)	RSSQRLVHSHGKTYLH (SEQ ID NO: 24)	KVSNRFS (SEQ ID NO: 25)	SQTAHFPYT (SEQ ID NO: 26)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
			NO: 51)	GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1	RFSGSGSGTDFTLKISR VEAEDLGVYFCSQTAH FPYTFGGGTKLEIK (SEQ ID NO: 7)		NO: 13)				
ACI–36	3A8E12H8*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49 y SEQ ID NO: 51)	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCAS YYAVGYWGQGTTLTVS S (SEQ ID NO: 1)	DIVMTQSPSSLAMSVG QKVTMSCKSSQSVFNS GNQKNSLAWYQQKPG QSPKLLVYFASTRESGV PDRFIGSGSGTDFSLTI SSVQAEDLADYFCQEH YTTPPTFGTGTKLELK (SEQ ID NO: 6)	GYTFTD YYMN (SEQ ID NO: 12	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	KSSQSVF NSGNQK NSLA (SEQ ID NO: 21)	FASTRE S (SEQ ID NO: 22)	QEHYTT PPT (SEQ ID NO: 23)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI–36	3A8E12H8*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_G (SEQ ID NO: 50 y SEQ ID NO: 51)	EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNRGGTTYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCASYYAVGYWGQGTTLTVSS (SEQ ID NO: 1)	DVVMTQTPLSLPVSLGDQASISCRSSQRLVHSHGKTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQTAHFPYTFGGGTKLEIK (SEQ ID NO: 7)	GYTFTDYYMN (SEQ ID NO: 12	DINPNRGGTTYNQKFKG (SEQ ID NO: 13)	YYAVGY (SEQ ID NO: 14)	RSSQRLVHSHGKTYLH (SEQ ID NO: 24)	KVSNRFS (SEQ ID NO: 25)	SQTAHFPYT (SEQ ID NO: 26)
ACI–36	2B6A10C11	SEQ ID NO: 46/SEQ ID NO: 52 y	SEQ ID NO: 50 y SEQ ID NO: 51	EVQLQQSGPELVKPGTSVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNRGGTTYNQKFKGKATLTVDKSSSTAYM	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSHGKTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRV	GYTFTDYYMN (SEQ ID NO: 12)	DINPNRGGTTYNQKFKG (SEQ ID NO: 13)	YYAVGY (SEQ ID NO: 14)	RSSQSLVHSHGKTYLH (SEQ ID NO: 27)	KVSNRFS (SEQ ID NO: 25)	SQTAHFPYT (SEQ ID NO: 26)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
		SEQ ID NO: 47		ELRSLTSEDSAVYYCASYYAVGYWGQGTTLTVSS (SEQ ID NO: 2)	EAEDLGVYFCSQTAHFPYTFGGGTKLEIK (SEQ ID NO: 8)						
ACI–36	2B6G7A12	SEQ ID NO: 46/SEQ ID NO: 52 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	EVQLQQSGPELVKPGTSVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNRGGTTYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCASYYAVGYWGQGTTLTVSS (SEQ ID NO: 2)	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSHGKTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQTAHFPYTFGGGTKLEIK (SEQ ID NO: 8)	GYTFTDYYMN (SEQ ID NO: 12)	DINPNRGGTTYNQKFKG (SEQ ID NO: 13)	YYAVGY (SEQ ID NO: 14)	RSSQSLVHSHGKTYLH (SEQ ID NO: 27)	KVSNRFS (SEQ ID NO: 25)	SQTAHFPYT (SEQ ID NO: 26)
ACI–36	6H1A11C11	SEQ ID NO: 46 y	SEQ ID NO: 50 y	EVQLQQSGPELVKPGASVKISCKASGYTFTDYY	DVVMTQTPLSLPVSLGDQASISCRSSQSLLHSH	GYTFTDYYMN	DINPNRGGTTYN	YYAVGY (SEQ	RSSQSLLHSHGNT	KVSNRFS (SEQ	SQTAHFPYT

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
		SEQ ID NO: 47	SEQ ID NO: 51	MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDTSSSTAYME LRSLTSEDSAVYYCASY YAVGYWGQGTTLTVSS (SEQ ID NO: 3)	GNTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ ID NO: 9)	(SEQ ID NO: 12)	QKFKG (SEQ ID NO: 13)	ID NO: 14)	YLH (SEQ ID NO: 28)	ID NO: 25)	(SEQ ID NO: 26))
ACI–36	6H1G6E6	SEQ ID NO: 46 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNRGGTTYNQKFK GKATLTVDTSSSTAYME LRSLTSEDSAVYYCASY YAVGYWGQGTTLTVSS	DVVMTQTPLSLPVSLG DQASISCRSSQSLLHSH GNTYLHWYLQKPGQSP KLLIYKVSNRFSGVPDR FSGSGSGTDFTLKISRV EAEDLGVYFCSQTAHF PYTFGGGTKLEIK (SEQ	GYTFTD YYMN (SEQ ID NO: 12)	DINPNR GGTTYN QKFKG (SEQ ID NO: 13)	YYAVG Y (SEQ ID NO: 14)	RSSQSLL HSHGNT YLH (SEQ ID NO: 28)	KVSNRF (SEQ ID NO: 25)	SQTAHF PYT (SEQ ID NO: 26)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
				(SEQ ID NO: 3)	ID NO: 9)						
ACI–33	6C10E5E9C12	SEQ ID NO: 53/SEQ ID NO:54 y SEQ ID NO: 47	SEQ ID NO: 48/SEQ ID NO:49 y SEQ ID NO: 51	EVQLVESGGGLVKPGGSLKLSCAPSGFTFSDYGMHWVRQAPEKGLEWVAYISSGSSTIYYGDTVKGRFTISRDNAKNTLFLQMTSLRSEDTAMYYCARRGQLRLRLFAYWGQGTLVTVSA (SEQ ID NO: 4)	DIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYTTPLTFGAGTKLELK (SEQ ID NO: 10)	GFTFSDYGMH (SEQ ID NO: 15)	YISSGSSTIYYGDTVKG (SEQ ID NO: 16)	RGQLRLRLFAY (SEQ ID NO: 17)	KASQDVSTAVA (SEQ ID NO: 29)	SASYRYT (SEQ ID NO: 30)	QQHYTTPLT (SEQ ID NO: 31)
ACI–33	6C10F9C12A11	SEQ ID NO: 53/SEQ	SEQ ID NO: 54 y SEQ ID	EVQLVESGGGLVKPGGSLKLSCAPSGFTFSDYGMHWVRQAPEKGLEWV	DIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIY	GFTFSDYGMH (SEQ ID	YISSGSSTIYYGDTVKG	RGQLRLRLFAY (SEQ ID	KASQDVSTAVA (SEQ ID	SASYRYT (SEQ ID NO:	QQHYTTPLT (SEQ ID NO:

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
		ID NO:54 y SEQ ID NO: 47	NO: 51	AYISSGSSTIYYGDTVKGRFTISRDNAKNTLFLQMTSLRSEDTAMYYCARRGQLRLRLFAYWGQGTLVTVSA (SEQ ID NO: 4)	SASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHYTTPLTFGAGTKLELK (SEQ ID NO: 10)	NO: 15)	(SEQ ID NO: 16)	NO: 17)	NO: 29)	30)	31)
ACI–41	7C2(1)F10C10D3	SEQ ID NO: 53/ SEQ ID NO:55 y SEQ ID NO: 47	SEQ ID NO: 49/SEQ ID NO:56/SEQ ID NO: 57 y SEQ ID NO: 51	EVKLMESGGGLVHPGASLRLYCAASGFTFTDYYMSWVRQPPGKAPEWLALIRNKANGYTTEYTASVKGRFTISRDNSQNILYLQMNTLRAEDSATYYCVKALGRYFDVWGTGTTVTVSS(SEQ ID NO: 5)	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYSYPFTFGSGTKLEIK (SEQ ID NO: 11)	GFTFTDYYMS (SEQ ID NO: 18)	LIRNKANGYTTEYTASVKG (SEQ ID NO: 19)	ALGRYFDV (SEQ ID NO: 20)	KSSQSLLYSSNQKNYLA (SEQ ID NO: 32)	WASTRES (SEQ ID NO: 33)	QQYYSYPFT (SEQ ID NO: 34)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI–41	7C2(2)B9F1 1D5	SEQ ID NO: 53/ SEQ ID NO:55 y SEQ ID NO: 47	SEQ ID NO: 57 y SEQ ID NO: 51	EVKLMESGGGLVHPGA SLRLYCAASGFTFTDYY MSWVRQPPGKAPEWL ALIRNKANGYTTEYTAS VKGRFTISRDNSQNILY LQMNTLRAEDSATYYC VKALGRYFDVWGTGTT VTVSS (SEQ ID NO: 5)	DIVMSQSPSSLAVSVGE KVTMSCKSSQSLLYSS NQKNYLAWYQQKPGQ SPKLLIYWASTRESGVP DRFTGSGSGTDFTLTIS SVKAEDLAVYYCQQYY SYPFTFGSGTKLEIK (SEQ ID NO: 11)	GFTFTD YYMS (SEQ ID NO: 18)	LIRNKAN GYTTEY TASVKG (SEQ ID NO: 19)	ALGRY FDV (SEQ ID NO: 20)	KSSQSLL YSSNQK NYLA(SE Q ID NO: 32)	WASTRE S (SEQ ID NO: 33)	QQYYSY PFT (SEQ ID NO: 34)
ACI–35	A4–4A6–18			QVQLQQPGAELLKPGA SVKLSCKASGYTFTSY WMHWVKQRPGRGLE WIGRIDPNSDRTKYNEK FKRKATLTVDKSSSTAY	DVLMTQTPLSLPVSLGD QASISCRSSQSIVHSNG NTYLEWYLQKPGQSPK LLIYKLSNRFSGVPDRF SGSGSGTDFTLKISRVE	GYTFTS YWMH (SEQ ID NO: 70)	RIDPNS DRTKYN EKFKR (SEQ ID NO: 71)	DDYAW FAY (SEQ ID NO: 72)	RSSQSIV HSNGNT YLE (SEQ ID NO: 73)	KLSNRF S (SEQ ID NO: 74)	FQGSHV PPT (SEQ ID NO: 75)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
				MQLSSLTSEDSAVYYCARDDYAWFAYWGQGTLVTVSA (SEQ ID NO: 68)	AEDLGVYYCFQGSHVPPTFGGGTKLEIK (SEQ ID NO: 69)						
ACI–35	A6–1D2–12			QVTLKESGPGILQSSQTLSLTCSFSGFSLSTSGMGVSWIRQPSGKGLEWLAHIYWDDDKRYNASLKSRLTISKDTSRNQVFLKITCVDTADTATYYCARLLRPYALDYWGQGTSVTVSS (SEQ ID NO: 76)	NILMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCLQYLSSLTFGAGTKLELK (SEQ ID NO: 77)	GFSLSTSGMGVS (SEQ ID NO: 78)	HIYWDDDKRYNASLKS (SEQ ID NO: 79)	LLRPYALDY (SEQ ID NO: 80)	KSSQSVLYSSNQKNYLA (SEQ ID NO: 81)	WASTRES (SEQ ID NO: 82)	LQYLSSLT (SEQ ID NO: 83)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI–35	A4–2A1–18			EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYCVREGRFAYWGHGTLVTVSA (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIHRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLKSPYTFGGGTKLEIK (SEQ ID NO: 116)	GYTFTDYYMN (SEQ ID NO: 89)	DINPNNGGTSYNQKFKG (SEQ ID NO: 90)	EGRFAY (SEQ ID NO: 91)	RSSKSLLHSNGNTYLY (SEQ ID NO: 93)	RMSNLAS (SEQ ID NO: 94)	MQHLKSPYT (SEQ ID NO: 95)
ACI–35	A4–2A1–40			EVQLQQSGPELVKPGASVKISCKASGYTFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYNQKFKGKATLTVDKSSSTAYM	DIX*MTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRV	GYTFTDYYMN (SEQ ID NO: 89)	DINPNNGGTSYNQKFKG (SEQ ID NO: 90)	EGRFAY (SEQ ID NO: 91)	RSSKSLLHSNGNTYLY (SEQ ID NO: 93)	RMSNLAS (SEQ ID NO: 94)	MQHLKSPYT (SEQ ID NO: 95)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
				ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	EAEDVGVYYCMQHLKS PYTFGGGTKLEIK (SEQ ID NO: 92) *X = M o V						
ACI–35	A4–4A6–48			EVQLQQSGPELVKPGA SVKISCKASGYTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYNQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGHGTLVTVS A (SEQ ID NO: 88)	DIVMTQAAPSVPVTPGE SVSISCRSSKSLLHSNG NTYLYWFLQRPGQSPQ LLIYRMSNLASGVPDRF SGSGSGTAFTLRISRVE AEDVGVYYCMQHLKSP YTFGGGTKLEIK (SEQ ID NO: 118)	GYTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYN QKFKG (SEQ ID NO: 90)	EGRFA Y (SEQ ID NO: 91)	RSSKSLL HSNGNT YLY (SEQ ID NO: 93)	RMSNLA S (SEQ ID NO: 94)	MQHLKS PYT (SEQ ID NO: 95)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
ACI–35	A6–2G5–08			QVQLKQSGAELVRPGASVKLSCKASGYTFTDYYINWVKQRPGQGLEWIARIYPGRGNIYYNEKFKGKATLTAEKSSSTAYMQLSSLTSEDSAVYFCARFWDVTYWGQGTLVTVSA (SEQ ID NO: 96)	DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWFLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGGGTKLEIK (SEQ ID NO: 97)	GYTFTDYYIN (SEQ ID NO: 98)	RIYPGRGNIYYNEKFKG (SEQ ID NO: 99)	FWDVTY (SEQ ID NO: 100)	RSSQSIVHSNGNTYLE (SEQ ID NO: 101)	KVSNRFS (SEQ ID NO: 102)	FQGSHVPYT (SEQ ID NO: 103)
ACI–35	A6–2G5–30			EVQLQQSGPELVKPGASVKISCKASGFTFTDYYMNWVKQSHGKSLEWIGDINPNNGGTSYHQKFKGKATLTVDKSSSTAYM	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSE	GFTFTDYYMN (SEQ ID NO: 89)	DINPNNGGTSYHQKFKG (SEQ ID NO: 115)	EGRFAY (SEQ ID NO: 91)	KASQNVGTNVA (SEQ ID NO: 106)	SASYRYS (SEQ ID NO: 107)	QQYNSYPYT (SEQ ID NO: 108)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK	VH CDR1	VH CDR2	VH CDR3	VK CDR1	VK CDR2	VK CDR3
				ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)						
ACI–35	A6–2G5–41			EVQLQQSGPELVKPGA SVKISCKASGFTFTDYY MNWVKQSHGKSLEWIG DINPNNGGTSYHQKFK GKATLTVDKSSSTAYM ELRSLTSEDSAVYYCVR EGRFAYWGQGTLVTVS A (SEQ ID NO: 104)	DIVMTQSQKFMSTSVG DRVSVTCKASQNVGTN VAWYQQKPGQSPKALI YSASYRYSGVPDRFTG SGSGTDFTLTISNVQSE DLAEYFCQQYNSYPYT FGGGTKLEIK (SEQ ID NO: 105)	GFTFTD YYMN (SEQ ID NO: 89)	DINPNN GGTSYH QKFKG (SEQ ID NO: 115)	EGRFA Y (SEQ ID NO: 91)	KASQNV GTNVA (SEQ ID NO: 106)	SASYRY S (SEQ ID NO: 107)	QQYNSY PYT (SEQ ID NO: 108)

