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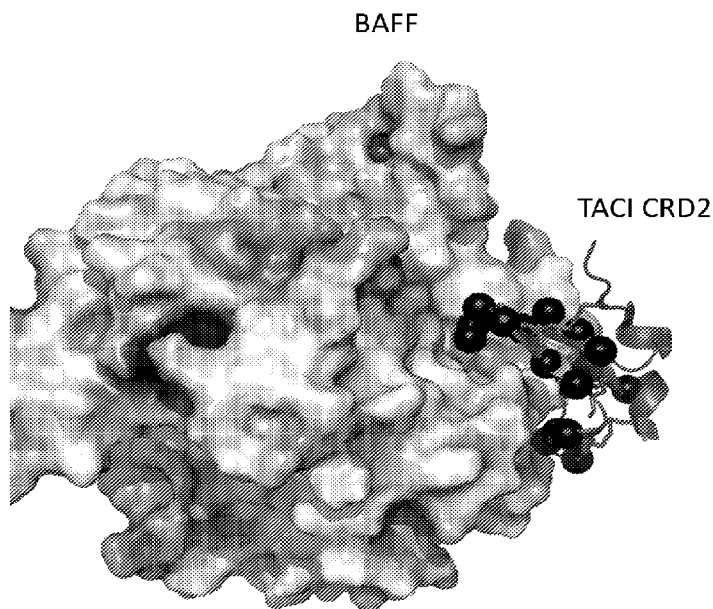
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(54) Title: TACI-FC FUSION PROTEINS FOR MULTIFUNCTIONAL INHIBITION OF BAFF, APRIL, AND NEONATAL FC RECEPTOR

FIG. 1



(57) Abstract: Methods and compositions TACI-Fc fusion proteins binding to and inhibiting the activity of BAFF, APRIL ligands to neutralize their activity and block or antagonize the activity of their corresponding B cell stimulatory receptors are provided. The TACI-Fc fusion proteins include a mutant Fc domain designed to mitigate one or more effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), and inhibit IgG recycling by neonatal Fc receptor (FcRn) blockade. The TACI-Fc fusion proteins can be used for the treatment of a variety of maladies, including diseases, disorders and conditions associated with a dysregulated immune response associated with inflammatory or autoimmune symptoms.



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**TACI-FC FUSION PROTEINS FOR MULTIFUNCTIONAL INHIBITION OF BAFF,
APRIL, AND NEONATAL FC RECEPTOR**

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. 63/430,265, filed December 5, 2022, the contents of which is incorporated by reference herein in its entirety.

BACKGROUND

10 B-cell proliferation, maturation, and survival depend on the signaling pathways of three receptors, TACI (transmembrane activator or calcium-modulating cyclophilin ligand-interactor), BCMA (B-cell maturation antigen), and BAFF-R (B-cell activating factor receptor). BCMA and BAFF-R are cell surface receptors of the TNF receptor superfamily, which are encoded by the *TNFRSF17* and *TNFRSF13C* genes, respectively.

15 TACI is a transmembrane protein of the TNF receptor superfamily (encoded by the *TNFRSF13B* gene), which is found predominantly on the surface of B cells. TACI recognizes three ligands, APRIL (A proliferation-inducing ligand encoded by the *TNFSF1* gene), BAFF (B-cell activating factor, encoded by the *TNFSF13B* gene), and CAML (calcium-modulating cyclophilin ligand). TACI is known to control T cell-independent B cell antibody responses,

20 isotype switching, and B cell homeostasis. APRIL is a proliferation inducing ligand, which is a ligand for both TACI and BCMA. BAFF is a ligand for TACI, BCMA, and BAFF-R (Marsters et al. Curr Biol 2000; 10(13): 785-788; Thompson et al. Science 2001; 293(2): 108-111). BAFF is a ligand for TACI, BCMA, and BAFF-R, is expressed in B lineage cells, acts as a potent B cell activator, and plays an important role in the proliferation and

25 differentiation of B cells.

 BAFF and APRIL are potent stimulators of B-cell maturation, proliferation, and survival (see, e.g., Gross et al., Nature 2000; 404: 995-999; Gross et al., Immunity 2001; 15(2): 289-302; and Groom et al., J Clin Invest 2002; 109(1): 59-68) and are known to independently form homodimers and BAFF/APRIL heterotrimers. BAFF and APRIL may be

30 necessary for persistence of autoimmune diseases, especially those involving B-cells. Transgenic mice engineered to express high levels of BAFF exhibit immune cell disorders and display symptoms like those seen in patients with Systemic Lupus Erythematosus (SLE) (see, e.g., Cheson et al. Blood 1996; 87:4990-4997; and Cheema et al. Arthritis Rheum 2001; 44(6):1313-1319). Similarly, increased levels of BAFF and APRIL have been measured in

serum samples taken from SLE patients and other patients with various autoimmune diseases like rheumatoid arthritis (see, e.g., Roschke et al. J. Immunol 2002; 169:4314-4321; Mariette et al. Ann Rheum Dis 2003; 62(2):168-171; and Hahne et al. J Exp Med 1998; 188(6): 1185-1190), extending the association of BAFF and/or APRIL and B-cell mediated diseases from
5 animal models to human.

The current approach for the treatment of autoimmune diseases is suppression of the unwanted immune reaction. For example, in the treatment of lupus nephritis (LN), a serious complication involving the kidney of patients suffering from systemic lupus nephritis (SLE), several immunosuppressive drugs have proven beneficial. These drugs include
10 cyclophosphamide (CYC), azathioprine (AZA), cyclosporine A (CSA), and mycophenolate mofetil (MMF) (see e.g., Mok et al. (2003) Ann Rheum Dis 62, 799-804 and Iaccarino et al. (2007) Autoimmunity Reviews 6, 190-195). Although these drugs are beneficial, there remains a need in the art for improved therapeutic agents to improve the response to immune suppressive drugs modulate immune responses, particularly B cell immune responses, to
15 better treat inflammatory or autoimmune diseases, disorder, and conditions. Described herein are anti-FcRn BAFF/APRIL inhibitor compositions and methods for their use in treating a variety of diseases and disorders.

The present disclosure provides immunomodulatory TACI-Fc fusion proteins exhibiting neutralizing activity of BAFF and APRIL (or BAFF/APRIL heterotrimers). The
20 TACI-Fc fusion proteins are anti-FcRn BAFF/APRIL inhibitor compositions containing variant extracellular DR1 and/or DR2 domains of TACI and variant Fc receptor binding domains. The present disclosure also provides nucleic acid molecules encoding the TACI-Fc fusion proteins, methods for making and using such proteins, and pharmaceutical compositions for treating a variety of maladies, including immunological diseases, disorders,
25 and conditions.

SUMMARY

Provided herein are TACI-Fc fusion proteins binding to and inhibiting the activity of BAFF, APRIL ligands to neutralize their activity and block or antagonize the activity of B
30 cell stimulatory receptors, TACI, BCMA, and BAFF-R. The TACI-Fc fusion proteins further include a mutant Fc domain containing mutations to block one or more effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), and/or inhibit one or more activities, including FcRn activity. As further described

below, the TACI-Fc fusion proteins can be used for the treatment of a variety of diseases, disorders or conditions associated with a dysregulated immune response.

In one aspect, the TACI-Fc fusion protein includes a TACI extracellular domain (ECD) portion and a hybrid immunoglobulin G (IgG) Fc domain *e.g.*, IgG1/IgG4. In some
 5 embodiments, the TACI ECD portion includes a TACI DR2 domain portion. In some embodiments, the TACI ECD portion includes a contiguous ECD portion spanning at least a portion of the DR1/DR2 regions. In one embodiment, the TACI ECD portion includes an amino acid sequence set forth in SEQ ID NO: 1 or 2.

In some embodiments, the TACI ECD portion includes one or more mutations. In one
 10 embodiment, the mutations include L82H alone or in combination with a single histidine substituted mutation in Table 1, where the amino acid identification and numbering is according to SEQ ID NO: 40. In another embodiment, the mutations include F78H and D80H. In another embodiment, the mutations include F78H, D80H, and L82H. In another
 15 embodiment, the mutations include Y79H, D80H, and L82H. In another embodiment, the mutations include D80H, L82H, and L83H. In another embodiment, the mutations include Y79H, D80H, and L83H. In another embodiment, the mutations include D80H, L83H, and I87H. The SPRs of **FIGS. 2A, 2B** and **3** show weakened affinity compared to WT for some specific variants, for example those having one or more mutations of: L82H, F78H/D80H/L82H, Y79H/D80H/L82H, D80H/L82H/L83H, Y79H/D80H/L83H,
 20 D80/L83H/I87H.

In one embodiment, the mutation is N434H. In another embodiment, the mutation is Y436L. In another embodiment, the mutations include L234F and L235E. In another
 embodiment, the mutations include M252Y, S254T, and T256E. In another embodiment, the mutations include L234F, L235E, and N434H. In another embodiment, the mutations include
 25 M252Y, S254T, T256E, N434H. In another embodiment, the mutations include L234F, L235E, M252Y, S254T, and T256E. In another embodiment, the mutations include L234F, L235E, M252Y, S254T, T256E, and N434H. In another embodiment, the mutations include T307A, E380A, and N434A.

In some embodiments, the mutant Ig Fc domain is derived from an IgG. In some
 30 embodiments, the mutant Ig Fc domain is derived from an IgG1, an IgG2, an IgG3, an IgG4, or a hybrid Fc domain thereof. In some embodiments, the mutant Ig Fc domain comprises a hybrid IgG1-IgG4 Fc domain.

In some embodiments, the mutant Ig Fc domain comprises an amino acid sequence at least 96%, 97%, 98%, or 99% identical to an amino acid sequence set forth in any one of

SEQ ID NOS: 16-24, and 72. In one embodiment, the mutant Ig Fc domain comprises an amino acid sequence identical to a mutant Ig Fc domain set forth in any one of SEQ ID NOS: 16-24, and 72.

5 In some embodiments, the TACI ECD portion is coupled to the mutant Ig Fc domain by a polypeptide linker. In one embodiment, the polypeptide linker contains an amino acid sequence selected from the group consisting of SEQ ID NOS: 3-15.

In some embodiments, the TACI-Fc fusion protein comprises an amino acid sequence at least 96%, 97%, 98%, or 99% identical to a TACI-Fc amino acid sequence set forth in any one of SEQ ID NOS: 25-38, 46-48, 55, 56, and 67. In one embodiment, the fusion protein
10 comprises an amino acid sequence identical to a TACI-Fc amino acid sequence set forth in any one of SEQ ID NOS: 25-38, 46-48, 55, 56, and 67. In another embodiment, the fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 25 or 67.

In some embodiments, the fusion protein exhibits a binding affinity (K_D) for FcRn at pH 6.0 that is less than 50 nM, less than 20 nM, less than 12 nM, less than 6 nM, or less than
15 4 nM.

In some embodiments, the fusion protein exhibits an increase in binding affinity (K_D) for FcRn of greater than 50-fold, greater than 100-fold, or greater than 200-fold at pH 6.0 relative to pH 7.4.

In another aspect, the present disclosure provides a pharmaceutical composition
20 comprising a TACI-Fc fusion protein disclosed herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a nucleic acid encoding a TACI-Fc fusion protein disclosed herein. In one embodiment, the nucleic acid encodes the fusion protein set forth in SEQ ID NO: 25 or 67. In another embodiment, the nucleic acid comprises
25 a nucleotide sequence set forth in SEQ ID NO: 69 or 70. In some embodiments, the nucleic acid encodes a codon-optimized TACI-Fc fusion protein.

In another aspect, the present disclosure provides an expression vector encoding a TACI-Fc fusion protein disclosed herein, such as the fusion protein set forth in SEQ ID NO: 25 or 67 or a codon-optimized TACI-Fc fusion protein. In one embodiment, the expression
30 vector is a viral vector. In another embodiment, the expression vector is an AAV vector.

In another aspect, the present disclosure provides a pharmaceutical composition comprising an expression vector encoding a TACI-Fc fusion protein disclosed herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides a host cell comprising a nucleic acid or expression vector encoding a TACI-Fc fusion protein disclosed herein.

In another aspect, a method of treating a malady comprises administering to a patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein or
5 expression vector encoding a TACI-Fc fusion protein in combination with a pharmaceutically acceptable carrier.

In some embodiments, the malady is an autoimmune disorder. In some embodiments, the autoimmune disorder is selected from the group consisting of Acute Antibody Mediated Rejection, Chronic Antibody Mediated Rejection primary Sjogren's syndrome, Autoimmune
10 Hemolytic Anemia, Antiphospholipid Syndrome (APS), Catastrophic APS, Refractory Rheumatoid Arthritis, Autoimmune Vasculitis, Cryoglobulinemia, Antineutrophil Cytoplasmic Autoantibodies (ANCA), IgA Vasculitis, Rheumatoid Vasculitis, Anti-Synthetase Syndrome, Primary Membranous Nephropathy, Juvenile Idiopathic Arthritis (JIA), Systemic Sclerosis, Guillain-Barre Syndrome, Autoimmune Pulmonary Alveolar
15 Proteinosis, Autoimmune Hemolytic Anemia (AIHA), Hemolytic Disease Of The Fetus And Newborn (HDFN), Acute Hemolytic Transfusion Reaction, Preventative Treatment with Adeno-Associated Virus (AAV), Thyroid Eye Disease (TED), Huntington's Disease, Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), Pemphigus Vulgaris (PV), Bullous Pemphigoid (BP), Immune Thrombocytopenic Purpura (ITP), Myasthenia Gravis (MG), and
20 Neuromyelitis Optica Spectrum Disorder (NMOSD).

In another aspect, a method of decreasing an immune response comprises administering to a patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein or expression vector encoding a TACI-Fc fusion protein in combination with a pharmaceutically acceptable carrier.

25 In another aspect, a method of inhibiting the activity of one or more pro-inflammatory cytokines comprises administering to a patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein or expression vector encoding a TACI-Fc fusion protein in combination with a pharmaceutically acceptable carrier. In some embodiments, the pro-inflammatory cytokines include BAFF and/or APRIL.

30 In another aspect, a method of enhancing clearance of IgG immunoglobulins comprises administering to a patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein or expression vector encoding a TACI-Fc fusion protein in combination with a pharmaceutically acceptable carrier.

In another aspect, a method of reducing levels of mature B-cells, IgG, IgM, IgA, or a combination thereof comprises administering to a patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein or expression vector encoding a TACI-Fc fusion protein in combination with a pharmaceutically acceptable carrier. In some embodiments, administration of the pharmaceutical composition does not significantly lower levels of IgD or IgE.

In another aspect, the present disclosure provides a kit for treating a malady in a patient in which the kit includes at least one dose of a pharmaceutical composition comprising a TACI-Fc fusion protein or expression vector encoding a TACI-Fc fusion protein and instructions for use.

In another aspect, the present disclosure provides a polypeptide linker comprising an amino acid sequence set forth in SEQ ID NOS: 4, 5, or 6.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a structural model based on 1XU1 and 1KD7 PBD entries showing positions of histidine residues (as black spheres) that were sequentially and combinatorially mutated at/near the predicted interface between TACI and BAFF using known contacts of TACI to BAFF and/or APRIL.

FIGS. 2A-2B show the effects of single histidine substitutions in the TACI domain on binding to BAFF (Figure 2A) and APRIL (Figure 2B) by Surface Plasmon Resonance (SPR).

FIG. 3 shows the effects of multiple histidine substitutions in the TACI domain on binding to BAFF by SPR.

FIG. 4 shows an exemplary sensorgram of TACI-Fc fusion proteins to BAFF, APRIL, or FcRn by bio-layer interferometry (BLI).

FIG. 5 is a sensorgram showing binding of TPP-6269 to BAFF at pH 6.0 and pH 7.4 by BLI.

FIG. 6 shows the results of a functional assay showing inhibition of BAFF binding to a BCMA/Nuclear Factor Kappa B (NF- κ B)-luciferase reporter HEK293 cell line by different concentrations of TACI-Fc fusion protein or a control (TPP-4217).

FIG. 7 shows the results of a functional assay showing inhibition of BAFF binding and NF- κ B-mediated activation in a BCMA/NF- κ B-luciferase reporter HEK293 cell line by different concentrations of TACI-Fc variants as indicated, including the corresponding IC50 values associated therewith.

FIG. 8 shows the results of a functional assay showing inhibition of BAFF binding and NF- κ B-mediated activation in a BCMA/NF- κ B-luciferase reporter HEK293 cell line by different concentrations of TACI-Fc fusion proteins TPP-4286 and a control (TPP-4291), including the corresponding IC₅₀ values associated therewith.

5 **FIGS. 9A-9H** depict bio-layer interferometry sensorgrams showing binding of TACI-Fc variants to FcRn at pH 6.0 (left panels) and pH 7.4 (right panels) using the Octet Red96 system. The TACI-Fc variants include: TPP-5260 (Figure 9A); TPP-5262 (Figure 9B); TPP-5317 (Figure 9C); TPP-5272 (Figure 9D); TPP-5273 (Figure 9E); TPP-5274 (Figure 9F); TPP-5275 (Figure 9G); and TPP-6269 (Figure 9H).

10 **FIG. 10** shows a crystal structure of a FcRn:Fc complex (PDB: 4N0U), showing acidic residues E115, E116, D130, and E133 in the processed human FcRn for targeting by the TACI-Fc fusion proteins of the present disclosure.

FIGS. 11A-11B show octet traces comparing FcRn binding of single-histidine substituted TACI-Fc variants (FIG. 11A) and both single and double-histidine substituted TACI-Fc variants (FIG. 11B) relative to a wild-type ("WT"; parental, non-histidine substituted TPP-5274) TACI-Fc fusion protein at pH 7.4. **FIGS. 11C-11D** show octet traces comparing FcRn binding of single-histidine substituted TACI-Fc fusion protein variants (FIG. 11C) and both single and double-histidine substituted TACI-Fc fusion protein variants (FIG. 11D) relative to a wild-type ("WT"; TPP-5274) TACI-Fc fusion protein at pH 6.0.

20 **FIG. 12A** shows octet traces comparing FcRn binding by TPP-5958 (L234F, L235E, M252Y, S254T, T256E, N434H) and TPP-5957 (L234F, L235E, M252Y, S254T, T256E) at pH 6.0. **FIG. 12B** shows octet traces comparing FcRn binding by TPP-5958 (L234F, L235E, M252Y, S254T, T256E, N434H) and TPP-5957 (L234F, L235E, M252Y, S254T, T256E) at pH 7.4.

25 **FIG. 13A** shows binding kinetics of TPP-6269 to various concentrations of FcRn at pH 6.0 by surface plasmon resonance (SPR)/Biacore. **FIG. 13B** shows binding kinetics of TPP-6269 to various concentrations of FcRn at pH 7.4 by SPR/Biacore. Figure 13C shows the corresponding equilibrium dissociation constants (K_D) determined by SPR/Biacore.

FIGS. 14A-14D show flow cytometry analyses demonstrating simultaneous binding of the TACI-Fc variants TPP-5954 (Figures 14A, 14B), TPP-5957 (Figures 14A, 14C) and TPP-5958 (Figures 14A, 14D) to BAFF and human FcRn in a human FcRn-expressing cell line.

FIGS. 15A-15B show flow cytometry analyses demonstrating the ability of TPP-6269 (Figure 15A) and other TACI-Fc variants (Figure 15B, as indicated) to block binding of human IgG1 to FcRn in a human FcRn-expressing cell line.

FIG. 16A is a graph showing the stability of selected TACI-Fc variants by accelerated stability testing using size exclusion chromatography to assay for percentage of TACI-Fc monomers as a function of time. **FIG. 16B** is a table showing the monomer percentage numbers for each of the selected TACI-Fc variants at time zero (T0) and following 1, 2, and 3 weeks of incubation at 37 °C.

FIG. 17A shows the *in-vivo* study design of an experiment to evaluate the ability of TPP-6269 to reduce levels of IgG, IgM, and mature B-cells following *in-vivo* treatment of mice with Keyhole Limpet Hemocyanin (KLH). **FIG. 17B** shows absolute numbers of B220+ cells and CD4+ T cells in mouse splenocytes following co-administration of TPP-6269 and KLH as compared to administration of a KLH control ("KLH + PBS") or no administration ("Healthy Control"), as determined by flow cytometry. **FIG. 17C** shows the resulting serum levels of anti-KLH IgM and IgG antibodies following the administrations. **FIG. 17D** shows the resulting serum levels of total IgM and total IgG antibodies following the administrations.

FIG. 18A shows the design of an experiment to evaluate clearance of human IgG (biotin-hIgG) antibodies following administration of TPP-6269 in a muFcRn^{-/-}, huFcRn Tg32 transgenic mouse *in vivo*. **FIG. 18B** shows the percent reduction of total human IgG antibodies as a function of time at dosages of 20 mg/kg and 60 mg/kg of TPP-6269 as compared to administration of PBS only.

FIG. 19A shows the design of an experiment to evaluate the pharmacokinetics of TPP-6269 over a period of 21 days.

FIG. 19B is a graph showing the results of this analysis.

FIG. 20A shows the design of an experiment to evaluate the pharmacokinetics and pharmacodynamics of TPP-6269 over a period of 10 days.

FIG. 20B is a graph showing the results of the pharmacokinetic analysis.

FIG. 20C is a graph showing the results of the pharmacodynamic analysis.

DETAILED DESCRIPTION

Definitions

5 As used herein, the word “a” or “plurality” before a noun represents one or more of the particular nouns. For example, the phrase “a mammalian cell” represents “one or more mammalian cells.”

The singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

10 The term “about”, particularly in reference to a given quantity or number, is meant to encompass deviations within plus or minus ten percent ($\pm 10\%$), five percent (*e.g.*, $\pm 5\%$), two percent ($\pm 2\%$), one percent ($\pm 1\%$), or one-half percent ($\pm 0.5\%$).

The terms “portion”, “portion of”, and “portion thereof” refer to a part or whole of the subject component. Thus, it can refer to the entirety of a component or part of the component.

15 The terms “polypeptide” and “protein” are used interchangeably herein and refer to a molecular chain of two or more amino acids linked through peptide bonds. The terms do not refer to a specific length of the product. Thus, “peptides,” and “oligopeptides,” are included within the definition of polypeptide. The terms include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. The
20 terms also include molecules in which one or more amino acid analogs, or non-canonical or unnatural amino acids are included as can be synthesized or expressed recombinantly using known protein engineering techniques. In addition, proteins can be derivatized as described herein by well-known organic chemistry techniques.

As used herein, the terms “domain” and “functional domain” are used with reference
25 to functional polypeptide or nucleic acid domain, typically containing or encoding an amino acid sequence of three or more, generally 5 or 7 or more amino acids, such as 10 to 200 amino acid residues that is structurally and/or functionally distinct from other portions of the molecule and is identifiable. For example, domains include those portions of a polypeptide chain that can form an independently folded structure within a protein made up of one or
30 more structural motifs and/or that is recognized by virtue of a functional activity, such as binding activity. A protein, such as the fusion proteins described herein, can include one or more distinct domains. A domain can be identified, defined, or distinguished by homology of the primary sequence or structure to related family members, such as homology to motifs. In another example, a domain can be distinguished by its function, such as an ability to interact

with a biomolecule, such as a cognate binding partner. A domain independently can exhibit a biological function or activity such that the domain independently or fused to another molecule can perform an activity, such as, for example binding. In some cases, adjacent N- and/or C-terminal amino acids of a given domain thereof (*e.g.*, cysteine rich domain (CRD)),
5 may be linked directly by a single peptide bond or a polypeptide linker containing one or more amino acid residues. Generally, the two portions and a polypeptide linker will be in reading frame with each other. Alternatively, the two portions may be covalently linked together using one or more cross-linkers known in the art.

As used herein, the term “TACI protein” (transmembrane activator or calcium-
10 modulating cyclophilin ligand-interactor) refers to a transmembrane protein of the TNF receptor superfamily (encoded by the *TNFRSF13B* gene), that antagonizes or blocks the activity of the B cell stimulatory receptors B cell maturation antigen (BCMA), B cell activation factor receptor (BAFF-R) by binding to their ligands APRIL (A proliferation-inducing ligand encoded by the *TNFSF1* gene) and BAFF (B-cell activating factor, encoded
15 by the *TNFSF13B* gene).

The terms “TACI extracellular domain (ECD)” and “TACI ECD portion” refer to a portion of the protein containing binding sites for APRIL and BAFF and are comprised of cysteine-rich TNFR repeat regions containing the TACI DR1/DR2 domains.

The terms “Fc region,” “Fc domain,” and “immunoglobulin (Ig) Fc” refers to the C-
20 terminal region of the heavy chain of an antibody, which includes Ig heavy chain constant region domains CH2 and CH3 and may include all of part of the Ig hinge domain. The Fc domain typically encodes various effector function(s) that can be modified or deleted. The Fc domain can form a monomer or dimer of two polypeptide chains joined via the hinge region cysteine residues forming inter-polypeptide disulfide bonds.

25 The terms “mutant” and “variant” refer to a protein obtained after modification of a parental protein in a protein modification process. Specifically, a mutant or variant is a protein that is derived from a parental protein by one or more amino acid substitution(s), amino acid deletion(s) and/or amino acid addition(s) relative to the parental protein, which may add, retain, remove, or enhance some or all of the functions of the parent.

30 As used herein, the terms “TACI mutant” and “TACI variant” are used interchangeably with reference to a TACI extracellular domain (ECD) portion that differs from the parent TACI ECD sequence due to at least one amino acid modification. The amino acid modification can be a substitution mutation, amino acid deletions and/or amino acid addition. A TACI mutant binds to APRIL and/or BAFF and may exhibit enhanced binding to

its ligands at low pH (*e.g.*, pH 6.0) compared to *e.g.*, a wild-type TACI ECD or a TACI reference protein from which the TACI mutant is derived. See *e.g.*, Tables 1, 2.

As used herein, the terms “Fc mutant”, “Fc variant” and “Ig Fc variant” are used interchangeably with reference to an Fc sequence that differs from the parent Fc sequence due to at least one amino acid modification. The Fc variant may comprise only the Fc region or may be present in the context of an antibody, Fc fusion, isolated Fc, Fc region, or other polypeptide that is substantially encoded by Fc. The Fc variant may refer to the Fc polypeptide itself, a composition comprising an Fc variant polypeptide, or an amino acid sequence encoding it.

The terms “TACI-Fc variant” and “TACI-Fc fusion protein” are used interchangeably with reference to a hybrid protein combining the binding specificity of a TACI-binding domain containing a TACI extracellular domain (ECD) portion comprised of TACI DR2- or TACI DR1/DR2 domain portions fused to or chemically conjugated to a mutant immunoglobulin (Ig) Fc domain exhibiting reduced effector functions due to inclusion of one or more mutations therein. The TACI ECD includes contiguous or non-contiguous amino acid sequences providing one or more binding sites for APRIL and BAFF. The APRIL and BAFF binding portions are derived from the TACI ECD without the transmembrane or cytoplasmic sequences of the TACI receptor.

The terms “Fc region,” “Fc domain,” and “Ig Fc” refers to the C-terminal region of the heavy chain of an antibody, which includes Ig heavy chain constant region domains CH2 and CH3 and may include all of part of the Ig hinge domain. The Fc domain typically encodes various effector function(s) that can be modified or deleted. The Fc domain can form a monomer or dimer of two polypeptide chains joined by one or more disulfide bonds. An Fc domain of the present disclosure is a variant or mutant form of a wild-type Fc domain exhibiting reduced effector functions (*e.g.*, reduced greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 2-fold, 5-fold or more).

As used herein, references to amino acid substitutions in the Fc region utilize the EU numbering system unless described with reference to a specific SEQ ID NO. EU numbering is known and is according to the most recently updated IMGT Scientific Chart (IMGT®, the international ImMunoGeneTics information System™, http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html (created: 17 May 2001, last updated: 10 Jan. 2013) and the EU index as reported in Kabat, E. A. et al. Sequences of Proteins of Immunological interest. 5th ed. US Department of Health and Human Services, NIH publication No. 91-3242 (1991).

The term "polypeptide linker" refers to a polypeptide defined by a sequence of amino acids that functions to connect the TACI domain in-frame to the Ig Fc domain, resulting in a TACI-Fc fusion protein.

As used herein, the term "hybrid Ig Fc" means a mutant Fc region comprises portions
5 from at least two IgG1 subclasses.

A "conjugate" refers to any hybrid molecule, including protein domains and/or other molecules that contain both amino acid or protein portions and non-protein portions. Conjugates may be synthesized by a variety of techniques known in the art including, for example, recombinant DNA techniques, solid phase synthesis, solution phase synthesis,
10 organic chemical synthetic techniques or a combination of these techniques. The choice of synthesis will depend upon the molecule to be generated. For example, a hybrid molecule not entirely "protein" in nature may be synthesized by a combination of recombinant techniques and solution phase techniques.

The terms "bind," "bound" or grammatical variations thereof are used with reference
15 to the participation of a molecule in any attractive interaction with another molecule, resulting in a stable association in which the two molecules are in close proximity to one another. Binding includes, but is not limited to, non-covalent bonds, covalent bonds (such as reversible and irreversible covalent bonds), and includes interactions between molecules such as, but not limited to, proteins, nucleic acids, carbohydrates, lipids, and small molecules, such
20 as chemical compounds including drugs.