Tabla 11. Secuencia de nucleótidos de las regiones variables de cadena pesada y cadena liviana (VH y VK)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	3A8A12G7*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49/ y SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 35)	GACATTGTGATGACACAGTCTCCATCCTCCCTGG CTATGTCAGTAGGACAGAAGGTCACTATGAGCTG CAAGTCCAGTCAGAGTGTTTTTAATAGTGGCAAT CAAAAGAACTCTTTGGCCTGGTACCAGCAGAAAC CAGGACAGTCTCCTAAACTTCTGGTATACTTTGC ATCCACTAGGGAATCTGGGGTCCCTGATCGCTTC ATAGGCAGTGGATCTGGGACAGATTTCAGTCTTA CCATCAGCAGTGTGCAGGCTGAGGACCTGGCAG ATTACTTCTGTCAGGAACATTATACCACTCCTCCC ACGTTCGGTACTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 40)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	3A8A12G7*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_G (SEQ ID NO: 50 y SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 35)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGGCTTGTACACAGTCATGGA AAAACCTATTTACATTGGTACCTGCAGAAGCCAG GCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTC CAACCGGTTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTTCTGTTCTCAAACTGCACATTTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 41)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	3A8E12H8*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_AD (SEQ ID NO: 48/ SEQ ID NO: 49 y SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 35)	GACATTGTGATGACACAGTCTCCATCCTCCCTGG CTATGTCAGTAGGACAGAAGGTCACTATGAGCTG CAAGTCCAGTCAGAGTGTTTTTAATAGTGGCAAT CAAAAGAACTCTTTGGCCTGGTACCAGCAGAAAC CAGGACAGTCTCCTAAACTTCTGGTATACTTTGC ATCCACTAGGGAATCTGGGGTCCCTGATCGCTTC ATAGGCAGTGGATCTGGGACAGATTTCAGTCTTA CCATCAGCAGTGTGCAGGCTGAGGACCTGGCAG ATTACTTCTGTCAGGAACATTATACCACTCCTCCC ACGTTCGGTACTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 40)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	3A8E12H8*	SEQ ID NO: 46 y SEQ ID NO: 47	VK_G (SEQ ID NO: 50 y SEQ ID NO: 51)	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 35)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGGCTTGTACACAGTCATGGA AAAACCTATTTACATTGGTACCTGCAGAAGCCAG GCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTC CAACCGGTTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTTCTGTTCTCAAACTGCACATTTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 41)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	2B6A10C11	SEQ ID NO: 46/SEQ ID NO: 52 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGACTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTTAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 36)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCCTTGTACACAGTCATGGA AAAACCTATTTACATTGGTACCTGCAGAAGCCAG GCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTC CAACCGGTTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTTCTGTTCTCAAACTGCACATTTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 42)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	2B6G7A12	SEQ ID NO: 46/SEQ ID NO: 52 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGACTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATATACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTTAAGGGCAAGGCCACGTTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAACTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 36)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCCTTGTACACAGTCATGGA AAAACCTATTTACATTGGTACCTGCAGAAGCCAG GCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTC CAACCGGTTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTTCTGTTCTCAAACTGCACATTTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 42)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	6H1A11C11	SEQ ID NO: 46 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATACACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACACGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 37)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCA GAGCCTTCTACACAGTCATGGAAACACCTATTTA CATTGGTACCTGCAGAAGCCAGGCCAGTCTCCA AAGCTCCTGATCTACAAAGTTTCCAACCGGTTTT CTGGGGTCCCAGACAGGTTCAGTGGCAGTGGAT CAGGGACAGATTTCACACTCAAGATCAGCAGAGT GGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCT CAAACTGCACATTTTCCGTACACGTTCGGAGGGG GGACCAAGCTGGAAATAAAA (SEQ ID NO: 43)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–36	6H1G6E6	SEQ ID NO: 46 y SEQ ID NO: 47	SEQ ID NO: 50 y SEQ ID NO: 51	GAGGTCCAGCTGCAACAATCTGGACCTGAA CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATACACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACCGTGGTGGAACTACTTACAAC CAGAAGTTCAAGGGCAAGGCCACGTTGACT GTAGACACGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGCAAGTTACTACGCCG TGGGCTACTGGGGCCAAGGCACCACTCTCA CAGTCTCCTCA (SEQ ID NO: 37)	GATGTTGTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCA GAGCCTTCTACACAGTCATGGAAACACCTATTTA CATTGGTACCTGCAGAAGCCAGGCCAGTCTCCA AAGCTCCTGATCTACAAAGTTTCCAACCGGTTTT CTGGGGTCCCAGACAGGTTCAGTGGCAGTGGAT CAGGGACAGATTTCACACTCAAGATCAGCAGAGT GGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCT CAAACTGCACATTTTCCGTACACGTTCGGAGGGG GGACCAAGCTGGAAATAAAA (SEQ ID NO: 43)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–33	6C10E5E9C12	SEQ ID NO: 53/SEQ ID NO:54 y SEQ ID NO: 47	SEQ ID NO: 48/SEQ ID NO:49 y SEQ ID NO: 51	GAGGTGCAGCTGGTGGAGTCTGGGGGAGG CTTAGTGAAGCCTGGAGGGTCCCTGAAACT CTCCTGTGCACCCTCTGGATTCACTTTCAGT GACTATGGAATGCACTGGGTTCGTCAGGCT CCAGAGAAGGGACTGGAGTGGGTTGCATAC ATTAGTAGTGGCAGTAGTACCATCTACTATG GAGACACAGTGAAGGGCCGATTCACCATCT CCAGAGACAATGCCAAGAACACCCTGTTCCT GCAAATGACCAGTCTGAGGTCTGAGGACAC GGCCATGTATTACTGTGCAAGAAGGGGACA GCTCAGGCTACGCCTGTTTGCTTACTGGGG CCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 38)	GACATTGTGATGACCCAGTCTCACAAATTCATGT CCACATCAGTAGGAGACAGGGTCAGCATCACCT GCAAGGCCAGTCAGGATGTGAGTACTGCTGTAG CCTGGTATCAACAGAAACCAGGACAATCTCCTAA ACTACTGATTTACTCGGCATCCTACCGGTACACT GGAGTCCCTGATCGCTTCACTGGCAGTGGATCT GGGACGGATTTCACTTTCACCATCAGCAGTGTGC AGGCTGAAGACCTGGCAGTTTATTACTGTCAGCA ACATTATACTACTCCGCTCACGTTCGGTGCTGGG ACCAAGCTGGAGCTGAAA (SEQ ID NO: 44)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–33	6C10F9C12 A11	SEQ ID NO: 53/SEQ ID NO:54 y SEQ ID NO: 47	SEQ ID NO: 54 y SEQ ID NO: 51	GAGGTGCAGCTGGTGGAGTCTGGGGGAGG CTTAGTGAAGCCTGGAGGGTCCCTGAAACT CTCCTGTGCACCCTCTGGATTCACTTTCAGT GACTATGGAATGCACTGGGTTCGTCAGGCT CCAGAGAAGGGACTGGAGTGGGTTGCATAC ATTAGTAGTGGCAGTAGTACCATCTACTATG GAGACACAGTGAAGGGCCGATTCACCATCT CCAGAGACAATGCCAAGAACACCCTGTTCCT GCAAATGACCAGTCTGAGGTCTGAGGACAC GGCCATGTATTACTGTGCAAGAAGGGGACA GCTCAGGCTACGCCTGTTTGCTTACTGGGG CCAAGGGACTCTGGTCACTGTCTCTGCA (SEQ ID NO: 38)	GACATTGTGATGACCCAGTCTCACAAATTCATGT CCACATCAGTAGGAGACAGGGTCAGCATCACCT GCAAGGCCAGTCAGGATGTGAGTACTGCTGTAG CCTGGTATCAACAGAAACCAGGACAATCTCCTAA ACTACTGATTTACTCGGCATCCTACCGGTACACT GGAGTCCCTGATCGCTTCACTGGCAGTGGATCT GGGACGGATTTCACTTTCACCATCAGCAGTGTGC AGGCTGAAGACCTGGCAGTTTATTACTGTCAGCA ACATTATACTACTCCGCTCACGTTCGGTGCTGGG ACCAAGCTGGAGCTGAAA (SEQ ID NO: 44)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–41	7C2(1)F10C 10D3	SEQ ID NO: 53/ SEQ ID NO:55 y SEQ ID NO: 47	SEQ ID NO: 49/SEQ ID NO:56/SEQ ID NO: 57 y SEQ ID NO: 51	GAGGTGAAGCTGATGGAATCTGGAGGAGGC TTGGTACACCCTGGGGCTTCTCTGAGACTCT ACTGTGCAGCTTCTGGATTCACCTTTACTGA TTACTACATGAGCTGGGTCCGCCAGCCTCC AGGGAAGGCACCTGAGTGGTTGGCTTTGAT TAGAAACAAAGCTAATGGTTACACAACAGAG TATACTGCATCTGTTAAGGGTCGGTTCACCA TCTCCAGAGATAATTCCCAAAACATCCTCTA TCTTCAAATGAACACCCTGAGGGCTGAGGA CAGTGCCACTTATTACTGTGTAAAAGCTCTG GGACGTTACTTCGATGTCTGGGGCACAGGG ACCACGGTCACCGTCTCCTCA (SEQ ID NO: 39)	GACATTGTGATGTCACAGTCTCCATCCTCCCTAG CTGTGTCAGTTGGAGAGAAGGTTACTATGAGCTG CAAGTCCAGTCAGAGCCTTTTATATAGTAGCAAT CAAAAGAACTACTTGGCCTGGTACCAGCAGAAAC CAGGGCAGTCTCCTAAACTGCTGATTTACTGGGC ATCCACTAGGGAATCTGGGGTCCCTGATCGCTTC ACAGGCAGTGGATCTGGGACAGATTTCACTCTCA CCATCAGCAGTGTGAAGGCTGAAGACCTGGCAG TTTATTACTGTCAGCAATATTATAGCTATCCATTC ACGTTCGGCTCGGGGACAAAGTTGGAAATAAAA (SEQ ID NO: 45)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–41	7C2(2)B9F1 1D5	SEQ ID NO: 53/ SEQ ID NO:55 y SEQ ID NO: 47	SEQ ID NO: 57 y SEQ ID NO: 51	GAGGTGAAGCTGATGGAATCTGGAGGAGGC TTGGTACACCCTGGGGCTTCTCTGAGACTCT ACTGTGCAGCTTCTGGATTCACCTTTACTGA TTACTACATGAGCTGGGTCCGCCAGCCTCC AGGGAAGGCACCTGAGTGGTTGGCTTTGAT TAGAAACAAAGCTAATGGTTACACAACAGAG TATACTGCATCTGTTAAGGGTCGGTTCACCA TCTCCAGAGATAATTCCCAAAACATCCTCTA TCTTCAAATGAACACCCTGAGGGCTGAGGA CAGTGCCACTTATTACTGTGTAAAAGCTCTG GGACGTTACTTCGATGTCTGGGGCACAGGG ACCACGGTCACCGTCTCCTCA (SEQ ID NO: 39)	GACATTGTGATGTCACAGTCTCCATCCTCCCTAG CTGTGTCAGTTGGAGAGAAGGTTACTATGAGCTG CAAGTCCAGTCAGAGCCTTTTATATAGTAGCAAT CAAAAGAACTACTTGGCCTGGTACCAGCAGAAAC CAGGGCAGTCTCCTAAACTGCTGATTTACTGGGC ATCCACTAGGGAATCTGGGGTCCCTGATCGCTTC ACAGGCAGTGGATCTGGGACAGATTTCACTCTCA CCATCAGCAGTGTGAAGGCTGAAGACCTGGCAG TTTATTACTGTCAGCAATATTATAGCTATCCATTC ACGTTCGGCTCGGGGACAAAGTTGGAAATAAAA (SEQ ID NO: 45)

Vacuna	**Hibridoma**	**Cebador VH (mezcla)**	**Cebador VK (mezcla)**	**VH**	**VK**
ACI–35	A4–4A6–18			CAGGTCCAACTGCAGCAGCCTGGGGCTGAG CTTCTGAAGCCTGGGGCTTCAGTGAAACTGT CCTGCAAGGCTTCTGGCTACACCTTCACCA GCTACTGGATGCACTGGGTGAAGCAGAGGC CTGGACGAGGCCTTGAGTGGATTGGAAGGA TTGATCCTAATAGTGATCGTACTAAGTACAAT GAGAAGTTCAAGCGCAAGGCCACACTGACT GTAGACAAATCCTCCAGCACAGCCTACATGC AGCTCAGCAGCCTGACATCTGAGGACTCTG CGGTCTATTATTGTGCAAGGGATGATTACGC CTGGTTTGCTTACTGGGGCCAAGGGACTCT GGTCACTGTCTCTGCA (SEQ ID NO: 84)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCATTGTACATAGTAATGGA AACACCTATTTAGAATGGTACCTGCAGAAACCAG GCCAGTCTCCAAAGCTCCTGATCTACAAACTTTC CAACCGATTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTACTGCTTTCAAGGTTCACATGTTCCTCCGAC GTTCGGTGGAGGCACCAAGCTGGAAATCAAA (SEQ ID NO: 85)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A6–1D2–12			CAGGTTACTCTGAAAGAGTCTGGCCCTGGG ATATTGCAGTCCTCCCAGACCCTCAGTCTGA CTTGTTCTTTCTCTGGGTTTTCACTGAGCAC TTCTGGTATGGGTGTGAGCTGGATTCGTCA GCCTTCAGGAAAGGGTCTGGAGTGGCTGGC ACACATTTACTGGGATGATGACAAGCGCTAT AACGCATCCCTGAAGAGCCGGCTCACAATC TCCAAGGATACCTCCAGAAACCAGGTATTCC TCAAGATCACCTGTGTGGACACTGCAGATAC TGCCACATACTACTGTGCTCGGTTACTGCGT CCTTATGCTTTGGACTACTGGGGTCAAGGAA CCTCAGTCACCGTCTCCTCA (SEQ ID NO: 86)	AACATTTTGATGACACAGTCGCCATCATCTCTGG CTGTGTCTGCAGGAGAAAAGGTCACTATGAGCTG TAAGTCCAGTCAAAGTGTTTTATACAGTTCAAATC AGAAGAACTACTTGGCCTGGTACCAGCAGAAACC AGGGCAGTCTCCTAAACTGCTGATCTACTGGGCA TCCACTAGGGAATCTGGTGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACAGATTTTACTCTTAC CATCAGCAGTGTACAAGCTGAAGACCTGGCAGTT TATTACTGTCTTCAATACCTCTCCTCGCTCACGTT CGGTGCTGGGACCAAGCTGGAGCTGAAA (SEQ ID NO: 87)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A4–2A1–18			GAGGTCCAGCTGCAACAATCTGGACCTGAG CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATACACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACAATGGTGGTACTAGCTACAAC CAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGTCTGACATCTGAGGACTCT GCAGTCTATTATTGTGTAAGAGAGGGGCGG TTTGCTTACTGGGGTCATGGGACTCTGGTCA CTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTAC CTGTCACTCCTGGAGAGTCAGTATCCATCTCCTG CAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGC AACACTTACTTGTATTGGTTCCTGCAGAGGCCAG GCCAGTCTCCTCAGCTCCTGATACATCGGATGTC CAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGT GGCAGTGGGTCAGGAACTGCTTTCACACTGAGA ATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTT TATTACTGTATGCAACATCTAAAATCTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 117)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A4–2A1–40			GAGGTCCAGCTGCAACAATCTGGACCTGAG CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATACACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACAATGGTGGTACTAGCTACAAC CAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGTCTGACATCTGAGGACTCT GCAGTCTATTATTGTGTAAGAGAGGGGCGG TTTGCTTACTGGGGTCATGGGACTCTGGTCA CTGTCTCTGCA (SEQ ID NO: 109)	GATATTR*TGATGACTCAGGCTGCACCCTCTGTA CCTGTCACTCCTGGAGAGTCAGTATCCATCTCCT GCAGGTCTAGTAAGAGTCTCCTGCATAGTAATGG CAACACTTACTTGTATTGGTTCCTGCAGAGGCCA GGCCAGTCTCCTCAGCTCCTGATATATCGGATGT CCAACCTTGCCTCAGGAGTCCCAGACAGGTTCA GTGGCAGTGGGTCAGGAACTGCTTTCACACTGA GAATCAGTAGAGTGGAGGCTGAGGATGTGGGTG TTTATTACTGTATGCAACATCTAAAATCTCCGTAC ACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 110) R* = A o G

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A4–4A6–48			GAGGTCCAGCTGCAACAATCTGGACCTGAG CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATACACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACAATGGTGGTACTAGCTACAAC CAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGTCTGACATCTGAGGACTCT GCAGTCTATTATTGTGTAAGAGAGGGGCGG TTTGCTTACTGGGGTCATGGGACTCTGGTCA CTGTCTCTGCA (SEQ ID NO: 109)	GATATTGTGATGACTCAGGCTGCACCCTCTGTAC CTGTCACTCCTGGAGAGTCAGTATCCATCTCCTG CAGGTCTAGTAAGAGTCTCCTGCATAGTAATGGC AACACTTACTTGTATTGGTTCCTGCAGAGGCCAG GCCAGTCTCCTCAGCTCCTGATATATCGGATGTC CAACCTTGCCTCAGGAGTCCCAGACAGGTTCAGT GGCAGTGGGTCAGGAACTGCTTTCACACTGAGA ATCAGTAGAGTGGAGGCTGAGGATGTGGGTGTT TATTACTGTATGCAACATCTAAAATCTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 119)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A6–2G5–08			CAGGTCCAGCTGAAGCAGTCTGGGGCTGAG CTGGTGAGGCCTGGGGCTTCAGTGAAACTG TCCTGCAAGGCTTCTGGCTACACTTTCACTG ACTACTATATAAACTGGGTGAAGCAGAGGCC TGGACAGGGACTTGAGTGGATTGCAAGGAT TTATCCTGGAAGAGGTAATATTTACTACAAT GAGAAGTTCAAGGGCAAGGCCACACTGACT GCAGAAAAATCCTCCAGCACTGCCTACATGC AGCTCAGCAGCCTGACATCTGAGGACTCTG CTGTCTATTTCTGTGCAAGATTCTGGGACGT GACTTACTGGGGCCAAGGGACTCTGGTCAC TGTCTCTGCA (SEQ ID NO: 111)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGC CTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTG CAGATCTAGTCAGAGCATTGTACATAGTAATGGA AACACCTATTTAGAATGGTTCCTGCAGAAACCAG GCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTC CAACCGATTTTCTGGGGTCCCAGACAGGTTCAGT GGCAGTGGATCAGGGACAGATTTCACACTCAAG ATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTACTGCTTTCAAGGTTCACATGTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 112)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A6–2G5–30			GAGGTCCAGCTGCAACAATCTGGACCTGAG CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATTCACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACAATGGTGGTACTAGCTACCAC CAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGTAAGAGAGGGAAGAT TTGCTTACTGGGGCCAAGGGACTCTGGTCA CTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGT CCACATCAGTAGGAGACAGGGTCAGCGTCACCT GCAAGGCCAGTCAGAATGTGGGTACTAATGTAG CCTGGTATCAACAGAAACCAGGGCAATCTCCTAA AGCACTGATTTACTCGGCATCCTACCGGTACAGT GGAGTCCCTGATCGCTTCACAGGCAGTGGATCT GGGACAGATTTCACTCTCACCATCAGCAATGTGC AGTCTGAAGACTTGGCAGAGTATTTCTGTCAGCA ATATAACAGCTATCCGTACACGTTCGGAGGGGG GACCAAGCTGGAAATAAAA (SEQ ID NO: 114)

Vacuna	Hibridoma	Cebador VH (mezcla)	Cebador VK (mezcla)	VH	VK
ACI–35	A6–2G5–41			GAGGTCCAGCTGCAACAATCTGGACCTGAG CTGGTGAAGCCTGGGGCTTCAGTGAAGATA TCCTGTAAGGCTTCTGGATTCACGTTCACTG ACTACTACATGAACTGGGTGAAGCAGAGCC ATGGAAAGAGCCTTGAGTGGATTGGAGATAT TAATCCTAACAATGGTGGTACTAGCTACCAC CAGAAGTTCAAGGGCAAGGCCACATTGACT GTAGACAAGTCCTCCAGCACAGCCTACATG GAGCTCCGCAGCCTGACATCTGAGGACTCT GCAGTCTATTACTGTGTAAGAGAGGGAAGAT TTGCTTACTGGGGCCAAGGGACTCTGGTCA CTGTCTCTGCA (SEQ ID NO: 113)	GACATTGTGATGACCCAGTCTCAAAAATTCATGT CCACATCAGTAGGAGACAGGGTCAGCGTCACCT GCAAGGCCAGTCAGAATGTGGGTACTAATGTAG CCTGGTATCAACAGAAACCAGGGCAATCTCCTAA AGCACTGATTTACTCGGCATCCTACCGGTACAGT GGAGTCCCTGATCGCTTCACAGGCAGTGGATCT GGGACAGATTTCACTCTCACCATCAGCAATGTGC AGTCTGAAGACTTGGCAGAGTATTTCTGTCAGCA ATATAACAGCTATCCGTACACGTTCGGAGGGGG GACCAAGCTGGAAATAAAA (SEQ ID NO: 114)

*Dos secuencias V_K productivas 6 y 7 en la Tabla 10; secuencias 40 y 41 en la Tabla 11) se aislaron de las líneas celulares 3A8A12G7 y 3A8E12H8; las secuencias "V_K_G" se prepararon de clones hechos usando mezcla de cebador "G" y secuencias "V_K_AD" de clones hechos usando mezclas de cebadores "A" y "D". Conforme a ello, dos anticuerpos con diferentes secuencias kappa son producidos por estos hibridomas.