With regard to binding of the TACI-Fc fusion proteins of the present disclosure, the terms "bind," "bound" or grammatical variations are used with reference to the TACI-Fc fusion protein or domain thereof specifically binding *in vitro* and/or *in vivo* to each of APRIL, BAFF, and the neonatal Fc receptor (FcRn) but not to other ligands or antigens.
25 Typically, the TACI-Fc fusion protein or domain specifically binds to each of APRIL, BAFF, and FcRn with an equilibrium dissociation constant (K_D) of approximately less than 10^{-7} M, such as approximately less than 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less, but not to another molecule with similar affinity when determined by, *e.g.*, surface plasmon resonance (SPR) technology in a BIACORE[®] 2000 surface plasmon resonance instrument using the
30 TACI-Fc fusion protein as the analyte and APRIL, BAFF or FcRn as the ligand. Accordingly, unless otherwise indicated, a TACI-Fc fusion protein binds to soluble or cell bound APRIL, BAFF, or FcRn with a K_D of 10^{-7} M or less, such as approximately less than 10^{-8} M, 10^{-9} M, 10^{-10} M, or lower.

The term “binding activity” refer to characteristics of a molecule, *e.g.*, a polypeptide or polypeptide domain relating to whether or how it binds one or more binding partners. A binding activity can include any measure of binding of one molecule for a binding partner. Binding activities include the ability to bind the binding partner(s), the affinity with which it binds to the binding partner (*e.g.*, high affinity), the avidity with which it binds to the binding partner, the strength of the bond with the binding partner and/or specificity or selectivity for binding with the binding partner.

The term “binding affinity” refers to the specific binding affinity of a protein for its binding partner (*i.e.*, its counter-structure) under specific binding conditions. The binding affinity refers to the strength of the interaction between two or more molecules, such as binding partners, typically the strength of the noncovalent interactions between two binding partners. An increase or attenuation in binding affinity of an affinity-modified domain, or a TACI-Fc fusion protein containing an affinity-modified domain, to a binding partner is determined relative to the binding affinity of the unmodified domain (*e.g.*, the native or wild-type TACI or Ig Fc domain). Methods for determining binding affinity, or relative binding affinity, are known in art, including Biacore measurements or flow cytometry, solid-phase ELISA immunoassays, ForteBio Octet etc. In some embodiments, binding affinity can be measured by flow cytometry, such as based on a Mean Fluorescence Intensity (MFI) in a flow binding assay.

The term “ k_{assoc} ” or “ k_a ”, as used herein, is intended to refer to the association rate of a particular protein-protein interaction, *e.g.*, TACI-Fc fusion protein or domain thereof to a ligand, such as APRIL, BAFF or FcRn, whereas the term “ k_{dis} ” or “ k_d ,” as used herein, is intended to refer to the dissociation rate of a particular protein-protein interaction. The term “ K_D ”, as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of k_d to k_a (*i.e.*, k_d/k_a) and is expressed as a molar concentration (M). K_D values can be determined using methods well established in the art. Methods for determining the K_D of the TACI-Fc fusion protein include surface plasmon resonance, preferably using a biosensor system such as a Biacore system, bio-layer interferometry, flow cytometry, and Scatchard analysis.

Such determinations are typically measured at, for example, 25° C or 37°C. For example, the kinetics of fusion protein binding to APRIL, BAFF or FcRn can be determined at pH 8.0, 7.4, 7.0, 6.5 and 6.0 via surface plasmon resonance (“SPR”) on a BIAcore 3000 instrument using a suitable capture method to immobilize the fusion protein or ligand binding thereto.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For
5 further descriptions, see Jonsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jonsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

The term, "corresponding to" with reference to positions of a protein or polynucleotide, whereby amino acid or nucleotide positions in a reference sequence
10 "correspond to" nucleotides or amino acid positions in a disclosed sequence, such as set forth in the Sequence Listing, such that the corresponding amino acid or nucleotide positions are identified upon structural alignment of two or more sequences using a standard alignment algorithm, such as the GAP algorithm. By aligning the sequences, one skilled in the art can identify corresponding residues, for example, using conserved and identical amino acid
15 residues as guides.

The term "sequence identity" as used herein refers to the sequence identity between genes or proteins at the nucleotide or amino acid level, respectively. "Sequence identity" is a measure of identity between proteins at the amino acid level and a measure of identity between nucleic acids at nucleotide level. The protein sequence identity may be determined
20 by comparing the amino acid sequence in each position when the sequences are aligned. Similarly, the nucleic acid sequence identity may be determined by comparing the nucleotide sequence in each position when the sequences are aligned. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software, FASTA and TFASTA.

25 The term "expression vector" refers to a nucleic acid molecule containing nucleic acid sequences necessary for expression in prokaryotes, including e.g., a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences, or expression in eukaryotic cells, including e.g., promoters, enhancers, termination and polyadenylation signals, and the like. A secretory signal peptide sequence can also, optionally, be encoded by
30 the recombinant expression vector, operably linked to the coding sequence so that the expressed protein can be secreted by the recombinant host cell, such as for its expression as a secreted protein or for more facile isolation or purification of the TACI-Fc fusion protein from the cell, if desired. The term includes the vector as a self-replicating nucleic acid

structure as well as the vector incorporated into the genome of a host cell into which it has been introduced.

Expression vectors include regulatory sequence(s), e.g., expression control sequences, sufficient to direct transcription of an operably linked nucleic acid under at least some conditions; other elements required or helpful for expression may be supplied by, e.g., the host cell or by an *in vitro* expression system. Such regulatory sequences typically include a promoter and may include enhancer sequences or upstream activator sequences. In some embodiments a vector may include sequences that encode a 5' untranslated region and/or a 3' untranslated region, which may comprise a cleavage and/or polyadenylation signal. In general, regulatory elements may be contained in a vector prior to insertion of a nucleic acid whose expression is desired or may be contained in an inserted nucleic acid or may be inserted into a vector following insertion of a nucleic acid whose expression is desired. Expression vectors include non-viral vectors, such as plasmid vectors, and viral vectors, such as adeno-associated virus (AAV) vectors, lentivirus vectors, and the like.

As used herein, the term "malady" is used with reference to any disease, disorder, or condition directly or indirectly causing or contributing to a state other than physiological homeostasis existing in the absence of such disease, disorder, or condition.

As used herein, the term "treating" includes prophylactic and/or therapeutic treatments of one or more maladies. The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the TACI- Fc fusion protein compositions described herein. If it is administered prior to clinical manifestation of a malady, the treatment is prophylactic, (*i.e.*, it protects the host against developing the malady or reduces the clinical manifestations that would otherwise result in the absence of treatment), whereas if it is administered after manifestation of the malady, the treatment is therapeutic, (*i.e.*, it is intended to diminish, ameliorate, or stabilize the existing malady or side effects thereof). Preferably, it is intended that the severity of the subject's condition is reduced or at least partially improved or modified and that some alleviation, mitigation, reversal or decrease in at least one clinical symptom (*e.g.*, reduced autoimmune symptoms, weight loss in subjects compared to normal subjects) is achieved.

The term "pharmaceutical composition" refers to preparations which are in such form as to permit the biological activity of the active ingredients to be unequivocally effective, and which contain no additional components which are significantly toxic to subjects in whom the formulation would be administered.

As used herein, the term “subject” and includes both human subjects and non-human subjects (*e.g.*, veterinary animal or wild animal). Preferably, “subjects” include human patients.

The term “effective amount” or “a therapeutically effective amount” refers to an amount of a TACI-Fc fusion protein, alone or in combination with one or more other therapeutic agents, that provides a desired biological, therapeutic, and/or prophylactic result. That result can be reduction, amelioration, palliation, lessening, delaying, and/or alleviation of one or more of the signs, symptoms, or causes of a disease in a subject, or any other desired alteration of a biological system. An effective amount can be administered in one or more administrations.

As used herein, “effective treatment” refers to treatment producing a beneficial effect, *e.g.*, amelioration of at least one symptom of a malady in a subject. A beneficial effect can take the form of an improvement over baseline, *i.e.*, an improvement over a measurement or observation made prior to initiation of therapy according to the method.

The term “biological half-life”, as used herein, refers to the amount of time it takes for the TACI-Fc fusion protein to lose half of its pharmacologic or physiologic activity or concentration. Biological half-life can be affected by elimination, excretion, degradation (*e.g.*, enzymatic degradation/digestion) of the fusion protein, or absorption and concentration in certain organs or tissues of the body. In some embodiments, biological half-life can be assessed by determining the time it takes for the blood plasma concentration of the substance to reach half its steady state level (“plasma half-life”).

I. TACI-Fc Fusion Proteins

Provided herein are TACI-Fc fusion proteins binding to and inhibiting the activity of BAFF, APRIL ligands to neutralize their activity and block or antagonize the activity of B cell stimulatory receptors, TACI, BCMA, and BAFF-R. The TACI-Fc fusion proteins further include a mutant Fc domain containing mutations to block one or more effector functions, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC), and/or inhibit one or more activities, including FcRn activity. As further described below, the TACI-Fc fusion proteins can be used for the treatment of a variety of maladies associated with a dysregulated immune response associated with inflammatory or autoimmune symptoms accompanying a wide range of inflammatory and autoimmune diseases and disorders.

The TACI-Fc fusion proteins of the present disclosure include a TACI extracellular domain (ECD) portion and a mutant Ig Fc domain.

In some embodiments, the TACI ECD portion includes a TACI DR2 domain. In some embodiments, the TACI ECD portion includes a contiguous ECD portion spanning at least a portion of the CRD1/CRD2 region. In some embodiments, the TACI ECD portion includes an amino acid sequence set forth in SEQ ID NO: 1 or 2.

In some embodiments, the TACI ECD portion includes one or more mutations, such as those identified in Tables 1-3, where the amino acids are numbered and identified according to the amino acid sequence set forth in SEQ ID NO: 40.

- 10 In another embodiment, the mutations include F78H and L83H.
In another embodiment, the mutations include F78H and D80H.
In another embodiment, the mutations include F78H, D80H, and L82H.
In another embodiment, the mutations include Y79H and L83H.
In another embodiment, the mutations include Y79H, D80H, and L83H.
15 In another embodiment, the mutations include Y79H, D80H, and L82H.
In one embodiment, the mutations include D80H.
In another embodiment, the mutations include D80H and L82H.
In another embodiment, the mutations include D80H and L83H.
In another embodiment, the mutations include D80H and I87H.
20 In another embodiment, the mutations include D80H, L82H, and L83H.
In another embodiment, the mutations include D80H, L83H, and I87H.
In one embodiment, the mutations include L82H.
In another embodiment, the mutations include L82H and I87H.
In another embodiment, the mutations include L82H and I92H.
25 In another embodiment, the mutations include L82H and Q95H.
In another embodiment, the mutations include L83H.
In another embodiment, the mutations include L83H and I87H.
In another embodiment, the mutations include I87H and I92H.

In some embodiments, the TACI-Fc fusion protein includes a mutant Ig Fc domain.

- 30 The mutant Fc domain may contain a variety of mutations serving to reduce Fc effector functions, including down-regulation of inflammatory functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC), and C1q binding; down-regulate B cell activation and B cell receptor expression; enhance binding to

the neonatal Fc receptor (FcRn); increase protein stability and/or render the mutant Fc domain inert for one or more of these functions.

The FcRn binding sequence in the Fc region plays an important in regulating serum IgG levels. The FcRn regulates serum IgG concentrations by binding to and protecting
5 endocytosed monomeric IgG from degradation in the lysosomal compartment and transporting the IgG to the cell surface for release at neutral extracellular pH. Through this mechanism, FcRn is responsible for the long serum half-life of IgG since IgG that is not bound by FcRn enters the lysosomal pathway and is degraded.

High dose administration of IgGs in autoimmune disease patients has a palliative
10 effect that can be explained at least partially by saturation of FcRn-mediated protection of IgG, shortening the half-life of pathogenic IgG (Jin & Balthasar, 2005, Hum. Immunol. 66:403-410; Akilesh et al., 2004, J. Clin. Invest. 113:1328-1333; Li et al., 2005, J. Clin. Invest. 115:3440-3450). Accordingly, specific blockade of FcRn-IgG interactions can be used to promote degradation of pathogenic IgG antibodies, for example to treat IgG mediated
15 autoimmune diseases. For example, in one embodiment, the TACI-Fc fusion proteins of the present disclosure are used in a method of enhancing clearance of IgG immunoglobulins in a patient with an inflammatory or autoimmune disease, disorder or condition.

The TACI-Fc fusion proteins of the present disclosure include n Ig Fc domain comprising one or more mutations that enhance binding of mutant Fc domain to the FcRn so
20 that the TACI-Fc fusion protein outcompetes IgGs for binding to the FcRn, thereby reducing IgG levels. The mutant Fc domain may further include one or more mutations conferring improved functional activities, anti-inflammatory functions, or pharmacokinetic properties associated with the TACI-Fc fusion protein of the present disclosure. The improved functional activities or anti-inflammatory functions relate to improvements associated with
25 Clq binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor); B cell activation; and/or increased protein stability.

In some embodiments, the TACI-Fc fusion protein includes an Ig Fc domain comprising an N434H mutation.

30 In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising an Y436L mutation.

In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising L234F and L235E mutations.

In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising M252Y, S254T, and T256E mutations.

In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising L234F/L235E/N434H mutations.

5 In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising M252Y, S254T, T256E, N434H mutations.

In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising L234F, L235E, M252Y, S254T, and T256E mutations.

10 In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising L234F, L235E, M252Y, S254T, T256E, and N434H mutations.

In another embodiment, the TACI-Fc fusion protein includes an Ig Fc domain comprising T307A, E380A, and N434A mutations.

15 Additionally useful mutations or mutation combinations for incorporation in the TACI-Fc fusion proteins of the present disclosure are disclosed in US 2015/0017164, US 2020/0095310, and US 2021/0388054, the disclosures of which are expressed incorporated by reference herein.

In some embodiments, the mutant Ig Fc domain comprises an amino acid sequence at least 96%, 97%, 98%, or 99% identical to an amino acid sequence set forth in SEQ ID NOS: 16-24, and 72. In other embodiments, the mutant Ig Fc domain comprises an amino acid
20 sequence identical to a mutant Ig Fc domain set forth in any one of SEQ ID NOS: 16-24, and 72. In some embodiments, the mutant Ig Fc domain according to any one of SEQ ID NOS: 16-24, and 72, and optionally including a terminal lysine residue attached thereto. In one embodiment, the mutant Ig Fc domain comprises the amino acid sequence set forth in SEQ ID NO: 19. In another embodiment, the mutant Ig Fc domain comprises the amino acid
25 sequence set forth in SEQ ID NO: 72.