Tabla 12. Cebadores usados para la secuenciación de CDR de regiones variables de anticuerpos

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
3A8A 12G7	IgG2b	Cebadores VH	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores VK	5’	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT	48
			AD	ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	49
			5’ G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
3A8E 12H8	IgG2b	Cebadores VH	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores VK	5’	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT	48
			AD	ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	49
			5’ G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
2B6A 10C11	IgG2b	Cebadores	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
		VH		ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT	52
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
2B6G 7A12	IgG2b	Cebadores	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
		VH		ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT	52
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6H1A11C11	IgG2b	Cebadores	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46
		VH	3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6H1G 6E6	IgG2b	Cebadores	5’	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	46

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
		VH	3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6C10F9	IgG3	Cebadores	5’	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	53
C12A11		VH		ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	54
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	54
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
6C10E5E	IgG3	Cebadores	5’	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	53
9C12		VH		ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	54
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores	5’	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT	48
		VK		ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	49
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
7C2(1)F10C 10D3	IgG2b	Cebadores VH	5’	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	53
				ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT	55
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores VK	5’	ACTAGTCGACATGGAGWCAGACACACISCTGYTATGGGT	56
				ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	49
				ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	57
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
7C2(2)B9F 11D5	IgG2b	Cebadores VH	5’	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	53
				ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT	55
			3’	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	47
		Cebadores VK	5’	ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	57
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6–2G5–08	IgG2a	Cebadores VH	5’	GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				GGGAATTCATGGAATGCAGCTGGGTCATTCTCTT	122
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGAAATGGAGCTGGGTCTTTTCTT	125
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTTC	127
			3’	CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAGTGGATAGACGGGTGG	132
				CCCAAGCTTCCAGGGACCAAGGGATAGATGATGG	133
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGGCCAATGGATAAACGATGG	135

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
		Cebadores VK	5’	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4–2A1–18	IgG2b	Cebadores VH	5’	GGGAATTCATGGAATGGAGCTGGGTCATTCTCTT	136
				GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGGAGCTGGGTTTTTCTCTT	123
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGAGCTGGGTCTTCCTCTT	138
				GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				ACTAGTCGACATGGGATGAGCTTATCATCCTCTT	140
			3’	CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGGATAAACGGGTGG	142
				CCCAAGCTTCCAGGGGCCAATGGATAAACGGGTGG	134
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGGTGG	143

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGATGG	145
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGGCCAGGGATAAACGGGTGG	146
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAGTGGATAAACGGTGG	148
		Cebadores	5’	ACTAGTCGACATGGTGTCCACAGCTCAGTTCCTTG	149
		VK	3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6–2G5–30	IgG2b	Cebadores	5’	GGGAATTCATGAAATGGAGCTGGGTCTTCCTCTT	150
		VH		GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTTATGGAATGGAGCTGGGTCTTCCTCTT	152
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127
				GGGAATTCATGGAATGCAGCTGGGTCATCCTCTT	153
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				ACTAGTCGACATGGGATGGAGCTATATCATCCTCTT	156
				ACTAGTCGACATGGGATGGAGCTTATCATCTTCTT	157
				ACTAGTCGACATGTAGATGTGGTTAAACTGGGT	158
			3’	CCCAAGCTTCCAGGGGCCAGGGGATAAACGGATGG	159
				CCCAAGCTTCCAGGGGCCAAGGGATAGACGGATGG	160
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGGTGG	161
				CCCAAGCTTCCAGGGACCAGGGGATAGACGGATGG	162
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGATGG	163
				CCCAAGCTTCCAGGGGCCAATGGATAACGATGG	164
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGATGG	165
				CCCAAGCTTCCAGGGACCAATGGATAAACGGTGG	130
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				CCCAAGCTTCCAGGGACCAATGGATAACGATGG	167
				CCCAAGCTTCCAGGGACCATGGATAAACGGGTGG	168
		Cebadores VK	5’	ACTAGTCGACATGGGCATCAAGATGAAGTCACATACTCTGG	169
				ACTAGTCGACATGGGCATCAAGATGAGTCACATACTCTGG	170
				ACTAGTCGACTGGGCATCAGATGAGTCACATACTCTGG	171
				ACTAGTCGACATGGGCATCAAGATGAAGTCACAGACCCAGG	172
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCTGG	173
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATATTCAGG	174
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4–2A1–40	IgG2b	Cebadores VH	5’	GGGAATTCATGGAATGGAGCTGGGTCATCCTCTT	139
				GGGAATTCATGGAATGCAGCTGGGTTTTCCTCTT	154
				GGGAATTCATGGAATGCAGCTGGGTCTTTCTCTT	155
				GGGAATTCATGGAATGGAGCTGGGTTTTCCTCTT	127

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121
				ACTAGTCGACATGGATGGAGCTTATCATCCTCTT	175
			3’	CCCAAGCTTCCAGGGACCAAGGGATAAACGGTGG	176
				CCCAAGCTTCCAGGGGCCAATGGATAAACCGGTGG	147
				CCCAAGCTTCCAGGGACCAATGGATAAACGATGG	129
				CCCAAGCTTCCAGGGGCCAGTGGATAAACGGGTGG	177
				CCCAAGCTTCCAGGGACCAATGGATAACGGGTGG	128
		Cebadores VK	5’	ACTAGTCGACATGAGGTACTCGGCTCAGTTCCTGGG	178
				ACTAGTCGACATGAGGTCCCCGGCTCAGTTCCTGGG	179
				ACTAGTCGACATGAGGACGTCGATTCAGTTCTTGGG	180
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A6–2G5–41	IgG2b	Cebadores VH	5’	GGGAATTCATGGAATGGACCTGGGTCATCCTCTT	181
				GGGAATTCATGGAATGCAGCTGGGTTTTTCTCTT	120
				GGGAATTCATGGAATGCAGCTGGGTTATCCTCTT	182

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				GGGAATTCATGGAATGGAGCTGGGTTATTCTCTT	124
				GGGAATTCATGGAATGCAGCTGGGTCTTCCTCTT	126
				GGGAATTCATGAATGGATCTGGGTTATTCTCTT	183
			3’	CCCAAGCTTCCAGGGACCAGGGGATAAACGGGTGG	184
				CCCAAGCTTCCAGGGACCAAGGGACGGGTGG	185
				CCCAAGCTTCCAGGGACCAATGGATAAACAGATGG	186
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
				CCCAAGCTTCCAGGGACCAGGGGATAAACGGATGG	145
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGGTGG	187
		Cebadores VK	5’	GGGAATTCATGGAGACACATTCCCAGGTCTTT	188
				GGGAATTCATGGAGTCACAGTCTCAGGTCTTT	189
				ACTAGTCGACATGGGCTTCAAGATGGAGTCACATTTTCAGG	190
				ACTAGTCGACATGGGCATCAAGATGAAGTCACATATTCAGG	191
				ACTAGTCGACATGGGCTTCAAGATGAAGTCACATTCTCAGG	192

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
				CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4–4A6–48	IgG2b	Cebadores VH	5’	ACTAGTCGACATGGGATGGAGCTTATCATGTTCTT	193
				ACTAGTCGACATGGGATGGAGCTTATCATGCTCTT	194
				GGGAATTCATGGAATGCACCTGGGTTTTCCTCTT	137
				GGGAATTCATGGAATGGACCTGGGTTTTCCTCTT	195
				GGGAATTCATGGAATGGACCTGGGTCTTTCTCTT	196
				GGGAATTCATGAAATGGAGCTGGGTTATTCTCTT	197
				GGGAATTCATGGAATGCAGCTGGGTTATTCTCTT	151
				GGGAATTCATGGAATGGAGCTGGGTCTTTCTCTT	121

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
			3’	CCCAAGCTTCCAGGGGCCAATGGATAGACGATGG	198
				CCCAAGCTTCCAGGGACCAAGGGATAGACGGATGG	199
				CCCAAGCTTCCAGGGACCAAGGGATAGACGATGG	200
				CCCAAGCTTCCAGGGGCCAATGGATAACGGTGG	141
				CCCAAGCTTCCAGGGACCAGTGGATAAACGATGG	166
				CCCAAGCTTCCAGGGACCAATGGATAAACGGATGG	131
				CCCAAGCTTCCAGGGACCAAGGGATAAACGGATGG	144
		Cebadores VK	5’	ACTAGTCGACATGATGTACCCGGCTCAGTTTCTGGG	201
				ACTAGTCGACATGAGGACTTCGATTCAGTTCTTGGG	202
				ACTAGTCTACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	203
				ACTAGTCGACATGAAGTTGTCTGTTAGGCTGTTGGTGCT	204
				ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	50
			3’	CCCAAGCTTACTGGATGGTGGGAAGATGGA	51
A4–4A6–18	IgG2b	Cebadores	5’	ATGGGATGGAGCTRTATCATSYTCTT	205

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
		VH		ATGAAGWTGTGGBTRAACTGGRT	206
				ATGGRATGGASCKKIRTCTTTMTCT	207
			3’	CCAGGGRCCARKGGATARACIGRTGG	208
		Cebadores VK	5’	ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3’	ACTGGATGGTGGGAAGATGGA	215
A6–1D2–12	IgG2a	Cebadores VH	5’	ATGAAATGCAGCTGGRTYATSTTCTT	216
				ATGGRCAGRCTTACWTYYTCATTCCT	217
				ATGATGGTGTTAAGTCTTCTGTACCT	218
			3’	CCAGGGRCCARKGGATARACIGRTGG	208

Subclón	Isotipo Ab	Secuencias de cebadores			SEQ ID NO
		Cebadores VK	5’	ATGRAGWCACAKWCYCAGGTCTTT	219
				ATGGAGACAGACACACTCCTGCTAT	209
				ATGGAGWCAGACACACTSCTGYTATGGGT	210
				ATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT	220
				ATGGGCWTCAAGATGRAGTCACAKWYYCWGG	221
				ATGAAGTTGCCTGTTAGGCTGTTGGTGCT	211
				ATGGATTTWCARGTGCAGATTWTCAGCTT	212
				ATGGTYCTYATVTCCTTGCTGTTCTGG	213
				ATGGTYCTYATVTTRCTGCTGCTATGG	214
			3’	ACTGGATGGTGGGAAGATGGA	215

Codones degenerados:

R = A o G S = C o G D = A o G o T B = C o G o T

Y = C o T M = A o C H = A o C o T

$K = G \circ T \qquad W = A \circ T \qquad V = A \circ G \circ C$

Tabla 13. Isoforma más larga de Tau humana (441aa), también denominada Tau40

Isoforma más larga de Tau humana (441aa), también denominada Tau40 (SEQ ID NO: 67) Isoforma 2 de proteína Tau asociada con microtúbulos [Homo sapiens] Secuencia de referencia NCBI: NP_005901.2	MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV DSPQLATLAD EVSASLAKQG L (SEQ ID NO: 67)

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Publicación de patente U.S. N.º 2004/0131692

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Patente U.S. N.º 5.112.596

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Patente U.S. N.º 5.506.206

Patente U.S. N.º 5.686.416

Patente U.S. N.º 5.004.697

REIVINDICACIONES

1. Un anticuerpo, o una de sus partes funcionales que reconoce y se une específicamente con un fosfo–epítope en la proteína tau de mamífero o en uno de sus fragmentos, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con la proteína tau soluble e insoluble y modula los niveles tau solubles e insolubles.

2. El anticuerpo de acuerdo con la reivindicación 1 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en el cerebro de un mamífero.

3. El anticuerpo de acuerdo con la reivindicación 2 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo modula los niveles de tau soluble e insoluble en la corteza cerebral y/o el hipocampo.

4. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total.

5. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau fosforilada soluble.

6. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de filamentos helicoidales en pares que contienen proteína tau hiperfosforilada.

7. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo reduce los niveles de proteína tau soluble total, proteína tau fosforilada soluble y filamentos helicoidales pTau en pares.

8. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho mamífero es un humano.

9. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 10 nM.

10. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de disociación de al menos 5 nM, al menos 2 nM; al menos 1 nM, al menos 500 pM, al menos 300 pM, al menos 200 pM, al menos 100 pM.

11. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una constante de tasa de asociación de 10^4 $M^{-1}s^{-1}$ o más; de 10^5 $M^{-1}s^{-1}$ o más, de 10^6 $M^{-1}s^{-1}$ o más.

12. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 4 nM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

13. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 3 nM y una constante de tasa de asociación de 10^6 $M^{-1}s^{-1}$ o más.

14. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de

disociación de al menos 2 nM y una constante de tasa de asociación de 10^4 $M^{-1}s^{-1}$ o más.

15. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 1 nM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

16. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 200 pM y una constante de tasa de asociación de 10^5 $M^{-1}s^{-1}$ o más.

17. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo tiene una alta afinidad de unión con una constante de disociación de al menos 100 pM y una constante de tasa de asociación de 10^6 $M^{-1}s^{-1}$ o más.

18. El anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope seleccionado del grupo que consiste en Tau aa 15–20 que comprende una Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende una Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende una Ser fosforilada en la posición 409 (pS409); Tau aa 208–218 que comprende una Thr fosforilada en la posición 212 (pT212) y una Ser fosforilada en la posición (pS214); Tau aa 393–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 394–400 que comprende una Ser fosforilada en la posición 396 (pS396),

Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404), y Tau aa 393–400 que comprende una Ser fosforilada en la posición 396 (pS396).

19. El anticuerpo de acuerdo con la reivindicación 12 ó 13 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 15–20 con una Tyr fosforilada en la posición 18 (Y18).

20. El anticuerpo de acuerdo con la reivindicación 14 ó 15 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 405–412 con una Ser fosforilada en la posición 409 (pS409).

21. El anticuerpo de acuerdo con la reivindicación 16 ó 17 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 405–411 con una Ser fosforilada en la posición 409 (pS409).

22. El anticuerpo de acuerdo con la reivindicación 12 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396).

23. El anticuerpo de acuerdo con la reivindicación 12 o uno de sus fragmentos funcionales, en donde dicho anticuerpo o fragmento de anticuerpo se une con un epítope que comprende Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404).

24. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene, en particular en secuencia, un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, 73, 81, 93, 101, 106, o una secuencia de aminoácido al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al

menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, 74, 82, 94, 102, 107, o una secuencia de aminoácido al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, 75, 83, 95, 103, 108, o una secuencia de aminoácido al menos 60%, en particular al menos 70%, en particular al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, 70, 78, 89, 98, o una secuencia de aminoácido al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%, en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, 71, 79, 90, 99, 115, o una secuencia de aminoácido al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, 72, 80, 91, 100, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

25. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 70% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 90% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 60% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 80% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 60% idéntica.

26. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 85% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 80% idéntica; y/o a dominio de anticuerpo que contiene un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 95% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 85% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 85% idéntica.

27. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que

contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, o una secuencia de aminoácido al menos 95% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica

28. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión (dominio de anticuerpo) que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 90% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 90% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

29. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 98% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y

un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 95% idéntica y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

30. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, o una secuencia de aminoácido al menos 98% idéntica; un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, o una secuencia de aminoácido al menos 98% idéntica;. y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, o una secuencia de aminoácido al menos 98% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20, o una secuencia de aminoácido al menos 90% idéntica.

31. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, 24, 27, 28, 29, 32, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, 25, 30, 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, 26, 31, 34, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, 15, 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, 16, 19, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, 17, 20.

32. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 21, o una secuencia de aminoácido al menos 76% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 22, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 23, o una secuencia de aminoácido al menos 66% idéntica, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, a DR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, o una secuencia de aminoácido al menos 88% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, o una secuencia de aminoácido al menos 66% idéntica.

33. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 24, o una secuencia de aminoácido al menos 88% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 25, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 26, o una secuencia de aminoácido al menos 66% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 12, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 13, o una secuencia de aminoácido al menos 88% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 14, o una secuencia de aminoácido al menos 66% idéntica

34. El anticuerpo de acuerdo con la reivindicación 33 o uno de sus fragmentos funcionales, que comprende un primer dominio de unión en donde el CDR1 tiene la secuencia de aminoácidos mostrada en SEQ ID NO: 27, o una secuencia de aminoácido al menos 88% idéntica.

35. El anticuerpo de acuerdo con la reivindicación 33 o uno de sus fragmentos funcionales, que comprende un primer dominio de unión en donde el

CDR1 tiene la secuencia de aminoácidos mostrada en SEQ ID NO: 28, o una secuencia de aminoácido al menos 88% idéntica.

36. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 29, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 30, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 31, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 15, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 16, o una secuencia de aminoácido al menos 94% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 17, o una secuencia de aminoácido al menos 36% idéntica.

37. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 32, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 33, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 34, y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 18, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 19, o una secuencia de aminoácido al menos 95% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 20, o una secuencia de aminoácido al menos 63% idéntica.

38. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 73, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 74, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 75, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en

particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 70, o una secuencia de aminoácido al menos 95%, en particular 98%, en particular 99% idéntica, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 71, o una secuencia de aminoácido al menos 94%, 95%, 96%, 97%, 98%, o 99% idéntica, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 72, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

39. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 81, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 82, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 83, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 78, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 79, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 80, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en

particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

40. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 93, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 94, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 95, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 89, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 90, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 91, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

41. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 101, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 102, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 103, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos

93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 98, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 99, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 100, o una secuencia de aminoácido al menos al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

42. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que comprende un primer dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 106, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 107, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 108, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica a cualquiera de los anteriores CDRs y/o un segundo dominio de unión que contiene en secuencia un CDR1 con la secuencia de aminoácidos mostrada en SEQ ID NO: 89, un CDR2 con la secuencia de aminoácidos mostrada en SEQ ID NO: 115, y un CDR3 con la secuencia de aminoácidos mostrada en SEQ ID NO: 91, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en

particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% % idéntica a cualquiera de los anteriores CDRs.

43. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 85% idéntica.

44. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 90% idéntica, y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 91% idéntica.

45. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión (dominio de anticuerpo) que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6, 7, 8, 9, 10, 11, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, 2, 3, 4, 5, o una secuencia de aminoácido al menos 91% idéntica.

46. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 69, 77, 116/92, 97, 105, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica, y/o un segundo dominio de unión que contiene la secuencia de

aminoácidos mostrada en SEQ ID NO: 68, 76, 88, 96, 104, o una secuencia de aminoácido al menos 60%, al menos 70%, al menos 80%, en particular al menos 85%, en particular al menos 90%, al menos 91%, al menos 92%, al menos 93%, al menos 94%,en particular al menos 95%, en particular al menos 96%, en particular al menos 97%, en particular al menos 98%, en particular al menos 99% o 100% idéntica.

47. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6 o SEQ ID NO: 7, o una secuencia de aminoácido al menos 90% y 94%, respectivamente, idénticas; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1, o una secuencia de aminoácido al menos 91% idéntica.

48. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 8, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 2, o una secuencia de aminoácido al menos 90% idéntica.

49. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión, que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 9, o una secuencia de aminoácido al menos 95% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 3, o una secuencia de aminoácido al menos 90% idéntica.

50. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 10, o una secuencia de aminoácido al menos 99% idéntica; y/o un segundo dominio de unión que contiene

la secuencia de aminoácidos mostrada en SEQ ID NO: 4, o una secuencia de aminoácido al menos 89% idéntica.

51. El péptido de unión de cualquiera de las reivindicaciones precedentes que comprende un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 11, o una secuencia de aminoácido al menos 98% idéntica; y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 5, o una secuencia de aminoácido al menos 87% idéntica.

52. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que comprende

a. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1; o

b. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 7 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 1; o

c. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 8 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 2; o

d. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 9 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 3; o

e. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 10 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 4; o

f. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 11 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 5; o

g. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 69 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 68; o

h. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 77 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 76; o

i. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 116 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 88; o

j. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 92 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 88; o

k. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 97 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 6; o

l. un primer dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 105 y/o un segundo dominio de unión que contiene la secuencia de aminoácidos mostrada en SEQ ID NO: 104.

53. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales, que es un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo completamente humano o uno de sus fragmentos funcionales.

54. El anticuerpo de cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales que es del isotipo IgG2b, IgG2a o IgG3.

55. Un péptido o proteína de unión del anticuerpo de acuerdo con cualquiera de las reivindicaciones 1–54.

56. El péptido o proteína de unión o el anticuerpo o uno de sus fragmentos funcionales de acuerdo con cualquiera de las reivindicaciones precedentes, en donde

dicho péptido de union, anticuerpo de proteína o fragmento de anticuerpo reconoce y específicamente se une con un fosfo–epítope en el mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular a un fosfo–epítope en la proteína Tau agregada, pero no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados.

57. Un polinucleótido que codifica el anticuerpo cualquiera de las reivindicaciones precedentes o uno de sus fragmentos funcionales.

58. El polinucleótido de acuerdo con la reivindicación 57 que comprende una molécula de ácido nucleico seleccionada del grupo que consiste en

a. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que codifica un polipéptido que comprende la secuencia de aminoácidos tal como se indica en SEQ ID NOs: 35–45;

b. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 85% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45, SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

c. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 90% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117;

d. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 95% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

e. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que tiene al menos el 98% de identidad de secuencia con la secuencia mostrada en SEQ ID NOs: 35–45; SEQ ID NOs: 84–87, SEQ ID NO: 109–112 y 117–119;

f. una molécula de ácido nucleico que comprende una secuencia de nucleótidos cuya cadena complementaria hibrida en la molécula de ácidos nucleicos de cualquiera de a) – f);

g. una molécula de ácido nucleico que comprende una secuencia de nucleótidos que se desvía de la secuencia de nucleótidos definida en cualquiera de a) – g) por degeneración del código genético:

en donde dicha molécula de ácido nucleico tal como se define en cualquiera de a) – g) reconoce y específicamente se une con un fosfo–epítope en un mamífero, en particular en la proteína humana Tau o en uno de sus fragmentos, en particular a un confórmero de proteína tau patológica, pero no se une con el correspondiente epítope no fosforilado y/o con epítopes no relacionados, en donde dicho anticuerpo tiene una alta afinidad de unión con la proteína tau agregada, y modula los niveles de tau soluble e insoluble.

59. Una composición farmacéutica que comprende un péptido o una proteína de unión, un anticuerpo o uno de sus fragmentos funcionales o un polinucleótido de acuerdo con cualquiera de las reivindicaciones anteriores, o una combinación de ellos, en una cantidad terapéuticamente efectiva junto con un portador farmacéuticamente aceptable.

60. La composición farmacéutica de acuerdo con la reivindicación 59, que comprende al menos dos péptidos o proteínas de union, anticuerpos o sus fragmentos funcionales o polinucleótidos de acuerdo con cualquiera de las reivindicaciones precedentes, que reconoce y se une con dos diferentes fosfo–epítopes en la proteína tau de mamífero.

61. La composición farmacéutica de acuerdo con la reivindicación 59 ó 60, que comprende un anticuerpo adicional o uno de sus fragmentos funcionales que reconoce y se une con una proteína o péptido amiloidogénico diferente.

62. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, un polinucleótido o una composición farmacéutica de acuerdo con

cualquiera de las reivindicaciones anteriores, o una combinación de ellos, para su uso en terapia, en particular, para su uso en el tratamiento de trastornos neurodegenerativos como ser tauopatías en un mamífero, en particular en un ser humano que necesita de dicho tratamiento.

63. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, o la composición farmacéutica de acuerdo con la reivindicación anterior, pasa su uso en el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano que sufre de una deficiencia como esta.

64. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior, donde el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano, lleva al detenimiento en el avance de las deficiencias cognitivas.

65. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior, donde el tratamiento o mitigación de deficiencias cognitivas en un mamífero, en particular en un ser humano, lleva al aumento de la retención, en particular a una restauración completa de la capacidad de memoria cognitiva en el sujeto tratado.

66. Un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior para su uso en el tratamiento de enfermedades y trastornos que son causados por la formación de lesiones neurofibrilares o están asociados con ella, patología cerebral predominante en tauopatía que comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de

Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica.

67. El anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, polinucleótido o la composición farmacéutica de acuerdo con la reivindicación anterior para su uso en el tratamiento de enfermedad de Alzheimer.

68. Un método para el tratamiento de una enfermedad o trastorno neurodegenerativo como ser una tauopatía que comprende la administración a un animal, en particular a un mamífero, pero en especial a un ser humano, que sufre de una enfermedad o trastorno neurodegenerativo como este, de un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, un polinucleótido o una composición farmacéutica de acuerdo una cualquiera de las reivindicaciones anteriores, o una combinación de ellas.