In some embodiments, the TACI-Fc fusion protein includes a TACI ECD portion having any of the above-described mutations or mutation combinations, and any of the above-described Ig Fc domain mutations or mutation combinations.

30 Preferably, the mutant Fc domain is derived from an IgG. Various IgG isotypes may be employed, including Fc domains derived from IgG1, IgG2, IgG3, IgG4, or a combination thereof. In preferred embodiments, the Fc domain comprises a hybrid IgG1-IgG4 Fc domain. In certain embodiments, the hybrid IgG1-IgG4 Fc domain is constructed from a nucleic acid encoding an IgG1 gamma-1 chain C region (*e.g.*, Accession No. P01857) and an IgG4 gamma-4 chain C region (*e.g.*, Accession No. P01861).

The TACI ECD portion is coupled (e.g., fused or conjugated) to the mutant Ig Fc domain. In some embodiments, the TACI ECD portion is fused to the mutant Ig Fc domain by a polypeptide linker. In some embodiments, the TACI ECD portion is chemically conjugated to the mutant Ig Fc domain.

5 In some embodiments, the polypeptide linker comprises an amino acid sequence with a length of at least 3, at least 5, at least 10, at least 15, at least 20, at least 30, at least 40, or at least 50 amino acids, including one or more amino acids selected from G (glycine), alanine (A), serine (S), proline (P), or a combination thereof.

In one embodiment, the polypeptide linker comprises the amino acid sequence
10 $(AG_x)_n$, $(GA_x)_n$, $(A_xG)_n$, or $(G_xA)_n$, where $x=1, 2, 3, 4, 5, 6, 7$, or 8 and $n=1, 2, 3, 4, 5, 6, 7$, or 8 .

In another embodiment, the polypeptide linker comprises the amino acid sequence $(SG_x)_n$, $(GS_x)_n$, $(S_xG)_n$, or $(G_xS)_n$, where $x=1, 2, 3, 4, 5, 6, 7$, or 8 and $n=1, 2, 3, 4, 5, 6, 7$, or 8 .

15 In another embodiment, the polypeptide linker comprises the amino acid sequence $(EA_xK)_n$, where $x=2, 3, 4, 5, 6, 7$, or 8 and $n=1, 2, 3, 4, 5, 6, 7$, or 8 .

In some embodiments, the TACI ECD portion is fused to the mutant Ig Fc domain by a polypeptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3-15.

20 In some embodiments, the present disclosure provides a polypeptide linker comprising an amino acid sequence selected from the group consisting of 4, 5, and 6.

In some embodiments, the TACI Fc fusion protein comprises a polypeptide linker according to any one of SEQ ID NOS: 3-15, which is fused to an Ig Fc domain portion according to any one of SEQ ID NOS: 16-24, and 72, and optionally containing a C-terminal
25 lysine attached thereto. For example, in one embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 61. In another embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 62. In another embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:
30 63. In another embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 64. In another embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 65. In another embodiment, the TACI Fc fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 66.

In some embodiments, the TACI Fc fusion protein comprises a TACI ECD portion with or without any of the above-described TACI mutations or TACI mutation combinations, wherein the TACI ECD portion is fused to any of the polypeptide linkers set forth in any one of SEQ ID NOS: 3-15.

5 In some embodiments, the TACI Fc fusion protein comprises a TACI ECD portion according to SEQ ID NO: 1 or SEQ ID NO: 2, which is fused to a polypeptide linker according to any one of SEQ ID NOS: 3-15.

In some embodiments, the TACI-Fc fusion protein includes a TACI ECD portion comprising any of the above-described TACI mutations or TACI mutation combinations in
10 combination with any of the above-described Ig Fc domain mutations or Ig Fc mutation combinations and with any of the above-described polypeptide linkers.

In some embodiments, the TACI ECD portion is conjugated to the Fc domain. In one embodiment, TACI ECD portion is cross-linked to the Fc domain using any of a number of known chemical cross linkers. Exemplary cross linkers are those which link two amino acid
15 residues via a linkage that includes a “hindered” disulfide bond. In these linkages, a disulfide bond within the cross-linking unit is protected (by hindering groups on either side of the disulfide bond) from reduction by the action, for example, of reduced glutathione or the enzyme disulfide reductase. One suitable reagent, 4-succinimidylloxycarbonyl- α -methyl- α (2-pyridyl)dithio) toluene (SMPT), forms such a linkage between two proteins utilizing a
20 terminal lysine on one of the proteins and a terminal cysteine on the other. Heterobifunctional reagents that cross-link by a different coupling moiety on each protein can also be used. Other useful cross-linkers include, without limitation, reagents which link two amino groups (e.g., N-5-azido-2-nitrobenzoyloxysuccinimide), two sulfhydryl groups (e.g., 1,4-bis-maleimidobutane), an amino group and a sulfhydryl group (e.g., m-maleimidobenzoyl-N-
25 hydroxysuccinimide ester), an amino group and a carboxyl group (e.g., 4-[p-azidosalicylamido]butylamine), and an amino group and a guanidinium group that is present in the side chain of arginine (e.g., p-azidophenyl glyoxal monohydrate).

In some embodiments, the TACI-Fc fusion protein is conjugated to polyethyleneglycol (PEG). The PEG may be attached to any amino acid side chain or
30 terminal amino acid functional group, e.g., a free amino, imino, thiol, hydroxyl, or carboxyl group. Methods of attaching PEG to antibodies known in the art may be employed.

In some embodiments, the fusion protein comprises an amino acid sequence at least 96%, 97%, 98%, or 99% identical to an amino acid sequence set forth in any one of SEQ ID

NOS: 25-38, 46-48, 55, 56, and 67. In some embodiments, the fusion protein comprises an amino acid sequence set forth in any one of SEQ ID NOS: 25-38, 46-48, 55, 56, and 67.

In one embodiment, the TACI-Fc fusion protein is TPP-6269, which comprises the amino acid sequence in SEQ ID NO: 25.

5 In another embodiment, the TACI-Fc fusion protein is TPP-6270, which comprises the amino acid sequence in SEQ ID NO: 26.

In another embodiment, the TACI-Fc fusion protein is TPP-6267, which comprises the amino acid sequence in SEQ ID NO: 27.

10 In another embodiment, the TACI-Fc fusion protein is TPP-5776, which comprises the amino acid sequence in SEQ ID NO: 28.

In another embodiment, the TACI-Fc fusion protein is TPP-5954, which comprises the amino acid sequence in SEQ ID NO: 29.

In another embodiment, the TACI-Fc fusion protein is TPP-5957, which comprises the amino acid sequence in SEQ ID NO: 30.

15 In another embodiment, the TACI-Fc fusion protein is TPP-5958, which comprises the amino acid sequence in SEQ ID NO: 31.

In another embodiment, the TACI-Fc fusion protein is TPP-5975, which comprises the amino acid sequence in SEQ ID NO: 32.

20 In another embodiment, the TACI-Fc fusion protein is TPP-5977, which comprises the amino acid sequence in SEQ ID NO: 33.

In another embodiment, the TACI-Fc fusion protein is TPP-5791, which comprises the amino acid sequence in SEQ ID NO: 34.

In another embodiment, the TACI-Fc fusion protein is TPP-5780, which comprises the amino acid sequence in SEQ ID NO: 35.

25 In another embodiment, the TACI-Fc fusion protein is TPP-5779, which comprises the amino acid sequence in SEQ ID NO: 36.

In another embodiment, the TACI-Fc fusion protein is TPP-5792, which comprises the amino acid sequence in SEQ ID NO: 37.

30 In another embodiment, the TACI-Fc fusion protein is TPP-5298, which comprises the amino acid sequence in SEQ ID NO: 38.

In another embodiment, the TACI-Fc fusion protein is TPP-7165, which comprises the amino acid sequence in SEQ ID NO: 46.

In another embodiment, the TACI-Fc fusion protein is TPP-7166, which comprises the amino acid sequence in SEQ ID NO: 47.

In another embodiment, the TACI-Fc fusion protein is TPP-7167, which comprises the amino acid sequence in SEQ ID NO: 48.

In another embodiment, the TACI-Fc fusion protein is TPP-6265, which comprises the amino acid sequence in SEQ ID NO: 55.

5 In another embodiment, the TACI-Fc fusion protein is TPP-6458, which comprises the amino acid sequence in SEQ ID NO: 56.

In another embodiment, the TACI-Fc fusion protein is TPP-6265, which comprises the amino acid sequence in SEQ ID NO: 67.

10 The TACI-Fc fusion proteins of the present disclosure have been engineered to exhibit increased binding at pH 6.0 and low or negligible binding at pH 7.4. In one embodiment, a TACI-Fc fusion protein is characterized by a binding affinity (K_D) for FcRn at pH 6.0 that is less than 50 nM, less than 20 nM, less than 12 nM, less than 6 nM, or less than 4 nM. The TACI-Fc fusion protein may be further characterized by a binding affinity (K_D) for FcRn that is greater than 50-fold, greater than 100-fold, or greater than 200-fold at pH 6.0
15 relative to pH 7.4. Moreover, inasmuch as the mutant Fc region in the TACI-Fc fusion protein exhibits increased binding to the FcRn compared to a wild-type Fc region, IgG levels in the circulation are reduced.

In certain embodiments, the TACI-Fc fusion proteins of the present disclosure exhibit *in vivo* half-life increases greater than or about 1.2-fold, about 1.5-fold, about 2.0-fold, about
20 3.0-fold, about 4.0-fold, about 5.0-fold, or about 6.0-fold. In some embodiments, the biological half-life is extended by more than 6 hours, more than 12 hours, more than 24 hours, more than 48 hours, more than 72 hours, more than 96 hours or more than 1 week after *in vivo* administration compared to a corresponding TACI-Fc fusion protein containing a wild-type or non-mutated parental Fc region. The biological half-life can be determined for
25 example, by using an ELISA assay or an activity assay.

In another aspect, the present application provides a fusion protein comprising a polypeptide linker fused to a hybrid Ig Fc domain. The polypeptide linker may be fused to the N-terminal or C-terminal end of the hybrid Ig Fc domain. The fusion protein may be further linked (or fused) to any TACI domain according to the present application. In one
30 embodiment, the polypeptide linker comprises an amino acid sequence set forth in any one of SEQ ID NOS: 3-15, and the hybrid Ig Fc domain comprises an amino acid sequence set forth in any one of SEQ ID NOS: 16-24 or 72, and optionally containing a lysine added at the C-terminal end. In some embodiments, the fusion protein comprises an amino acid sequence set

forth in any of SEQ ID NOS: 61-66, and optionally containing a lysine added at the C-terminal end.

II. Nucleic Acids, Expression Vectors, and Cells

5 In another aspect, the present disclosure provides nucleic acids encoding the TACI-Fc fusion proteins, expression vectors for expressing the TACI-Fc fusion proteins, as host cells, including stably transformed cell cells expressing the TACI-Fc fusion proteins. Cell lines expressing the TACI-Fc fusion proteins can be used for manufacturing the TACI-Fc fusion proteins used in the pharmaceutical compositions for treatment.

10 In one aspect, the present disclosure provides expression vectors for expressing the TACI-Fc fusion proteins in appropriate cells under conditions suitable for expressing the TACI-Fc fusion protein. A nucleic acid molecule or expression vector includes the DNA molecule that encodes the TACI-Fc fusion protein operatively linked to appropriate expression control sequences. Expression control sequences include promoters, activators,
15 enhancers, operators, ribosomal binding sites, start signals, stop signals, cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation. The choice and design of an appropriate vector and regulatory element(s) is within the ability and discretion of one of ordinary skill in the art. For example, one of ordinary skill in the art can select an appropriate promoter (or other expression control
20 sequences) for expression in a desired species (*e.g.*, a mammalian species) or cell type.

In some embodiments, the expression vector includes a promoter capable of directing expression in mammalian cells, such as a suitable viral promoter, *e.g.*, from a cytomegalovirus (CMV), retrovirus, simian virus (*e.g.*, SV40), papilloma virus, herpes virus or other virus that infects mammalian cells, or a mammalian promoter from, *e.g.*, a gene such
25 as EF1 α , ubiquitin (*e.g.*, ubiquitin B or C), globin, actin, phosphoglycerate kinase (PGK), etc., or a composite promoter such as a CAG promoter (combination of the CMV early enhancer element and chicken beta-actin promoter). In some embodiments a human promoter may be used. In some embodiments, a promoter that ordinarily directs transcription by a eukaryotic RNA polymerase II (a “pol II promoter”) or a functional variant thereof is
30 used. In some embodiments, a promoter that ordinarily directs transcription by a eukaryotic RNA polymerase I promoter, *e.g.*, a promoter for transcription of ribosomal RNA (other than 5S rRNA) or a functional variant thereof is used. In some embodiments, a promoter that ordinarily directs transcription by a eukaryotic RNA polymerase III (a “pol III promoter”), *e.g.*, (a U6, H1, 7SK or tRNA promoter or a functional variant thereof) may be used.

Exemplary promoters include, but are not limited to, the cytomegalovirus (CMV) immediate early promoter, an RSV LTR, a MoMLV LTR, a phosphoglycerate kinase-1 (PGK) promoter, a simian virus 40 (SV40) promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a neuron-selective promoter, such as the human synapsin promoter, a muscle-specific promoter, such as the human creatine kinase (MCK) promoter, a liver-specific promoter, such as the human phosphoenolpyruvate carboxykinase (PEPCK) promoter, a rhodopsin kinase promoter, an opsin promoter, a U6 promoter, an E2F promoter, a telomerase (hTERT) promoter, an H1 promoter, a cytomegalovirus enhancer/chicken beta-actin/rabbit β -globin promoter (CAG) promoter, an elongation factor 1-alpha promoter (EF1- α) promoter, a human β -glucuronidase promoter, a chicken β -actin (CBA) promoter, a retroviral Rous sarcoma virus (RSV) LTR promoter, a dihydrofolate reductase promoter, and a 13-actin promoter.

In some embodiments, the expression vector includes a codon-optimized TACI-Fc fusion protein. The terms “codon optimized” and “codon optimization” refer to a process for modifying a nucleic acid sequence according to one or more of the following: (1) to match codon frequencies in a host organism target; (2) to promote increased expression; (3) to ensure proper folding; (4) to provide a GC content suitable for increasing mRNA stability or reducing secondary structures; (5) to minimize tandem repeat codons or base runs that may impair gene construction or expression; (6) to customize transcriptional and translational control regions; (7) to insert or remove protein trafficking sequences; (8) to remove/add post translation modification sites in an encoded protein (*e.g.* glycosylation sites); (9) to add, remove or shuffle protein domains; (10) to insert or delete restriction sites; (11) modify ribosome binding sites and mRNA degradation sites; (12) to adjust translational rates to allow the various domains of the protein to fold properly; or (13) to reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art—non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park Calif.) and/or proprietary methods.

In one embodiment, the nucleic acid encodes the fusion protein set forth in SEQ ID NO: 25 or 67.

In another embodiment, the nucleic acid comprises a nucleotide sequence set forth in SEQ ID NO: 69 or 70.

In certain embodiments, a nucleic acid encoding the TACI-Fc fusion protein is provided, which is codon optimized for expression in humans. The codon-optimized

polynucleotide may be prepared by replacing the codons of the polynucleotide encoding the TACI-Fc fusion protein with *e.g.*, codons appearing more frequently in highly expressed human genes. Codon optimization methods are known in the art and may be used as provided herein. In some embodiments,

5 In some embodiments, a codon optimized polynucleotide sequence shares less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55% or less than 50% sequence identity to the corresponding naturally occurring or wild-type sequences from which the TACI-Fc fusion protein is derived. In some embodiments, a codon optimized polynucleotide sequence shares between
10 50% and 95%, between 50% and 90%, between 50% and 85%, between 50% and 80%, between 50% and 75%, between 50% and 70%, between 50% and 65%, between 50% and 60%, between 50% and 55%, between 55% and 95%, between 55% and 90%, between 55% and 85%, between 55% and 80%, between 55% and 75%, between 55% and 70%, between 55% and 65%, between 55% and 60%, between 60% and 95%, between 60% and 90%,
15 between 60% and 85%, between 60% and 80%, between 60% and 75%, between 60% and 70%, between 60% and 65%, between 65% and 95%, between 65% and 90%, between 65% and 85%, between 65% and 80%, between 65% and 75%, between 65% and 70%, between 70% and 95%, between 70% and 90%, between 70% and 85%, between 70% and 80%, between 70% and 75%, between 75% and 95%, between 75% and 90%, between 75% and
20 85%, between 75% and 80%, between 80% and 95%, between 80% and 90%, between 80% and 85%, between 85% and 95%, between 85% and 90%, or between 90% and 95% sequence identity to the corresponding naturally occurring or wild-type sequences from which the TACI-Fc fusion protein is derived.

 In some embodiments, a heterologous (non-native) signal peptide is added to or
25 utilized in a suitable expression encoding the TACI-Fc fusion protein. The signal peptide operably linked to the nucleic acid encoding a TACI-Fc fusion protein such that a resultant soluble TACI-Fc fusion protein is recovered from the culture medium, host cell, or host cell periplasm. In some embodiments, the signal peptide is a signal peptide from an immunoglobulin (such as IgG heavy chain or IgG-kappa light chain), a cytokine (such as
30 interleukin-2 (IL-2), or CD33), a serum albumin protein (*e.g.* HSA or albumin), a human azurocidin preprotein signal sequence, a luciferase, a trypsinogen (*e.g.*, chymotrypsinogen or trypsinogen) or other signal peptides able to efficiently express and/or secrete the TACI-Fc fusion protein.

The expression vectors may be in the form of viral vectors or non-viral vectors, such as plasmids. In some embodiments, the expression vectors are viral vectors. Viral vectors for expression of the antibody may be derived from, e.g., adenoviruses, adeno-associated viruses (AAV), retroviruses (including lentiviruses, such as HIV-1 and HIV-2), vaccinia viruses and
5 other poxviruses, herpesviruses (*e.g.*, herpes simplex virus Types 1 and 2), polioviruses, Sindbis and other RNA viruses, alphaviruses, astroviruses, coronaviruses, orthomyxoviruses, papovaviruses, paramyxoviruses, parvoviruses, picornaviruses, togaviruses and others. The viral vectors may or may not contain sufficient viral genetic information and/or structural components for production of infectious virus when introduced into host cells, i.e., viral
10 vectors may be replication-competent or replication-defective. In some embodiments, *e.g.*, where structural components for production of infectious virus are lacking, the necessary functional components may be supplied in trans by a host cell or by another vector introduced into the cell when production of recombinant virus is desired. In preferred embodiments, replication-defective recombinant viruses are administered for treatment. A nucleic acid for
15 delivery may be incorporated into a naturally occurring or modified viral genome (or a portion thereof) or may be present within a viral capsid as a separate nucleic acid molecule.

In certain cases, the viral vectors may be engineered to target specific cells affected by disease, disorder, or conditions, as appropriate, by using the targeting characteristics inherent to the virus vector or engineered into the virus vector. Specific cells may be
20 “targeted” for delivery and expression of polynucleotides. Thus, “targeting,” in this case, relates to the use of endogenous or heterologous binding agents in the form of capsids, envelope proteins, antibodies for delivery to specific cells, the use of tissue-specific regulatory elements for restricting expression to specific subset(s) of cells, or both.

In some embodiments, the viral vectors are AAV vectors. AAV vectors provide a
25 preferred delivery system for the nucleic acid therapeutics of the present application since they can allow for long lasting and continuous expression of functional alleles and silencing of the corresponding mutant alleles. AAV vectors can include or can be modified to control expression of the first and second active agents on account of a variety of regulatory elements, including various promoter and/or enhancer elements for constitutive or cell-type
30 specific expression.

In another aspect, a method for producing the TACI-Fc fusion protein includes the steps of culturing a host cell comprising an expression vector encoding TACI-Fc and collecting TACI-Fc fusion protein from the cell or from a culture supernatant thereof.

A large number of well-known, publicly available host cells can be used in preparing the polypeptides from *e.g.* engineered cells or cell lines. The selection of a cell or cell line is dependent upon a number of factors recognized in the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of transformation, ease of recovery of the peptides, expression characteristics, biosafety, and costs.

In some embodiments, the host cell is a mammalian cell. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

In some embodiments, the host cells are Chinese hamster ovary (CHO) or HEK293 cells. In some embodiments, the host cell is a suspension cell, and the polypeptide is engineered or produced in cultured suspension, such as in cultured suspension CHO cells, *e.g.*, CHO-S cells. In some examples, the cell line is a CHO cell line that is deficient in DHFR (DHFR-), such as DG44 and DUXB11. In some embodiments, the cell is deficient in glutamine synthase (GS), *e.g.*, CHO-S cells, CHOK1 SV cells, and CHOZN((R)) GS-/- cells. In some embodiments, the CHO cells, such as suspension CHO cells, may be CHO-S-2H2 cells, CHO-S-clone 14 cells, or ExpiCHO-S cells. In other embodiments, the host cells are yeast cells.

In some embodiments, host cells are prokaryotic cells, such as *E. coli*. The transformed prokaryotic cell is cultured under polypeptide expressing conditions, and then purified to obtain a soluble protein. The host cells can be cultured under conventional fermentation conditions so that the desired polypeptides are expressed. The TACI-Fc fusion proteins can be recovered and purified from recombinant cell cultures by methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion, or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, and affinity chromatography. Protein refolding steps can be used, as desired, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed in the final purification steps.

III. Pharmaceutical Compositions

In another aspect, a pharmaceutical composition comprises the TACI-Fc fusion according to the present disclosure and a pharmaceutically acceptable carrier. The pharmaceutical composition can contain, for example, one or more excipients for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Such compositions may include buffers, such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates, such as glucose, mannose, sucrose or dextrans, mannitol; polypeptides or amino acids, such as glycine; antioxidants; chelating agents, such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); preservatives; or combinations thereof.

In some embodiments, the pharmaceutical composition is a solid, such as a powder, capsule, or tablet. For example, the components of the pharmaceutical composition can be lyophilized. In some embodiments, the solid pharmaceutical composition is reconstituted or dissolved in a liquid prior to administration.

In some embodiments, the pharmaceutical composition is a liquid, for example TACI-Fc fusion proteins dissolved in an aqueous solution (such as physiological saline or Ringer's solution). In some embodiments, the pH of the pharmaceutical composition is between about 4.0 and about 8.5 (such as between about 4.0 and about 5.0, between about 4.5 and about 5.5, between about 5.0 and about 6.0, between about 5.5 and about 6.5, between about 6.0 and about 7.0, between about 6.5 and about 7.5, between about 7.0 and about 8.0, or between about 7.5 and about 8.5).

In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable excipient, for example a filler, binder, coating, preservative, lubricant, flavoring agent, sweetening agent, coloring agent, a solvent, a buffering agent, a chelating agent, or stabilizer. Exemplary pharmaceutically acceptable fillers include cellulose, dibasic calcium phosphate, calcium carbonate, microcrystalline cellulose, sucrose, lactose, glucose, mannitol, sorbitol, maltol, pregelatinized starch, corn starch, or potato starch. Exemplary pharmaceutically acceptable binders include polyvinylpyrrolidone, starch, lactose, xylitol, sorbitol, maltitol, gelatin, sucrose, polyethylene glycol, methyl cellulose, or cellulose. Exemplary pharmaceutically acceptable coatings include hydroxypropyl methylcellulose (HPMC), shellac, corn protein zein, or gelatin. Exemplary pharmaceutically acceptable disintegrants include polyvinylpyrrolidone, carboxymethyl cellulose, or sodium starch

glycolate. Exemplary pharmaceutically acceptable lubricants include polyethylene glycol, magnesium stearate, or stearic acid. Exemplary pharmaceutically acceptable preservatives include methyl parabens, ethyl parabens, propyl paraben, benzoic acid, or sorbic acid. Exemplary pharmaceutically acceptable sweetening agents include sucrose, saccharine, aspartame, or sorbitol. Exemplary pharmaceutically acceptable buffering agents include carbonates, citrates, gluconates, acetates, phosphates, or tartrates.

In some embodiments, the pharmaceutical composition further comprises an agent for the controlled or sustained release of the product, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes.

Preferably, the pharmaceutical composition is sterile. Sterilization may be accomplished by filtration through sterile filtration membranes or radiation. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The pharmaceutically acceptable carrier may be a pharmaceutically acceptable material, composition, or vehicle. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

IV. Articles of Manufacture and Kits

Articles of manufacture that comprise the pharmaceutical compositions described herein in suitable packaging. Suitable packaging for compositions (such as ophthalmic compositions) described herein are known in the art, and include, for example, vials (such as sealed vials), vessels, ampules, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. These articles of manufacture may further be sterilized and/or sealed.

Further provided are kits comprising the pharmaceutical compositions (or articles of manufacture) described herein, which may further comprise instruction(s) on methods of

using the composition, such as uses described herein. The kits described herein may also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

5

V. Methods of Treatment and Therapeutic Indications

In another aspect, a method of treating a malady in a patient comprises administering to the patient in need thereof an effective amount of a pharmaceutical composition comprising the TACI-Fc fusion protein of the present disclosure. In certain preferred
10 embodiments, the malady is an autoimmune disease or disorder.

A pharmaceutical composition in accordance with the present disclosure can be used to treat a variety of inflammatory or autoimmune disorders, cancer, organ transplantation, viral infections, and/or bacterial infections in a mammal, particularly where a reduced immune response is desired. Thus, in certain embodiments the pharmaceutical composition
15 may be useful in the treatment of inflammatory or autoimmune disorders, or organ transplantation.

Prophylactic or therapeutic methods may be used for treating the therapeutic indications disclosed herein. In a therapeutic context, the mammalian subject is typically one with a malady, such as an immune system disease or condition, and administration is
20 conducted to prevent further progression of the disease or condition. In addition, suppression of an immune response can be beneficial in prophylactic and/or therapeutic methods for inhibiting rejection of a tissue, cell, or organ transplant in a donor by a recipient.

The TACI-Fc fusion proteins may be used for the treatment of one or more maladies, including autoimmune diseases, B cell cancers, antibody-mediated pathologies (*e.g.*, ITCP, myasthenia gravis and the like), renal diseases, indirect T cell immune response, graft
25 rejection, and graft versus host disease. Administration of the TACI-Fc fusion proteins can specifically regulate B cell responses during the immune response. Additionally, administration of the TACI-Fc fusion proteins can be used to modulate B cell development, development of other cells, antibody production, and cytokine production. Administration or
30 use of provided TACI-Fc fusion proteins can also modulate B cell communication, such as by neutralizing the proliferative effects of BAFF or APRIL.

In certain embodiments, the pharmaceutical composition is used to treat a malady, such as an autoimmune disease, disorder, or condition. In some embodiments, the

administration of the pharmaceutical composition suffering from the malady can result in suppression or inhibition of such immune system attack or biological responses associated therewith. By suppressing this immune system attack on healthy body tissues, the resulting physical symptoms (*e.g.*, pain, joint inflammation, joint swelling, or tenderness) resulting from or associated with such attack on healthy tissues can be decreased or alleviated, and the biological and physical damage resulting from or associated with the immune system attack can be decreased, retarded, or stopped. In a prophylactic context, the subject may be one with, susceptible to, or believed to present a malady (*e.g.*, immune system disease, disorder or condition), and administration is typically conducted to prevent progression of the malady, disease, disorder, or condition; inhibit, or alleviate symptoms, signs, or biological responses associated therewith; prevent bodily damage potentially resulting therefrom; and/or maintain or improve the subject's physical functioning.

In some embodiments, the malady that can be treated by the pharmaceutical composition described herein is any disease mediated by immune complex deposition (*e.g.* lupus nephritis, vasculitis); direct interference with a pathway (*e.g.* catastrophic antiphospholipid antibody syndrome, myasthenia gravis crisis; anti-Jo-1 disease); opsonization or direct damage to cells (*e.g.*, Idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia); antibody-mediated rejection of an allograft (*e.g.*, highly-sensitized renal transplant patients); or anti-drug antibodies to biologic replacement factors, vectors (*e.g.*, anti-Factor 8).

In some embodiments, the malady is Systemic lupus erythematosus (SLE), including flare prevention without glucocorticoids; Sjogren's syndrome; Primary biliary cirrhosis (PBC); Systemic sclerosis; Polymyositis; Diabetes prevention; IgA nephropathy; IgA vasculitis; B cell cancers, for example myeloma; Multiple sclerosis or Optic neuritis.

In some embodiments, the pharmaceutical composition may be used to treat pre-B or B-cell leukemias, such as plasma cell leukemia, chronic or acute lymphocytic leukemia, myelomas such as multiple myeloma, plasma cell myeloma, endothelial myeloma and giant cell myeloma, and lymphomas such as non-Hodgkin's lymphoma. In some of any embodiments, the type of myeloma includes multiple myeloma, plasmacytoma, multiple solitary plasmacytoma, and/or extramedullary myeloma. In some of any embodiments, the type of myeloma includes light chain myeloma, nonsecretory myeloma, and/or IgD or IgE myeloma.

In some embodiments, the provided TACI-Fc fusion proteins can be used as immunosuppressants to selectively block the action of B-lymphocytes for use in treating

disease. For example, certain autoimmune diseases are characterized by production of autoantibodies, which contribute to tissue destruction and exacerbation of disease.