69. El método de acuerdo con la reivindicación anterior para el tratamiento de enfermedades y trastornos que son causados por la formación de lesiones neurofibrilares o están asociados con ella, patología cerebral predominante en tauopatía que comprende un grupo heterogéneo de enfermedades o trastornos neurodegenerativos que incluyen enfermedades o trastornos que muestran coexistencia de patologías tau y amiloides que incluyen, sin que la enumeración sea

taxativa, enfermedad de Alzheimer, enfermedad de Creutzfeldt–Jacob, demencia pugilística, síndrome de Down, enfermedad de Gerstmann–Sträussler–Scheinker, miositis por cuerpos de inclusión y angiopatía amiloide cerebral por proteína priónica, lesión cerebral traumática y otras enfermedades o trastornos que no muestran una patología amiloide distinta que incluyen, pero sin que la enumeración sea taxativa, esclerosis lateral amiotrófica/complejo parkinsonismo–demencia de Guam, enfermedad neuromotriz no Guam con ovillos neurofibrilares, demencia con gránulos argirofílicos, degeneración corticobasal, ovillos neurofibrilares difusos con calcificación, demencia frontotemporal con parkinsonismo vinculado al cromosoma 17, enfermedad de Hallevorden–Spatz, atrofia de múltiples sistemas, enfermedad de Niemann–Pick, tipo C, degeneración palido–ponto–nigral, enfermedad de Pick, gliosis subcortical progresiva, parálisis supranuclear progresiva, demencia por ovillos neurofibrilares, parkinsonismo postencefálico, distrofia miotónica.

70. Un método para inducir una respuesta inmune pasiva en un animal, en particular en un mamífero o ser humano, que sufre de una enfermedad o trastorno neurodegenerativo como ser una tauopatía mediante la administración a dicho animal o ser humano de un anticuerpo o uno de sus fragmentos funcionales, un péptido o proteína de unión, o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones anteriores, o una combinación de ellas.

71. Un método de diagnosis de una enfermedad, trastorno o condición asociados con la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un anticuerpo o uno de sus fragmentos activos a un epítope de la proteína tau en una muestra o in situ que incluye las etapas de

a. poner en contacto la muestra o una parte del cuerpo específica o área corporal sospechada de contener la proteína tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con la proteína tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de la proteína tau en la muestra o parte o área específica del cuerpo.

72. Un método de diagnosis de una predisposición a una enfermedad, trastorno o condición asociado a la proteína tau en un paciente que comprende detectar la unión inmunoespecífica de un anticuerpo o uno de sus fragmentos activos a un epítope de la proteína tau en una muestra o in situ que incluye las etapas de

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico a un valor de control normal,

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente padece o está en riesgo de desarrollar una enfermedad o condición asociados con la proteína Tau.

73. Un método de control de una enfermedad residual mínima en un paciente después del tratamiento con un anticuerpo o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones precedentes, que comprende:

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico a un valor de control normal,

en donde un aumento en la cantidad de dicho agregado en comparación con un valor de control normal indica que dicho paciente todavía padece de una enfermedad residual mínima.

74. Un método para predecir la respuesta de un paciente en tratamiento con un anticuerpo o una composición farmacéutica de acuerdo con cualquiera de las reivindicaciones precedentes que comprende

a. poner en contacto la muestra o una parte corporal o área corporal específicas sospechadas de contener el antígeno tau con un anticuerpo o uno de sus fragmentos de acuerdo con cualquiera de las reivindicaciones precedentes, cuyo péptido o su fragmento se une con un epítope de la proteína tau;

b. permitir que el anticuerpo se una con el antígeno tau para formar un complejo inmunológico;

c. detectar la formación del complejo inmunológico; y

d. correlacionar la presencia o ausencia del complejo inmunológico con la presencia o ausencia de antígeno tau en la muestra o parte o área específica del cuerpo,

e. comparar la cantidad de dicho complejo inmunológico antes y después del inicio del tratamiento,

en donde una disminución de la cantidad de dicho agregado indica que dicho paciente tiene un alto potencial de responder al tratamiento.

75. Kits de ensayo para la detección y diagnosis de enfermedades, trastornos o condiciones asociadas con la proteína Tau que comprenden un anticuerpo de acuerdo con cualquier reivindicación precedente.

76. Kit de ensayo de acuerdo con la reivindicación precedente que comprende un recipiente que porta uno o varios anticuerpos o uno de sus fragmentos funcionales de acuerdo con la presente invención e instrucciones de uso de los anticuerpos a los fines de unirse con el antígeno tau para formar un complejo inmunológico y detectar la formación del complejo inmunológico de modo que la presencia o la ausencia del complejo inmunológico se correlacione con la presencia o ausencia del antígeno tau.

77. Un epítope seleccionado del grupo que consiste en Tau aa 15–20 que comprende Tyr fosforilada en la posición 18 (Y18), Tau aa 405–412 que comprende Ser fosforilada en la posición 409 (pS409), Tau aa 405–411 que comprende Ser fosforilada en la posición 409 (pS409); y Tau aa 208–218 que comprende Thr fosforilada en la posición 212 (pT212) y Ser fosforilada en la posición (pS214), Tau aa 393–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 396–401 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 394–400 que comprende una Ser fosforilada en la posición 396 (pS396), Tau aa 402–406 que comprende una Ser fosforilada en la posición 404 (pS404), y Tau aa 393–400 que comprende una Ser fosforilada en la posición 396 (pS396).

78. Una línea celular que produce un anticuerpo de acuerdo con cualquier reivindicación precedente.

79. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079.

80. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081.

81. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080.

82. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088.

83. Una línea celular de acuerdo con la reivindicación 77, que es la línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084.

84. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 2B6G7A12 depositada el 10 de marzo de 2010 como DSM ACC3087.

85. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086.

86. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085.

87. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082.

88. Una línea celular de acuerdo con la reivindicación 78, que es la línea celular de hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083.

89. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6C10F9C12A11 depositada el 25 de agosto de 2010 como DSM ACC3079 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

90. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6C10E5E9C12 depositada el 25 de agosto de 2010 como DSM ACC3081 usando

a. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 54 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

91. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de

hibridoma 6H1A11C11 depositada el 25 de agosto de 2010 como DSM ACC3080 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

92. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 6H1G6E6 depositada el 25 de agosto de 2010 como DSM ACC3088 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

93. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 2B6A10C11 depositada el 25 de agosto de 2010 como DSM ACC3084 usando

a. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5’ de SEQ ID NO: 46 y SEQ ID NO: 52 y un cebador 3’ de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

94. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 2B6G7A12 depositada el 25 de agosto de 2010 como DSM ACC3087 usando

a. un par de cebadores que comprende un cebador 5’ de SEQ ID NO: 50 y un cebador 3’ de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5’ de SEQ ID NO: 46 y SEQ ID NO: 52 y un cebador 3’ de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

95. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 3A8A12G7 depositada el 25 de agosto de 2010 como DSM ACC3086 usando

a_1. una mezcla de cebadores que comprende un cebador 5’ de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3’ de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5’ de SEQ ID NO: 50 y un cebador 3’ de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5’ de SEQ ID NO: 46 y un cebador 3’ de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

96. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 3A8E12H8 depositada el 25 de agosto de 2010 como DSM ACC3085 usando

a_1. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 48 y SEQ ID NO: 49 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; o

a_2. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 50 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. un par de cebadores que comprende un cebador 5' de SEQ ID NO: 46 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

97. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de hibridoma 7C2(1)F10C10D3 depositada el 25 de agosto de 2010 como DSM ACC3082 usando

a. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 49; SEQ ID NO: 56 y SEQ ID NO: 57 y un cebador 3' de SEQ ID NO: 51 para la amplificación de un primer dominio de unión;

b. una mezcla de cebadores que comprende un cebador 5' de SEQ ID NO: 53 y SEQ ID NO: 55 y un cebador 3' de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

98. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación de PCR de ADN de línea celular de

hibridoma 7C2(2)B9F11D5 depositada el 25 de agosto de 2010 como DSM ACC3083 usando

a. un par de cebadores que comprende un cebador 5’ de SEQ ID NO: 57 y un cebador 3’ de SEQ ID NO: 51 para la amplificación de un primer dominio de unión;

b. una mezcla de cebadores que comprende un cebador 5’ de SEQ ID NO: 53 y SEQ ID NO: 55 y un cebador 3’ de SEQ ID NO: 47 para la amplificación de un segundo dominio de unión.

99. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139 usando

a. un par de cebadores que comprende un cebador 5’ de SEQ ID NO: 149 y un cebador 3’ de SEQ ID NO: 51 para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEQ ID NOs: 120, 123, 124, 136, 137, 138, 139, y 140 y un cebador 3’ seleccionado del grupo que consiste en SEQ ID NOs: 131, 134 y 141–148, para la amplificación de un segundo dominio de unión.

100. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137 usando

a. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEQ ID NOs: 51 y 169–174 y un cebador 3’

de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 124, 127 y 150–158 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 130 y 159–168, para la amplificación de un segundo dominio de unión.

101. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 178, 179 y 180 y un cebador 3' de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 121, 127, 139, 154, 155 y 175 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 128, 129, 147, 176 y 177, para la amplificación de un segundo dominio de unión.

102. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 51 y 188–192 y un cebador 3'

de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 120, 124, 126, 181, 182 y 183 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 144, 145 y 184–187, para la amplificación de un segundo dominio de unión.

103. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 50 y 201–204 y un cebador 3' de SEQ ID NO: 51, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 121, 137, 151 y 193–197 y un cebador 3' seleccionado del grupo que consiste en SEQ ID NOs: 131, 141, 144, 166, 198, 199 y 200, para la amplificación de un segundo dominio de unión.

104. Un anticuerpo o uno de sus fragmentos funcionales de acuerdo con la reivindicación 1 que está codificado por un polinucleótido ubicado en un fragmento de nucleótido que se puede obtener por amplificación por PCR de ADN de línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141 usando

a. una mezcla de cebadores que comprende un cebador 5' seleccionado del grupo que consiste en SEQ ID NOs: 209–214, y 219–221 un

cebador 3’ de SEQ ID NO: 215, para la amplificación de un primer dominio de unión; y/o

b. una mezcla de cebadores que comprende un cebador 5’ seleccionado del grupo que consiste en SEQ ID NOs: 216, 217 y 218 y un cebador 3’ de SEQ ID NOs: 208, para la amplificación de un segundo dominio de unión.

105. El anticuerpo o uno de sus fragmentos funcionales de acuerdo con cualquiera de las reivindicaciones precedentes, en donde dicho anticuerpo es un anticuerpo del isotipo IgG2a, IgG2b o IgG3, un anticuerpo policlonal, un anticuerpo monoclonal, un anticuerpo quimérico, un anticuerpo humanizado o un anticuerpo completamente humano.

106. Un fragmento de union o proteína o un anticuerpo de acuerdo con cualquiera de las reivindicaciones precedentes.

107. Una línea celular que produce el anticuerpo de acuerdo con cualquiera de las reivindicaciones 1–54, 56 o cualquiera de las reivindicaciones 89 – 105.

108. Una línea celular que comprende el polinucleótido de acuerdo con la reivindicación 57 ó 58.

109. Un método para producir el anticuerpo de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o con una cualquiera de las reivindicaciones 89 a 105, que comprende el paso de cultivo de la línea celular de acuerdo con la reivindicación 106 en un medio de cultivo adecuado.

110. Un método para producir un anticuerpo de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, que comprende el paso de cultivo de la línea celular de acuerdo con cualquiera de las reivindicaciones 78–88 en un medio de cultivo adecuado.

111. El método de acuerdo con la reivindicación 100 ó 110, que comprende, además, el paso de purificación del anticuerpo a partir de la línea celular o medio de cultivo.

112. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, con una cualquiera de las reivindicaciones 88 a 99, en la preparación de un medicamento para su uso en el tratamiento de tauopatías, un grupo de enfermedades y trastornos asociados con la proteína tau.

113. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, en la preparación de un medicamento para su uso en el tratamiento o mitigación de los síntomas asociados con tauopatías, un grupo de enfermedades y trastornos asociados con la proteína tau.

114. El uso del anticuerpo que incluye cualquier parte funcional de él de acuerdo con cualquiera de las reivindicaciones 1 a 54, 56 o cualquiera de las reivindicaciones 89–105, en la preparación de un medicamento para su uso en la retención o aumento de la capacidad de memoria cognitiva en un mamífero que sufre de una tauopatía.

115. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–4A6–48 depositada el 30 de agosto de 2011 como DSM ACC3136.

116. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–2G5–30 depositada el 30 de agosto de 2011 como DSM ACC3137.

117. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–2G5–41 depositada el 30 de agosto de 2011 como DSM ACC3138.

118. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–2A1–18 depositada el 30 de agosto de 2011 como DSM ACC3139.

119. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A4–2A1–40 depositada el 30 de agosto de 2011 como DSM ACC3140.

120. Una línea celular de acuerdo con la reivindicación 78, que es una línea celular de hibridoma A6–1D2–12 depositada el 6 de septiembre de 2011 como DSM ACC3141.

p.p.de. AC IMMUNE S.A. * K.U. LEUVEN RESEARCH & DEVELOPMENT

RESUMEN

La presente invención se refiere a métodos y composiciones para el uso terapéutico y de diagnóstico en el tratamiento de enfermedades y trastornos que son causados por ovillos neurofibrilares o están asociados con ellos. En particular, la invención se refiere a anticuerpos, que específicamente reconocen y se unen a conformadores de proteína tau patológicos fosforilados y con métodos y composiciones que incluyen dichos anticuerpos para el uso terapéutico y diagnóstico en el tratamiento de tauopatías entre las que encuentra la enfermedad de Alzheimer (EA).

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K.U. Leuven Research & Development

<120> COMPOSICIÓN FARMACÉUTICA

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Gln Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Phe Asn Ser
            20                  25                  30
Gly Asn Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45
Ser Pro Lys Leu Leu Val Tyr Phe Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60
Pro Asp Arg Phe Ile Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Thr
65                  70                  75                  80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr Phe Cys Gln Glu
                85                  90                  95
His Tyr Thr Thr Pro Pro Thr Phe Gly Thr Gly Thr Lys Leu Glu Leu
            100                 105                 110
Lys


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<211> 112
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20 25 30
His Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
85 90 95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

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<220>
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<400> 8
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20 25 30
His Gly Lys Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
85 90 95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

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<211> 112
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<220>
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1 5 10 15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser

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            20                  25                  30
His Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Thr
                85                  90                  95
Ala His Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

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1               5                   10                  15
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            20                  25                  30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
        35                  40                  45
Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Val Gln Ala
65                  70                  75                  80
Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Leu
                85                  90                  95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105

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Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30
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        35                  40                  45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95
Tyr Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
            100                 105                 110
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Lys

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<223> /mol_type="proteína"
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<210> 14
<211> 6
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1 5

<210> 15
<211> 10
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<220>
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1 5 10

<210> 16

<211> 17
<212> PRT
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<220>
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<222> 1..17
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1 5 10 15
Gly

<210> 17
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<220>
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<400> 17
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<220>
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<400> 18
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1 5 10

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<211> 19
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<220>
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<400> 19
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1 5 10 15
Val Lys Gly

<210> 20
<211> 8

<212> PRT
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1 5

<210> 21
<211> 17
<212> PRT
<213> Mus musculus

<220>
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<211> 7
<212> PRT
<213> Mus musculus

<220>
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<400> 22
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1 5

<210> 23
<211> 9
<212> PRT
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<220>
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<400> 23

Gln Glu His Tyr Thr Thr Pro Pro Thr
1 5

<210> 24
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<220>
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<210> 25
<211> 7
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<220>
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1 5

<210> 26
<211> 9
<212> PRT
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<220>
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<223> /mol_type="proteína"
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<400> 26
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1 5

<210> 27
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<220>
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<400> 27
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<210> 28
<211> 16
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<223> /mol_type="proteína"
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<400> 28
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<210> 29
<211> 11
<212> PRT
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<220>
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1 5 10

<210> 30
<211> 7
<212> PRT
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<220>
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<223> /mol_type="proteína"
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<400> 30
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<210> 31
<211> 9
<212> PRT
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<220>
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<222> 1..9
<223> /mol_type="proteína"
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<400> 31
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1 5

<210> 32
<211> 17
<212> PRT
<213> Mus musculus

<220>
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<222> 1..17
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 32
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1 5 10 15
Ala

<210> 33
<211> 7
<212> PRT
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<220>
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<223> /mol_type="proteína"
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<400> 33
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<210> 34
<211> 9
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<220>
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<223> /mol_type="proteína"
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<400> 34
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<210> 35
<211> 345
<212> DNA
<213> Mus musculus

<220>
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<222> 1..345
<223> /mol_type="DNA"
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catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt tcaagggcaa ggccacgttg actgtagaca agtcctccag cacagcctac 240

atggaactcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 36
<211> 345
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<213> Mus musculus

<220>
<221> source
<222> 1..345
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tcctgtaagg cttctggata tacgttcact gactactaca tgaactgggt gaagcagagc 120

catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt ttaagggcaa ggccacgttg actgtagaca agtcctccag cacagcctac 240

atggaactcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 37
<211> 345
<212> DNA
<213> Mus musculus

<220>
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<223> /mol_type="DNA"
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tcctgtaagg cttctggata cacgttcact gactactaca tgaactgggt gaagcagagc 120

catggaaaga gccttgagtg gattggagat attaatccta accgtggtgg aactacttac 180

aaccagaagt tcaagggcaa ggccacgttg actgtagaca cgtcctccag cacagcctac 240

atggagctcc gcagcctgac atctgaggac tctgcagtct attactgtgc aagttactac 300

gccgtgggct actggggcca aggcaccact ctcacagtct cctca 345

<210> 38
<211> 360
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<220>
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ccagagaagg gactggagtg ggttgcatac attagtagtg gcagtagtac catctactat 180

ggagacacag tgaagggccg attcaccatc tccagagaca atgccaagaa caccctgttc 240

ctgcaaatga ccagtctgag gtctgaggac acggccatgt attactgtgc aagaagggga 300

cagctcaggc tacgcctgtt tgcttactgg ggccaaggga ctctggtcac tgtctctgca 360

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<211> 357

<212> DNA
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<220>
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<222> 1..357
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ccagggaagg cacctgagtg gttggctttg attagaaaca aagctaatgg ttacacaaca 180

gagtatactg catctgttaa gggtcggttc accatctcca gagataattc ccaaaacatc 240

ctctatcttc aaatgaacac cctgagggct gaggacagtg ccacttatta ctgtgtaaaa 300

gctctgggac gttacttcga tgtctggggc acagggacca cggtcaccgt ctcctca 357

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<220>
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<223> /mol_type="DNA"
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atgagctgca agtccagtca gagtgttttt aatagtggca atcaaaagaa ctctttggcc 120

tggtaccagc agaaaccagg acagtctcct aaacttctgg tatactttgc atccactagg 180

gaatctgggg tccctgatcg cttcataggc agtggatctg ggacagattt cagtcttacc 240

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cctcccacgt tcggtactgg gaccaagctg gagctgaaa 339

<210> 41
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
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<223> /mol_type="DNA"
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tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tatttctgtt ctcaaactgc acattttccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 42
<211> 336
<212> DNA
<213> Mus musculus

<220>
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<222> 1..336
<223> /mol_type="DNA"
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atctcttgca gatctagtca gagccttgta cacagtcatg gaaaaaccta tttacattgg 120

tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tatttctgtt ctcaaactgc acattttccg 300

tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 43
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<212> DNA
<213> Mus musculus

<220>
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<223> /mol_type="DNA"
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tacctgcaga agccaggcca gtctccaaag ctcctgatct acaaagtttc caaccggttt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

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tacacgttcg gaggggggac caagctggaa ataaaa 336

<210> 44
<211> 321
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atcacctgca aggccagtca ggatgtgagt actgctgtag cctggtatca acagaaacca 120

ggacaatctc ctaaactact gatttactcg gcatcctacc ggtacactgg agtccctgat 180

cgcttcactg gcagtggatc tgggacggat ttcactttca ccatcagcag tgtgcaggct 240

gaagacctgg cagtttatta ctgtcagcaa cattatacta ctccgctcac gttcggtgct 300

gggaccaagc tggagctgaa a 321

<210> 45
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<212> DNA
<213> Mus musculus

<220>
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<223> /mol_type="DNA"
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tggtaccagc agaaaccagg gcagtctcct aaactgctga tttactgggc atccactagg 180

gaatctgggg tccctgatcg cttcacaggc agtggatctg ggacagattt cactctcacc 240

atcagcagtg tgaaggctga agacctggca gtttattact gtcagcaata ttatagctat 300

ccattcacgt tcggctcggg gacaaagttg gaaataaaa 339

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<211> 34
<212> DNA
<213> Secuencia Artificial

<220>
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<222> 1..34
<223> /mol_type="DNA"
/nota="Descripción de Secuencia Artificial: cebador"
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<210> 47
<211> 35
<212> DNA
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<220>
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/nota="Descripción de Secuencia Artificial: cebador"
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<220>
<221> variación
<222> 30..30
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<210> 48
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<220>
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<222> 1..32
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<210> 49
<211> 41
<212> DNA
<213> Secuencia Artificial

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<210> 50
<211> 39
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<220>
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<211> 30
<212> DNA
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<210> 52
<211> 36
<212> DNA
<213> Secuencia Artificial

<220>
<221> source
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<223> /mol_type="DNA"
/nota="Descripción de Secuencia Artificial: cebador"
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<210> 53
<211> 33
<212> DNA
<213> artificial seuqence

<220>
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<222> 1..33
<223> /mol_type="DNA"
/nota="Descripción de Secuencia Artificial: cebador"
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<400> 53
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<210> 54
<211> 39
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<213> Secuencia Artificial

<220>
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<212> DNA
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<220>
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<210> 56
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<212> DNA
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<222> 1..39
<223> /mol_type="DNA"
/nota="Descripción de Secuencia Artificial: cebador"
/organismo="Secuencia Artificial"

<220>
<221> misc_feature
<222> 27..27
<223> /nota="n=i"

<400> 56
actagtcgac atggagwcag acacacnsct gytatgggt 39

<210> 57
<211> 37
<212> DNA
<213> Secuencia Artificial

<220>
<221> source
<222> 1..37
<223> /mol_type="DNA"
/nota="Descripción de Secuencia Artificial: cebador"
/organismo="Secuencia Artificial"

<400> 57
actagtcgac atggtyctya tvttrctgct gctatgg 37

<210> 58
<211> 30
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..30
<223> /mol_type="proteína"
/nota="Description of artifiial sequence: ACI-37"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 18..18
<223> serina fosforilada

<220>
<221> VARIANTE
<222> 26..26
<223> serina fosforilada

<400> 58
Arg Glu Asn Ala Lys Ala Lys Thr Asp His Gly Ala Glu Ile Val Tyr
1 5 10 15
Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu
20 25 30

<210> 59
<211> 16
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-33"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 14..14
<223> serina fosforilada

<400> 59
Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly Thr Tyr Gly Leu
1 5 10 15

<210> 60
<211> 16
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-39"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 7..7
<223> treonina fosforilada

<220>
<221> VARIANTE
<222> 9..9
<223> serina fosforilada

<400> 60
Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg
1 5 10 15

<210> 61
<211> 16
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-39"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 7..7
<223> serina fosforilada

<220>
<221> VARIANTE
<222> 10..10
<223> treonina fosforilada

<400> 61
Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg
1 5 10 15

<210> 62
<211> 16
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-35"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 4..4
<223> serina fosforilada

<220>
<221> VARIANTE
<222> 12..12
<223> serina fosforilada

<400> 62
Val Tyr Lys Ser Pro Val Val Ser Gly Asp Thr Ser Pro Arg His Leu
1 5 10 15

<210> 63
<211> 18
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..18
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-36"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 4..4
<223> serina fosforilada

<220>
<221> VARIANTE
<222> 9..9
<223> serina fosforilada

<400> 63
Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser
1 5 10 15
Ile Asp

<210> 64
<211> 17
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-34"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 3..3
<223> serina fosforilada

<220>
<221> VARIANTE
<222> 6..6
<223> treonina fosforilada

<220>
<221> VARIANTE
<222> 13..13
<223> treonina fosforilada

<220>
<221> VARIANTE
<222> 15..15
<223> serina fosforilada

<400> 64
Pro Gly Ser Pro Gly Thr Pro Gly Ser Arg Ser Arg Thr Pro Ser Leu
1 5 10 15
Pro

<210> 65
<211> 12
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..12
<223> /mol_type="proteína"
/nota="Descripción de Secuencia Artificial: ACI-42"
/organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 3..3
<223> serina fosforilada

<400> 65
His Leu Ser Asn Val Ser Ser Thr Gly Ser Ile Asp
1 5 10

<210> 66
<211> 10
<212> PRT
<213> Secuencia Artificial

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="proteína"
      /nota="Descripción de Secuencia Artificial: ACI-43"
      /organismo="Secuencia Artificial"