Autoantibodies can also lead to the occurrence of immune complex deposition complications and lead to many symptoms of systemic lupus erythematosus, including kidney failure,

5 neuralgic symptoms, and death. Modulating antibody production independent of cellular response would also be beneficial in many disease states. B cells have also been shown to play a role in the secretion of arthritogenic immunoglobulins in rheumatoid arthritis. Methods and uses of the provided TACI-Fc fusion proteins to inhibit, block or neutralize action of B cells to thereby suppress antibody production would be beneficial in treatment of
10 autoimmune diseases such as myasthenia gravis, rheumatoid arthritis, polyarticular-course juvenile rheumatoid arthritis, and psoriatic arthritis.

 In some embodiments, the TACI-Fc fusion proteins may be used to block or neutralize the actions of B-cells in association with end stage renal diseases, which may or may not be associated with autoimmune diseases. Such methods would also be useful for
15 treating immunologic renal diseases. Additionally, such methods would be useful for treating glomerulonephritis associated with diseases such as membranous nephropathy, IgA nephropathy or Berger's Disease, IgM nephropathy, IgA Vasculitis, Goodpasture's Disease, post-infectious glomerulonephritis, mesangioproliferative disease, chronic lymphoid leukemia, minimal-change nephrotic syndrome. Such methods would also serve as
20 therapeutic applications for treating secondary glomerulonephritis or vasculitis associated with such diseases as lupus, polyarteritis, Henoch-Schonlein, Scleroderma, HTV-related diseases, amyloidosis, or hemolytic uremic syndrome. The provided methods would also be useful as part of a therapeutic application for treating interstitial nephritis or pyelonephritis associated with chronic pyelonephritis, analgesic abuse, nephrocalcinosis, nephropathy
25 caused by other agents, nephrolithiasis, or chronic or acute interstitial nephritis. The methods provided herein also include use of the provided TACI-Fc fusion proteins in the treatment of hypertensive or large vessel diseases, including renal artery stenosis or occlusion and cholesterol emboli or renal emboli. The provided methods and uses also can be used for treatment of renal or urological neoplasms, multiple myelomas, lymphomas, light chain
30 neuropathy or amyloidosis.

 In some embodiments, the provided TACI-Fc fusion proteins may be used for the treatment of asthma and other chronic airway diseases such as bronchitis and emphysema. In some embodiments, the TACI-Fc fusion proteins may be used for immunosuppression in graft-versus-host disease and graft rejection. In some embodiments, methods and uses of the

TACI-Fc fusion proteins include treatment of autoimmune diseases, such as insulin dependent diabetes mellitus (IDDM) and Crohn's Disease. Additional applications include treatment of chronic inflammatory diseases to *e.g.* lessen joint pain, swelling, anemia and other associated symptoms as well as treating septic shock.

- 5 In some embodiments, the inflammatory and autoimmune disorders include Achalasia; Addison's disease; Adult Still's disease; Agammaglobulinemia; Alopecia areata; Amyloidosis; Ankylosing spondylitis; Anti-GBM/Anti-TBM nephritis; Antiphospholipid syndrome; Autoimmune adrenalitis (Addison's disease); Autoimmune angioedema; Autoimmune dysautonomia; Autoimmune encephalomyelitis; Autoimmune hepatitis;
- 10 Autoimmune inner ear disease (AIED); Autoimmune myocarditis; Autoimmune oophoritis; Autoimmune orchitis; Autoimmune pancreatitis; Autoimmune polyglandular syndrome type II (APS II); Autoimmune retinopathy; Autoimmune thyroid disease (AITD), i.e. Hashimoto's disease; Autoimmune urticarial; Axonal & neuronal neuropathy (AMAN); Balo disease; Behcet's disease; Benign mucosal pemphigoid; Bullous pemphigoid; Castleman disease
- 15 (CD); Celiac disease; Chagas disease; Chronic inflammatory demyelinating polyneuropathy (CIDP); Chronic recurrent multifocal osteomyelitis (CRMO); Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA); Cicatricial pemphigoid; Cogan's syndrome; Cold agglutinin disease; Congenital heart block; Coxsackie myocarditis; CREST syndrome; Crohn's disease; Dermatitis herpetiformis; Dermatomyositis; Devic's disease (neuromyelitis
- 20 optica); Discoid lupus; Dressler's syndrome; Endometriosis; Eosinophilic esophagitis (EoE); Eosinophilic fasciitis; Erythema nodosum; Essential mixed cryoglobulinemia; Evans syndrome; Fibromyalgia; Fibrosing alveolitis; Giant cell arteritis (temporal arteritis); Giant cell myocarditis; Glomerulonephritis; Goodpasture's syndrome; Granulomatosis with Polyangiitis; Graves' disease; Guillain-Barre syndrome; Hashimoto's thyroiditis; Hemolytic
- 25 anemia; Henoch-Schonlein purpura (HSP); Herpes gestationis or pemphigoid gestationis (PG); Hidradenitis Suppurativa (HS) (Acne Inversa); Hypogammaglobulinemia; IgA Nephropathy; IgA Vasculitis; IgG4-related sclerosing disease; Immune thrombocytopenic purpura (ITP); Inclusion body myositis (IBM); Interstitial cystitis (IC); Juvenile arthritis; Juvenile diabetes (Type 1 diabetes); Juvenile myositis (JM); Kawasaki disease; Lambert-
- 30 Eaton syndrome; Leukocytoclastic vasculitis; Lichen planus; Lichen sclerosus; Ligneous conjunctivitis; Linear IgA disease (LAD); Lupus; Lyme disease chronic; Meniere's disease; Microscopic polyangiitis (MPA); Mixed connective tissue disease (MCTD); Mooren's ulcer; Mucha-Habermann disease; Multifocal Motor Neuropathy (MMN) or MMNCB; Multiple sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neonatal Lupus; Neuromyelitis optica;

Neutropenia; Ocular cicatricial pemphigoid; Optic neuritis; Palindromic rheumatism (PR);
 PANDAS; Paraneoplastic cerebellar degeneration (PCD); Paroxysmal nocturnal
 hemoglobinuria (PNH); Parry Romberg syndrome; Pars planitis (peripheral uveitis);
 Parsonage-Turner syndrome; Pemphigus, Pemphigus vulgaris; Peripheral neuropathy;
 5 Perivenous encephalomyelitis; Pernicious anemia (PA); POEMS syndrome; Polyarteritis
 nodosa; Polyglandular syndromes type I, II, III; Polymyalgia rheumatic; Polymyositis;
 Postmyocardial infarction syndrome; Postpericardiotomy syndrome; Primary biliary
 cirrhosis; Primary sclerosing cholangitis; Progesterone dermatitis; Psoriasis; Psoriatic
 arthritis; Pure red cell aplasia (PRCA); Pyoderma gangrenosum; Raynaud's phenomenon;
 10 Reactive Arthritis; Reflex sympathetic dystrophy; Relapsing polychondritis; Restless legs
 syndrome (RLS); Retroperitoneal fibrosis; Rheumatic fever; Rheumatoid arthritis;
 Sarcoidosis; Schmidt syndrome; Scleritis; Scleroderma; Sjogren's syndrome; Sperm &
 testicular autoimmunity; Stiff person syndrome (SPS); Subacute bacterial endocarditis (SBE);
 Susac's syndrome; Sympathetic ophthalmia (SO); Takayasu's arteritis; Temporal
 15 arteritis/Giant cell arteritis; Thrombocytopenic purpura (TTP); Tolosa-Hunt syndrome (THS);
 Transverse myelitis; Type 1 diabetes; Ulcerative colitis (UC); Undifferentiated connective
 tissue disease (UCTD); Uveitis; Vasculitis; Vitiligo or Vogt-Koyanagi-Harada Disease,
 Antiphospholipid Ab syndrome; Multiple sclerosis; IgG4-related disease; Type I diabetes;
 Rheumatoid arthritis, including glucocorticoid therapy (GC) RA or Acute lupus nephritis,
 20 Amyotrophic lateral sclerosis, Neuromyelitis optica, Transverse myelitis, CNS autoimmunity,
 Guillain-Barre syndrome, Neurocystercosis, Sarcoidosis (T/seroneg), Churg-Strauss
 Syndrome, Hashimoto's thyroiditis, Grave's disease, immune thrombocytopenia (ITP),
 Addison's Disease, Polymyositis, or Dermatomyositis; IgA nephropathy; chronic
 inflammatory demyelinating polyneuropathy (CIDP); antisyntetase disease, such as Jo-1
 25 syndrome and ANCA vasculitis.

In some embodiments, the provided TACI-Fc fusion proteins can be used to treat a B
 cell cancer. In some embodiments, the B cell cancer is a cancer in which BAFF and APRIL
 are involved or implicated in providing an autocrine survival loop to the B cells. In some
 embodiments, the cancer is B cell chronic lymphocytic leukemia, non-Hodgkin's lymphoma,
 30 or myeloma. In some embodiments, the cancer is myeloma.

In preferred embodiments, the autoimmune disorder is selected from the group
 consisting of acute antibody mediated rejection, primary Sjogren's syndrome, autoimmune
 hemolytic anemia, antiphospholipid syndrome (APS), catastrophic APS, refractory
 rheumatoid arthritis, autoimmune vasculitis, cryoglobulinemia, antineutrophil cytoplasmic

autoantibodies (ANCA), IgA vasculitis, rheumatoid vasculitis, anti-synthetase syndrome, primary membranous nephropathy, juvenile idiopathic arthritis, systemic sclerosis, Guillain-Barre syndrome, autoimmune pulmonary alveolar proteinosis, autoimmune hemolytic anemia, hemolytic disease of the fetus and newborn, antibody mediated rejection, and acute
5 hemolytic transfusion reaction.

In other aspects, a method of enhancing clearance of IgG immunoglobulins in a patient in need thereof comprises administering to the patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein of the present disclosure.

In another aspect, a method of reducing levels of mature B-cells, IgG, IgM, IgA (or a
10 combination thereof) in a patient in need thereof comprises administering to the patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein of the present disclosure. Further, in some embodiments, administration of the pharmaceutical composition does not significantly lower levels of IgD or IgE.

In another aspect, a method of inhibiting the activity of one or more pro-inflammatory
15 cytokines in a patient in need thereof comprises administering to the patient an effective amount of a pharmaceutical composition comprising a TACI-Fc fusion protein of the present disclosure. In one embodiment, the pro-inflammatory cytokines include BAFF and/or APRIL.

Generally, dosages and routes of administration of the pharmaceutical composition
20 are determined according to the size and condition of the subject, according to standard pharmaceutical practice. For example, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, pigs, or monkeys. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to
25 determine useful doses and routes for administration in humans. The exact dosage can be determined in view of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors to consider for treatment include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and
30 frequency of administration, drug combination(s), reaction sensitivities, and response to therapy.

Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the formulation. The frequency of dosing will depend upon the pharmacokinetic parameters of the molecule in the

formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made.

5 Appropriate dosages may be ascertained through use of appropriate dose-response data.

In some embodiments, the pharmaceutical composition is administered to a subject through any route, including orally, transdermally, by inhalation, intravenously, intra-arterially, intramuscularly, direct application to a wound site, application to a surgical site, intraperitoneally, by suppository, subcutaneously, intradermally, transcutaneously, by
10 nebulization, intrapleurally, intraventricularly, intraarticularly, intraocularly, or intraspinally. In some embodiments, the pharmaceutical composition is administered in a form suitable for intravenous infusion.

In some embodiments, an administered dose of the pharmaceutical composition is about 1 mg of protein per kg subject body mass or more (such as about 2 mg of protein per kg
15 subject body mass or more, about 5 mg of protein per kg subject body mass or more, about 10 mg of protein per kg subject body mass or more, about 25 mg of protein per kg subject body mass or more, about 50 mg of protein per kg subject body mass or more, about 100 mg of protein per kg subject body mass or more, about 250 mg of protein per kg subject body mass or more, about 500 mg of protein per kg subject body mass or more, about 1000 mg of
20 protein per kg subject body mass or more, about 2000 mg of protein per kg subject body mass or more, or about 5000 mg of protein per kg subject body mass or more).

A dosage of the pharmaceutical composition may be administered as a single dose or a repeated dose. In some embodiments, the doses are given to a subject once per day, twice per day, three times per day, or four or more times per day. In some embodiments, about 1 or
25 more (such as about 2 or more, about 3 or more, about 4 or more, about 5 or more, about 6 or more, or about 7 or more) doses are given in a week. In some embodiments, multiple doses are given over the course of days, weeks, months, or years. In some embodiments, a course of treatment is about 1 or more doses (such as about 2 or more does, about 3 or more doses, about 4 or more doses, about 5 or more doses, about 7 or more doses, about 10 or more doses,
30 about 15 or more doses, about 25 or more doses, about 40 or more doses, about 50 or more doses, or about 100 or more doses).

VI. Evaluating Immune Responses to TACI-Fc Fusion Protein Compositions

The TACI-Fc fusion proteins modulate B cell activity, such B cell proliferation, differentiation, or survival. Evaluation of these activities can be carried out in vitro or in vivo to monitor responses to TACI-Fc therapy.

5 The functional activity of the TACI-Fc fusion proteins can be examined using a variety of approaches to assess the ability of the proteins to bind to cognate binding partners. For example, TACI-Fc fusion proteins may be assessed for binding to APRIL or BAFF. A variety of assays are known for assessing binding affinity and/or determining whether the TACI-Fc fusion protein specifically binds to APRIL, BAFF or FcRn. A number of binding assays well
10 known in the art may be utilized, including, but not limited to methods involving BIAcore®, OCTET®, and other surface plasmon resonance (SPR) devices and methodologies; flow cytometry, kinetic exclusion assay (KinExA), and enzyme linked immunosorbent assays (ELISA). For example, in some embodiments, a BIAcore® instrument can be used to determine the binding kinetics and constants of a complex between the TACI-Fc fusion
15 protein and APRIL, BAFF or FcRn using surface plasmon resonance analysis. SPR measures changes in the concentration of molecules at a sensor surface as molecules bind to or dissociate from the surface. The change in the SPR signal is directly proportional to the change in mass concentration close to the surface, thereby allowing measurement of binding kinetics between two molecules. The dissociation constant for the complex can be determined
20 by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays, such as ELISA assays, radioimmunoassays (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance
25 (NMR).

 The TACI-Fc fusion proteins also can be evaluated in any of a variety of assays well known in the art to assess modulation of B cell activity. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound (*e.g.*, TACI-Fc fusion protein), and cell proliferation is detected by, for example, measuring incorporation of
30 tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. Numerous reporter genes that are easily

assayed for in cell extracts are known in the art, for example, the *E. coli* lacZ, chloroamphenicol acetyl transferase (CAT) and serum response element (SRE). An exemplary reporter gene is a luciferase gene. Expression of the luciferase gene is detected by luminescence using methods and kits well known in the art

5 The TACI-Fc fusion proteins can be characterized by the ability to inhibit the stimulation of human B cells by soluble APRIL or BAFF, as described by Gross et al, international publication No. WO00/40716. Briefly, human B cells are isolated from peripheral blood mononuclear cells, such as using CD19 magnetic beads separation (*e.g.*, Miltenyi Biotec Auburn, Calif.). The purified B cells can be incubated under conditions of
10 stimulation, *e.g.*, in the presence of soluble APRIL, and further in the presence of titrated concentration of TACI-Fc fusion protein. The B cells can be labeled with a proliferation dye or can be labeled with 1 μ Ci ^{3} H-thymidine to measure proliferation. The number of B cells can be determined over time.

Reporter cell lines that express a reporter gene under the operable control of a
15 transcription factor, such as NF- κ B, NFAT-1 and AP-1, can be made that express TACI or BCMA. For example, Jurkat and other B Lymphoma cell lines may be used as suitable reporter cells. Incubation of these cells with soluble BAFF or APRIL ligands signal through the reporter genes in these constructs. The effect of provided the TACI-Fc fusion proteins to modulate this signaling can be assessed.

20 For example, in some embodiments, mouse models for Sjogren's syndrome can be used. The Sjogren's syndrome disease, as well as an accelerated onset of diabetes, can be induced in female diabetes-prone non-obese diabetic (NOD) mice using repeat dosing with anti-mouse (m) PD-L1 antibody, based on a modified version of a protocol published by Zhou et al., 2016; Sci. Rep. 6, 39105. Starting at 6 weeks of age, mice are injected
25 intraperitoneally (IP) on Study Days 0, 2, 4, and 6 with 100 μ g of anti-PD-L1 antibody and are treated on various days with provided TACI-Fc fusion proteins. Naive mice are included as controls for the endpoint analyses. All mice are typically terminated on Study Day 10 and submandibular glands (SMG) and the pancreas from each mouse are collected for histopathology evaluation to assess for signs and severity of sialadenitis and insulinitis. Blood
30 glucose levels can be measured on various days.

In some embodiments, a collagen-induced arthritis (CIA) model can be used in which mice develop chronic inflammatory arthritis which closely resembles human rheumatoid arthritis (RA). Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compositions.

Another advantage in using the CIA model is that the mechanisms of pathogenesis are known. The T and B cell epitopes on type II collagen have been identified, and various immunological (delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-
5 mediating arthritis have been determined and can be used to assess test compound efficacy in the models (Wooley, *Curr. Opin. Rheum.* 3:407-20, 1999; Williams et al., *Immunol.* 89:9784-788, 1992; Myers et al., *Life Sci.* 61:1861-78, 1997; and Wang et al., *Immunol.* 92:8955-959, 1995). Administration of TACI-Fc fusion proteins to ameliorate symptoms and alterations to the course of a malady or disease can be assessed.

10 Pharmacokinetic studies can be used in association with radiolabeled TACI-Fc fusion proteins to determine the distribution and half-life of such polypeptides in vivo.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The list of sequences, figures and all references, patents, and published patent applications cited throughout this application are expressly incorporated
15 herein by reference. Lists of the sequences referenced throughout the application are described following the Examples and in Figures 20A-20K.

EXAMPLES

20 **EXAMPLE 1: ENGINEERING OF THE TACI EXTRACELLULAR DOMAIN TO ENHANCE THE pH DEPENDENT BINDING OF BAFF AND APRIL CYTOKINES**

The transmembrane activator or Calcium-Modulating Cyclophilin Ligand-interactor (TACI) receptor has a high affinity to BAFF and APRIL cytokines. The extracellular domain
25 of TACI has been demonstrated to block BAFF and ARPIL as an Fc fusion, the blockade of BAFF and APRIL prevents activation of the receptors in this family (Merrill et al. (2018) *Arthritis & Rheumatology* Vol. 70, No. 2, pp 266–276).

Guided by the published crystal structure of the TACI cysteine-rich CRD2 domain in complex with mouse APRIL (PDB: 1XU1), single-added histidine mutants (fused to an Fc
30 domain with C-terminal Flag tag) were generated to sequentially replace all non-cysteine residues at or near the binding interface formed with APRIL (Figure 1). These histidine mutants were expressed from pCDNA3.1 (containing a signal sequence) in Expi293 cells and

purified from cell supernatants using HiTrap MabSelect SuRe 1 mL affinity columns on an AKTApure 25 M2 purification system with protein elution at 100 mM citrate pH 3.2.

The pH dependence on binding of the TACI histidine mutants to BAFF and APRIL was determined using Surface Plasmon Resonance (SPR) (Biacore™ T200 instrument) as shown in Figures 2A-2B. After immobilizing biotinylated BAFF (Figure 2A) or biotinylated APRIL (Figure 2B) to a Streptavidin sensor, BAFF-Biotin and APRIL-Biotin were flown over at 100nM in PBS + 0.05% Tween® at pH 7.4. Dissociation rates were then measured at pH 7.4, PBS + 0.05% Tween®20 pH 6.0 was then injected, and the new dissociation rates were measured as shown in Figure 2. By comparing the relative rates of dissociation at pH 6.0 to pH 7.4 (Figure 2), the most promising single-added histidine TACI-DR1/DR2 Fc variants (with relatively faster dissociation at pH 6.0) were identified as summarized in Table 1. TACI variants are named based on the amino acid position in the native TACI amino acid sequence (SEQ ID NO: 39; NCBI Reference Sequence NP_036584).

Table 1. SPR binding results from single histidine substituted TACI mutants at pH 6.0 and 7.4.

Sample ID	His Mutation	BAFF Off rate pH 7.4	BAFF Off rate pH 6.0	Fold change	APRIL Off rate pH 7.4	APRIL Off rate pH 6.0	Fold change
TPP-	Parent	-0.0183	-0.0269	1.47	-0.014	-0.06151	4.39
TPP-4287	K77H	-0.0118	-0.0214	1.81	-0.009602	-0.03442	3.58
TPP-4288	F78H	-0.0219	-0.0544	2.49	-0.01385	-0.05775	4.17
TPP-4289	Y89H	-0.0132	-0.0391	2.97	-0.01037	-0.0308	2.97
TPP-4290	D80H	-0.027	-0.07	2.60	-0.02558	-0.0559	2.18
TPP-4291	L82H	-0.006	-0.1106	18.32	-0.007817	-0.06348	8.12
TPP-4292	L83H	-0.0138	-0.0586	4.25	-0.01175	-0.03185	2.71
TPP-4293	R84H	-0.0197	-0.0275	1.40	-0.01094	-0.0364	3.33
TPP-4294	D85H	-0.0313	-0.0575	1.84	-0.03452	-0.05319	1.54
TPP-4295	I87H	-0.0202	-0.0515	2.54	-0.01377	-0.02647	1.92
TPP-4296	S88H	-0.0332	-0.0739	2.22	-0.02895	-0.04626	1.60
TPP-4297	S91H	-0.0213	-0.0505	2.37	-0.01564	-0.03406	2.18
TPP-4298	I92H	-0.0155	-0.0659	4.25	-0.01403	-0.04342	3.09
TPP-4299	Q95H	-0.0247	-0.0798	3.23	-0.02726	-0.06321	2.32
TPP-4300	P97H	-0.0224	-0.0498	2.22	-0.0168	-0.03858	2.30
TPP-4301	K98H	-0.0149	-0.0201	1.34	-0.008894	-0.01039	1.17
TPP-4302	Q99H	-0.0272	-0.0454	1.67	-0.02152	-0.04155	1.93
TPP-4303	A101H	-0.0194	-0.0467	2.41	-0.01843	-0.03889	2.11
TPP-4313	G94H	-0.0201	-0.0451	2.24	-0.01471	-0.03796	2.58

Next, a second-round panel of double- and triple-added-histidine TACI-Fc variants were designed based on combining the most effective mutations identified in the first round.

These new variants were analogously tested for BAFF binding by the same Biacore method as described above to determine relative off-rate at pH 6.0 and pH 7.4, as well as overall binding stability (Figure 3).

Table 2. SPR binding results from multiple histidine substituted TACI mutants at pH 6.0 and 7.4.

Sample ID	His Mutations	Off rate pH 7.4	Off rate pH 6.0	Fold Change	Binding Stability
TPP-4543	D80H, L82H	-0.009351	-0.03705	3.96	24.87
TPP-4549	F78H, D80H	-0.002507	-0.03875	15.46	27.72
TPP-4554	D80H, L83H	-0.006068	-0.0262	4.32	20.27
TPP-4555	D80H, I87H	-0.009252	-0.03223	3.48	21.10
TPP-4563	F78H, D80H, L82H	0.002041	-0.03224	15.80	27.44
TPP-4566	Y79H, D80H, L82H	-8.80E-06	-0.04192	4764.18	27.98
TPP-4568	D80H, L82H, L83H	-0.003081	-0.03421	11.10	25.27
TPP-4577	Y79H, D80H, L83H	0.0009488	-0.03859	40.67	26.49
TPP-4578	D80H, L83H, I87H	0.0009337	-0.0414	44.34	23.53

The entire panel of single-, double-, and triple-added histidine TACI-Fc variants was also tested in an ELISA assay to determine pH dependence of BAFF binding. Biotinylated BAFF (1 µg/mL in 1X PBST) was immobilized to Streptavidin-coated pre-blocked 96-well plates. Wells were washed 3 times, and then serial dilutions of the TACI-Fc panel (0.003 to 10 µg/mL in 1X PBST at either pH 5.5 or 7.4) were applied for 45 min. Wells were washed 3 times, an HRP-conjugated anti-FLAG M2 mouse antibody (diluted 1:20,000 in 1X PBST) was applied, the wells were washed 3 times, and the TMB substrate solution was added and then quenched with 2N sulfuric acid after 15 minutes before absorbance at 450 nm was read on a Spectramax i3X plate reader.

Table 3 summarizes the relative shifts in the observed BAFF binding affinity compared to the WT at pH 5.5 and 7.4 for the His mutants as determined by the ELISA assay. A higher net pH dependence shift indicates a larger difference in affinity from pH 7.4 to pH 5.5 (weaker affinity at pH 5.5). These results demonstrate reduced affinity of the TACI-Ig Fc fusion proteins to BAFF under the low pH conditions (*e.g.*, pH 5.5) with a higher affinity under neutral pH conditions (*e.g.*, pH 7.4). The results of this analysis allowed for the identification of several promising double- and triple-added histidine TACI-Fc variants for further characterization.

Table 3: ELISA binding assay of TACI histidine substitution mutants at pH 5.5 and 7.4.

ID#	TACI_D1/D2 variant	Fold decrease in affinity at pH 7.4 (orders of magnitude)	Fold decrease in affinity at pH 5.5 (orders of magnitude)	Net pH dependence shift
TPP-4286	WT TACI-D2	--	--	1
TPP-4287	K77H	1	2.2	2.2
TPP-4288	F78H	2.2	5	2.2
TPP-4289	Y79H	2.2	5	2.2
TPP-4290	D80H	5	25	5
TPP-4291	L82H	5	11	2.2
TPP-4292	L83H	1	5	5
TPP-4293	R84H	1	2.2	2.2
TPP-4294	D85H	1	1	1
TPP-4295	I87H	1	1	1
TPP-4296	S88H	1	1	1
TPP-4297	S91H	1	1	1
TPP-4298	I92H	1	1	1
TPP-4299	Q95H	1	1	1
TPP-4300	P97H	2.2	2.2	1
TPP-4301	K98H	1	1	1
TPP-4302	Q99H	1	1	1
TPP-4303	A101H	1	1	1
TPP-4313	G94H	2.2	5	2.2
TPP-4541	F78H, L82H	25	280	11
TPP-4542	Y79H, L82H	25	280	11
TPP-4543	D80H, L82H	5	25	5
TPP-4544	L82H, L83H	25	280	11
TPP-4545	L82H, I87H	11	125	11
TPP-4546	L82H, I92H	11	280	25
TPP-4547	L82H, Q95H	11	280	25
TPP-4548	F78H, Y79H	11	25	2.2
TPP-4549	F78H, D80H	5	25	5
TPP-4550	F78H, L83H	11	56	5
TPP-4551	F78H, I87H	5	25	5
TPP-4552	Y79H, D80H	2.2	5	2.2
TPP-4553	Y79H, L83H	5	25	5
TPP-4554	D80H, L83H	5	25	5
TPP-4555	D80H, I87H	56	280	5
TPP-4556	L83H, I87H	5	56	11
TPP-4557	I87H, I92H	2.2	11	5
TPP-4558	S88H, S91H	1	2.2	2.2
TPP-4559	S91H, I92H	1	2.2	2.2
TPP-4560	I92H, Q95H	1	2.2	2.2
TPP-4561	G94H, Q95H	2.2	5	2.2

TPP-4562	F78H, Y79H, L82H	280	625	2.2
TPP-4563	F78H, D80H, L82H	5	25	5
TPP-4564	F78H, L82H, L83H	280	280	1
TPP-4565	F78H, L82H, I87H	280	280	1
TPP-4566	Y79H, D80H, L82H	5	25	5
TPP-4567	Y79H, L82H, L83H	280	280	1
TPP-4568	D80H, L82H, L83H	5	25	5
TPP-4569	D80H, L82H, I87H	5	25	5
TPP-4570	L82H, L83H, I87H	125	625	5
TPP-4571	L82H, I87H, I92H	125	625	5
TPP-4572	L82H, S88H, S91H	25	280	11
TPP-4573	L82H, S91H, I92H	125	625	5
TPP-4574	L82H, I92H, Q95H	25	125	5
TPP-4575	L82H, G94H, Q95H	25	125	5
TPP-4576	F78H, Y79H, L83H	125	625	5
TPP-4577	Y79H, D80H, L83H	5	25	5
TPP-4578	D80H, L83H, I87H	5	11	2.2
TPP-4579	L83H, I87H, I92H	56	625	11
TPP-4580	Y79H, L82H, Q95H	56	625	11
TPP-4581	Y79H, L82H, I92H	125	625	5

EXAMPLE 2: TACI-Fc FUSION PROTEINS BIND TO BAFF AND APRIL IN A pH-DEPENDENT MANNER

Fusion proteins comprised of TACI and Ig Fc regions were designed using Geneious software (Biomatters LLC), submitted to GeneArt for codon optimization, cloned into pCDNA3.1 (with a signal sequence included) using HindIII and ApaI restriction enzymes, expressed using ExpiCHO-S or Expi293F (Thermo Fisher Scientific) cell expression systems, and purified on an AKTApure 25 M2 purification system (Cytiva) by MabSelect SuRe Protein A affinity chromatography (Cytiva) with a citrate pH 3.8 elution, followed by size-exclusion chromatography in PBS on a column packed with Superdex 200 pg resin (Cytiva).