<220>
<221> VARIANTE
<222> 6..6
<223> serina fosforilada

<400> 66
Val Ser Gly Asp Thr Ser Pro Arg His Leu
1   5   10

<210> 67
<211> 441
<212> PRT
<213> Homo sapiens

<220>
<221> SOURCE
<222> 1..441
<223> /mol_type="proteína"
      /organismo="Homo sapiens"

<400> 67
Met Ala Glu Pro Arg Gln Glu Phe Glu Val Met Glu Asp His Ala Gly
1   5   10   15
Thr Tyr Gly Leu Gly Asp Arg Lys Asp Gln Gly Gly Tyr Thr Met His
            20   25   30
Gln Asp Gln Glu Gly Asp Thr Asp Ala Gly Leu Lys Glu Ser Pro Leu
        35   40   45
Gln Thr Pro Thr Glu Asp Gly Ser Glu Glu Pro Gly Ser Glu Thr Ser
    50   55   60
Asp Ala Lys Ser Thr Pro Thr Ala Glu Asp Val Thr Ala Pro Leu Val
65   70   75   80
Asp Glu Gly Ala Pro Gly Lys Gln Ala Ala Ala Gln Pro His Thr Glu
                85   90   95
Ile Pro Glu Gly Thr Thr Ala Glu Glu Ala Gly Ile Gly Asp Thr Pro
            100   105   110
Ser Leu Glu Asp Glu Ala Ala Gly His Val Thr Gln Ala Arg Met Val
        115   120   125
Ser Lys Ser Lys Asp Gly Thr Gly Ser Asp Asp Lys Lys Ala Lys Gly
    130   135   140
Ala Asp Gly Lys Thr Lys Ile Ala Thr Pro Arg Gly Ala Ala Pro Pro
145   150   155   160
Gly Gln Lys Gly Gln Ala Asn Ala Thr Arg Ile Pro Ala Lys Thr Pro
                165   170   175
Pro Ala Pro Lys Thr Pro Pro Ser Ser Gly Glu Pro Pro Lys Ser Gly
            180   185   190
Asp Arg Ser Gly Tyr Ser Ser Pro Gly Ser Pro Gly Thr Pro Gly Ser
        195   200   205
Arg Ser Arg Thr Pro Ser Leu Pro Thr Pro Pro Thr Arg Glu Pro Lys
    210   215   220
Lys Val Ala Val Val Arg Thr Pro Pro Lys Ser Pro Ser Ser Ala Lys
225   230   235   240
Ser Arg Leu Gln Thr Ala Pro Val Pro Met Pro Asp Leu Lys Asn Val
                245   250   255
Lys Ser Lys Ile Gly Ser Thr Glu Asn Leu Lys His Gln Pro Gly Gly
            260   265   270
Gly Lys Val Gln Ile Ile Asn Lys Lys Leu Asp Leu Ser Asn Val Gln
        275   280   285
Ser Lys Cys Gly Ser Lys Asp Asn Ile Lys His Val Pro Gly Gly Gly
    290   295   300

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Ser Val Gln Ile Val Tyr Lys Pro Val Asp Leu Ser Lys Val Thr Ser
305                 310                 315                 320
Lys Cys Gly Ser Leu Gly Asn Ile His His Lys Pro Gly Gly Gly Gln
                325                 330                 335
Val Glu Val Lys Ser Glu Lys Leu Asp Phe Lys Asp Arg Val Gln Ser
            340                 345                 350
Lys Ile Gly Ser Leu Asp Asn Ile Thr His Val Pro Gly Gly Gly Asn
        355                 360                 365
Lys Lys Ile Glu Thr His Lys Leu Thr Phe Arg Glu Asn Ala Lys Ala
    370                 375                 380
Lys Thr Asp His Gly Ala Glu Ile Val Tyr Lys Ser Pro Val Val Ser
385                 390                 395                 400
Gly Asp Thr Ser Pro Arg His Leu Ser Asn Val Ser Ser Thr Gly Ser
                405                 410                 415
Ile Asp Met Val Asp Ser Pro Gln Leu Ala Thr Leu Ala Asp Glu Val
            420                 425                 430
Ser Ala Ser Leu Ala Lys Gln Gly Leu
        435                 440

<210> 68
<211> 117
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..117
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 68
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Leu Lys Pro Gly Ala
1               5                   10                  15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30
Trp Met His Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45
Gly Arg Ile Asp Pro Asn Ser Asp Arg Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60
Lys Arg Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Asp Asp Tyr Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110
Val Thr Val Ser Ala
        115

<210> 69
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 69
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
            20                  25                  30
Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
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Pro Lys Leu Leu Ile Tyr Lys Leu Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95
Ser His Val Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

<210> 70
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 70
Gly Tyr Thr Phe Thr Ser Tyr Trp Met His
1               5                   10

<210> 71
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 71
Arg Ile Asp Pro Asn Ser Asp Arg Thr Lys Tyr Asn Glu Lys Phe Lys
1               5                   10                  15
Arg


<210> 72
<211> 8
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..8
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 72
Asp Asp Tyr Ala Trp Phe Ala Tyr
1               5

<210> 73
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
      /organismo="Mus musculus"
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<400> 73
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1   5   10   15

<210> 74
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 74
Lys Leu Ser Asn Arg Phe Ser
1   5

<210> 75
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 75
Phe Gln Gly Ser His Val Pro Pro Thr
1   5

<210> 76
<211> 119
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..119
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 76
Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln Ser Ser Gln
1   5   10   15
Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser Thr Ser
            20   25   30
Gly Met Gly Val Ser Trp Ile Arg Gln Pro Ser Gly Lys Gly Leu Glu
        35   40   45
Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Ala Ser
    50   55   60
Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg Asn Gln Val
65   70   75   80
Phe Leu Lys Ile Thr Cys Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
                85   90   95
Cys Ala Arg Leu Leu Arg Pro Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
            100   105   110
Thr Ser Val Thr Val Ser Ser
        115

<210> 77
<211> 112

<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 77
Asn Ile Leu Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30
Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Leu Gln
85 90 95
Tyr Leu Ser Ser Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105 110

<210> 78
<211> 12
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..12
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 78
Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Ser
1 5 10

<210> 79
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 79
His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr Asn Ala Ser Leu Lys Ser
1 5 10 15

<210> 80
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 80
Leu Leu Arg Pro Tyr Ala Leu Asp Tyr
1 5

<210> 81
<211> 17
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 81
Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
1 5 10 15
Ala

<210> 82
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 82
Trp Ala Ser Thr Arg Glu Ser
1 5

<210> 83
<211> 8
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..8
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 83
Leu Gln Tyr Leu Ser Ser Leu Thr
1 5

<210> 84
<211> 351
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..351
<223> /mol_type="DNA"
/organismo="Mus musculus"

<400> 84
caggtccaac tgcagcagcc tggggctgag cttctgaagc ctggggcttc agtgaaactg 60

tcctgcaagg cttctggcta caccttcacc agctactgga tgcactgggt gaagcagagg 120

cctggacgag gccttgagtg gattggaagg attgatccta atagtgatcg tactaagtac 180

aatgagaagt tcaagcgcaa ggccacactg actgtagaca aatcctccag cacagcctac 240

atgcagctca gcagcctgac atctgaggac tctgcggtct attattgtgc aagggatgat 300

tacgcctggt ttgcttactg gggccaaggg actctggtca ctgtctctgc a 351

<210> 85
<211> 336
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..336
<223> /mol_type="DNA"
/organismo="Mus musculus"

<400> 85
gatgttttga tgacccaaac tccactctcc ctgcctgtca gtcttggaga tcaagcctcc 60

atctcttgca gatctagtca gagcattgta catagtaatg gaaacaccta tttagaatgg 120

tacctgcaga aaccaggcca gtctccaaag ctcctgatct acaaactttc caaccgattt 180

tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

agcagagtgg aggctgagga tctgggagtt tattactgct ttcaaggttc acatgttcct 300

ccgacgttcg gtggaggcac caagctggaa atcaaa 336

<210> 86
<211> 357
<212> DNA
<213> Mus musculus

<220>
<221> source
<222> 1..357
<223> /mol_type="DNA"
/organismo="Mus musculus"

<400> 86
caggttactc tgaaagagtc tggccctggg atattgcagt cctcccagac cctcagtctg 60

acttgttctt tctctgggtt ttcactgagc acttctggta tgggtgtgag ctggattcgt 120

cagccttcag gaaagggtct ggagtggctg gcacacattt actgggatga tgacaagcgc 180

tataacgcat ccctgaagag ccggctcaca atctccaagg atacctccag aaaccaggta 240

ttcctcaaga tcacctgtgt ggacactgca gatactgcca catactactg tgctcggtta 300

ctgcgtcctt atgctttgga ctactggggt caaggaacct cagtcaccgt ctcctca 357

<210> 87
<211> 336
<212> DNA
<213> Mus musculus

<220>

<221> source
<222> 1..336
<223> /mol_type="DNA"
/organismo="Mus musculus"

<400> 87
aacattttga tgacacagtc gccatcatct ctggctgtgt ctgcaggaga aaaggtcact 60

atgagctgta agtccagtca aagtgtttta tacagttcaa atcagaagaa ctacttggcc 120

tggtaccagc agaaaccagg gcagtctcct aaactgctga tctactgggc atccactagg 180

gaatctggtg tccctgatcg cttcacaggc agtggatctg ggacagattt tactcttacc 240

atcagcagtg tacaagctga agacctggca gtttattact gtcttcaata cctctcctcg 300

ctcacgttcg gtgctgggac caagctggag ctgaaa 336

<210> 88
<211> 115
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..115
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 88
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45
Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Val Arg Glu Gly Arg Phe Ala Tyr Trp Gly His Gly Thr Leu Val Thr
100 105 110
Val Ser Ala
115

<210> 89
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 89
Gly Phe Thr Phe Thr Asp Tyr Tyr Met Asn
1 5 10

<210> 90
<211> 17
<212> PRT

<213> Mus musculus

<220>
<221> SOURCE
<222> 1..17
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 90
Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15
Gly

<210> 91
<211> 6
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..6
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 91
Glu Gly Arg Phe Ala Tyr
1 5

<210> 92
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SITE
<222> 3..3
<223> Xaa is Met or Val

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 92
Asp Ile Xaa Met Thr Gln Ala Ala Pro Ser Val Pro Val Thr Pro Gly
1 5 10 15
Glu Ser Val Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
20 25 30
Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95
Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 93
<211> 16
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..16
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 93
Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Asn Thr Tyr Leu Tyr
1 5 10 15

<210> 94
<211> 7
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..7
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 94
Arg Met Ser Asn Leu Ala Ser
1 5

<210> 95
<211> 9
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..9
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 95
Met Gln His Leu Lys Ser Pro Tyr Thr
1 5

<210> 96
<211> 115
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..115
<223> /mol_type="proteína"
/organismo="Mus musculus"

<400> 96
Gln Val Gln Leu Lys Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30
Tyr Ile Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45
Ala Arg Ile Tyr Pro Gly Arg Gly Asn Ile Tyr Tyr Asn Glu Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ala Glu Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95
Ala Arg Phe Trp Asp Val Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

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Val Ser Ala
        115

<210> 97
<211> 112
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..112
<223> /mol_type="proteína"
      /organismo="Mus musculus"

<400> 97
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
            20                  25                  30
Asn Gly Asn Thr Tyr Leu Glu Trp Phe Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
                85                  90                  95
Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110

<210> 98
<211> 10
<212> PRT
<213> Mus musculus

<220>
<221> SOURCE
<222> 1..10
<223> /mol_type="proteína"
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<400> 98
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Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35   40   45
Gly Asp Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr His Gln Lys Phe
    50   55   60
Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65   70   75   80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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            20   25   30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
        35   40   45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
    50   55   60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65   70   75   80
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catggaaaga gccttgagtg gattggagat attaatccta acaatggtgg tactagctac 180

aaccagaagt tcaagggcaa ggccacattg actgtagaca agtcctccag cacagcctac 240

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cctggacagg gacttgagtg gattgcaagg atttatcctg gaagaggtaa tatttactac 180

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tctggggtcc cagacaggtt cagtggcagt ggatcaggga cagatttcac actcaagatc 240

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caccagaagt tcaagggcaa ggccacattg actgtagaca agtcctccag cacagcctac 240

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cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcagcaa tgtgcagtct 240

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Asn Gly Asn Thr Tyr Leu Tyr Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile His Arg Met Ser Asn Leu Ala Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
85 90 95
Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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35 40 45
Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro

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    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Ala Phe Thr Leu Arg Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln His
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Leu Lys Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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<213> Secuencia Artificial

<220>
<221> source
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/nota="Descripción de Secuencia Artificial: cebador"
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