Octet binding analysis

Bio-layer interferometry was performed using an Octet RED96e system (ForteBio) to examine the kinetics of TACI-Fc fusion protein binding to the TACI ligands BAFF and APRIL, as well as binding to the neonatal Fc receptor (FcRn) as described in Example 3 below. In this assay, the addition of protein mass to a biosensor is detected by biolayer interferometry (BLI) as a deflection or increase in detectable signal observed in a sensorgram. Where there is no mass change, a flat line or baseline is observed, as shown in the buffer only steps. Further, when proteins dissociate from the biosensor, a negative slope is observed in

the sensorgram. A sensorgram may depict the following temporal phases in an exemplary assay shown in Figure 4: (1) baseline (buffer only); (2) loading biotinylated analyte; (3) baseline (buffer only); (4) TACI-Fc fusion protein association; and (5) TACI-Fc fusion protein dissociation.

5 To demonstrate binding of an exemplary TACI-Fc fusion protein (i.e., TPP-6269, SEQ ID NO: 22) to BAFF, TPP-6269 (20 µg/mL) was diluted in phosphate buffered saline (PBS) pH 6.0 or 7.4 and analyzed for binding to human biotinylated BAFF (Biotechne Catalog # 7537-BF-025/CF) loaded on Streptavidin biosensors (Sartorius, Catalog # 18-5019) using an Octet RED96e system (ForteBio). Negative control assays were carried out at pH
10 6.0 and pH 7.4 using PBS buffer only. The results of this binding experiment are shown in the sensorgram depicted in Figure 5.

The sensorgram in Figure 5 depicts the latter three phases shown in Figure 4, including the buffer only baseline, the TACI-Fc / BAFF association phase, and the TACI-Fc / BAFF dissociation phase. From these traces the on-rate and off-rate binding profiles of the
15 variant TACI-Fc fusion proteins to BAFF were determined.

BAFF inhibition by TPP-6269 was further tested in a functional assay carried out in a BCMA/Nuclear Factor Kappa B (NF-κB)-luciferase reporter HEK293 cell line. This cell line includes a stably integrated full-length human BCMA cDNA (Genbank #NM_001192) under control of a CMV promoter (Amsbio Llc) and reporter in which expression of a firefly
20 luciferase gene (in relative light units (RLU)) is under the control of four copies of an NF-κB response element upstream of a minimal promoter. In this assay, binding of BAFF ligand to BCMA activates the NF-κB signaling pathway, leading to expression of the NF-κB-controlled luciferase reporter. Inhibition of BAFF binding by the TACI-Fc fusion protein inhibits NF-κB signaling, which is reflected in a reduced luciferase signal (i.e., RLUs).

25 TPP-6269 (SEQ ID NO: 25) contains a TACI extracellular domain portion consisting of a DR1-DR2 region lacking an N-terminal portion of DR1 and a C-terminal portion of DR2. For comparison, TPP-6269 was tested against a TACI-Fc fusion protein (TPP-4217) containing a full-length TACI extracellular domain. The results from this assay are shown in Figure 6. In this assay, TPP-6269 was found to completely inhibit luciferase expression at a
30 concentration of 5.3 nM and at a greater level of inhibition compared to TPP-4217. As expected, a control Fc protein without a TACI domain showed no inhibition.

Additional TACI-Fc variants were similarly found to exhibit potent inhibition of BAFF binding to BCMA-HEK293 cells (Figure 7). Further, in an analogous functional assay using the using BCMA/NF-κB-luciferase reporter HEK293 cell line with APRIL (Biotechne,

Catalog # 5860-AP/CF) as the BCMA ligand, TACI variants TPP-4286 (SEQ ID NO: 57) and TPP-4291 (SEQ ID NO: 58) were found to exhibit potent inhibition of APRIL-mediated BCMA activation, as shown in Figure 8.

5 **EXAMPLE 3: HUMAN IgG1, IgG2, IgG3, AND IgG4 FRAGMENT
CRYSTALLIZABLE (Fc) SEQUENCE VARIANTS ENHANCE BINDING TO THE
NEONATAL Fc RECEPTOR**

To investigate whether enhanced binding profiles and pH dependent properties could be engineered, a series of TACI-Fc fusion proteins comprising chimeric Fc regions
10 assembled from different IgG1/IgG2/IgG4 subclass portions were designed and purified, essentially as described in Example 2. The resulting TACI-Fc chimeric IgG subclass fusion proteins comprising the amino acid sequences set forth in SEQ ID NOS: 20 and 39-45 were diluted in phosphate buffered saline (PBS) pH 6.0 or 7.4 and analyzed for binding to human biotinylated FcRn (Biotechne, Catalog # 8639-FC) loaded on Streptavidin biosensors
15 (Sartorius, Catalog # 18-5019) essentially as described in Example 2. Negative control assays were carried out at pH 6.0 and pH 7.4 using PBS buffer only.

These variant chimeric Fc fusion proteins were similarly evaluated for binding and pH dependence to neonatal FcRn by bio-layer interferometry using the Octet Red96 system (ForteBio) as described in Example 2. The results of these assays are shown in Figures 9A-
20 9H. Additional assays employed surface plasmon resonance (SPR/Biacore). Like the assays in Figure 5, the sensorgrams in Figures 9A-9G depict the buffer only baseline phase, the Fc variant association phase, and the Fc dissociation phase. From this data, the on-rate and off-rate binding profiles of the variant TACI-Fc fusion proteins to the FcRn can be determined.

As shown in Figures 9A-9H, the TACI-Fc fusion variants demonstrated various levels
25 of binding to FcRn with observable differences in the on-rate and off-rate profiles at pH 6.0 and pH 7.4. For example, fusion of different IgG1/IgG2/IgG4 subclass regions with M252Y, S254T, and T256E mutations exhibited enhanced binding as illustrated by TPP-5260 (SEQ ID NO: 50) compared to TPP-5272 (SEQ ID NO: 51) as shown in Figures 9A and 9D, respectively. An example demonstrating this observation for an IgG1/IgG4 Fc subclass
30 variant is illustrated by TPP-5262 (SEQ ID NO: 49), which was found to exhibit an increased on-rate and amplitude at pH 7.4 compared to TPP-5274 (SEQ ID NO: 45) (see Figures 9B and 9F, respectively).

The TACI-Fc variants in Figures 9A-9H illustrate slower off-rates to human FcRn when the M252Y, S254T, and T256E mutations are present in the different hybrid subclass

fusion proteins. TPP-6269 (SEQ ID NO: 25), which contains a hybrid IgG1/IgG4 Fc domain in combination with L234F, L235E, M252Y, S254T, T256E, and N434H mutations exhibited exceptional pH dependence with an observed decreased in on-rate and total amplitude of binding and a faster off-rate at pH 7.4 compared to pH 6.0 (Figure 9H) as confirmed by
5 Surface Plasmon Resonance (SPR/ Biacore) in Figure 13 of Example 4.

EXAMPLE 4: RATIONALLY DESIGNED MUTAGENESIS OF HUMAN IgG1/IgG4 Fc TO ENHANCE pH-DEPENDENT BINDING TO FcRn

Guided by the published crystal structure of FcRn.Fc complex (PDB: 4N0U; Figure
10 10), a series of single- and double-substituted histidine IgG1/4 Fc mutants (as TACI-D1/D2 Fc fusions with C-terminal His-tags) were designed for targeting the interface with FcRn, especially the acidic residues E115, E116, D130, and E133 in the processed human FcRn (E138, E139, D253, E256 in UniProtKB/Swiss-Prot: P55899.1). The selected mutations were introduced into constructs containing a TACI D1/D2 domain region, a chimeric IgG1/IgG4
15 Fc region, and a 6x His tag. These proteins were expressed from pCDNA3.1 in Expi293 cells and purified from cell supernatants using HisTrap HP 1 mL nickel affinity columns on an AKTApure 25 M2 purification system using an imidazole gradient to separate monomeric proteins from aggregates.

The relative effect of the mutations on FcRn binding at pH 7.4 and 6.0 was assessed
20 using the Octet system. Streptavidin tips were soaked in PBS for 15 minutes, a 60 second baseline was recorded, biotinylated FcRn (10 µg/mL in PBS) was loaded for 200 seconds, then after a 30 second dip into PBS the Fc variants (400 nM in PBS) were associated for 120 seconds followed by a 120 second dissociation, with all steps done at either pH 6.0 or 7.4.

Figures 11A-11D show Octet traces depicting binding kinetics of single- and double-
25 added histidine TACI-Fc variants compared to TPP-5274 “WT” (SEQ ID NO: 45), which contains a parental IgG1/IgG4 hybrid Fc without the added mutations in the variants. The Octet traces in Figures 11A-11B were carried out at pH 7.4; the Octet traces in Figures 11C-11D were carried out pH 6.0. The results from this study identified a novel N434H variant, TPP-5721 (SEQ ID NO: 60) as improving FcRn binding affinity in a pH-dependent manner
30 (see Figures 11A, 11C).

To further investigate the improved pH dependent binding properties conferred by the N434H mutation, further binding studies were carried out with the Octet RED96e system (ForteBio) using TPP-5957 (SEQ ID NO: 30) and TPP-5958 (SEQ ID NO: 31) at pH 6.0 (Figure 12A) and pH 7.4 (Figure 12B). Both TPP-5957 and TPP-5958 contain the amino acid

substitutions, L234F, L235E, M252Y, S254T, and T256E. However, TPP-5958 further includes the N434H mutation. The results of this comparison are consistent with the N434H mutation conferring improved pH-dependent binding properties.

Binding properties of TACI-Fc variants were further evaluated by SPR/ Biacore analysis. In these experiments, TACI-Fc variants were directly immobilized on the surface of a CM5 chip exposed to injections of FcRn (Biotechne, Catalog # 8639-FC) at various concentrations at pH 7.4 and pH 6.0 using a Biacore T200 system. Surfaces were regenerated with 50 mM NaOH and binding kinetics parameters were determined. All Biacore analyses was done following WPD-PC-050-02D, "Kinetic analysis of protein-protein interactions with Direct Ligand Immobilization using Surface Plasmon Resonance". The pH 7.4 and pH 6.0 data were processed according to the Biacore protocol using the Biacore evaluation software with double referencing.

In an exemplary embodiment, TPP-6269 was evaluated by SPR/Biacore. TPP-6269 (SEQ ID NO: 25) was engineered with a hybrid IgG1/ IgG4 Fc region with the aforementioned mutations in TPP-5958 (L234F, L235E, M252Y, S254T, T256E, and N434H). From this analysis, TPP-6269 was found to exhibit an exceptional pH dependent binding to Human FcRn by SPR/ Biacore (Figures 13A, 13B). At a pH of 7.4 in Phosphate Buffered Saline (PBS), TPP-6269 was bound to FcRn at a binding affinity (K_D) of 928 nM at pH 7.4 and a binding affinity (K_D) of 3.82 nM at pH 6.0, a 244-fold differential (Figure 13C).

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EXAMPLE 5: SIMULTANEOUS BINDING OF TACI-Fc VARIANTS TO A HUMAN FcRn EXPRESSING CELL LINE AND BAFF BY FLOW CYTOMETRY

A flow cytometry analysis was carried out to determine whether TACI-Fc variants can simultaneously bind to BAFF (Biotechne, Catalog # 7537-BF-025/CF, biotinylated) and human FcRn using a CHO-K1 human FcRn stable cell line (Genscript, Cat. No. M00603). CHO-K1/FcRn cells were plated at 100,000 cells per well in PBS (pH 6.0), combined with 1 μ M of TACI-Fc variants in 0.5% BSA (pH 6.0), and incubated for 60 minutes at 4°C. The cells were washed 3x with 100 μ L 0.5% BSA (pH 6.0) and 10 μ g/ml BAFF-Biotin in 0.5% BSA (pH 6.0) was added to the cells which were then incubated for 60 minutes at 4°C. The cells were washed 3x and 5 μ g/ml of 0.5% BSA (pH 6.0) was added to the cells, which were then incubated for 30 minutes on ice. Cells were washed, labeled with 5 μ g/mL Streptavidin-Alexa-Fluor™ 488 (Invitrogen, Catalog # 532354) in 0.5% BSA (pH 6.0), and incubated for

30

30 minutes on ice. The cells were washed 3x, resuspended in 100 µl 0.5% BSA (pH 6.0) and analyzed using a LSRII Fortessa (BD, custom configuration) flow cytometer.

In this assay, a positive Median Fluorescence Intensity (MFI) greater than the labeled BAFF demonstrates simultaneous binding of the TACI-Fc variants to BAFF as well as FcRn on the cells. Figure 14A shows a graph summarizing the mean fluorescence intensity (MFI) ratio, consistent with simultaneous binding of the TACI domain to both labeled BAFF and FcRn in a human FcRn-expressing cell line. Figures 14B-14D are histograms showing simultaneous binding of TPP-5954 (Figure 14B), TPP-5957 (Figure 14C) and TPP-5958 (Figure 14D) to FcRn and BAFF binding compared to labeled BAFF (i.e., BAFF-biotin + Streptavidin-Alexa-Fluor™ 488 (SA-488)) binding to the TACI-Fc variants only.

EXAMPLE 6: TACI-Fc VARIANTS BLOCK IgG BINDING TO A HUMAN FcRn EXPRESSING CELL LINE

A flow cytometry analysis was employed to examine the capacity of TACI-Fc variants to block IgG1 binding to FcRn in a CHO-K1/hu FcRn cell line. Briefly, HO-K1/hu FcRn cells were plated at 100,000 cells per well in PBS (pH 6.0), combined with 1 µM of FcRn Fc variants in 0.5% BSA (pH 6.0) and then incubated for 60 minutes at 4°C. Cells were washed 3x with 100 µL 0.5% BSA (pH 6.0). Human IgG1 labeled with Phycoerythrin (IgG1-PE, Southern Biotech, Catalog # 0151K-09) at 100 µg/ml in 0.5% BSA (pH 6.0) was added in various amounts and incubated for 30 minutes at 4°C. The cells were washed 2x, resuspended in 100 µl 0.5% BSA (pH 6.0) and analyzed using a LSRII Fortessa (BD, custom configuration) flow cytometer.

The FcRn-expressing CHO-K1 cells treated with IgG-exhibited a reference level of fluorescent (MFI) reflecting binding of IgG1-PE to cellular FcRn. However, as shown in Figures 15A and 15B, when TACI-Fc fusion proteins were titrated into the cell cultures at increasing concentration, they reduced (i.e., outcompeted) the signal produced from IgG1-PE binding to FcRn. The reduction of signal from IgG1-PE was plotted as a ratio of mean fluorescence intensity (MFI) to background signal (Figure 15A) or raw MFI (Figure 15B). The different binding profiles of the TACI-Fc variants to FcRn in Figure 15B show that

weaker affinities to FcRn translate into lower potencies in this cell-based human IgG1 competition assay.

The results in Figures 15A and 15B confirm that the TACI-Fc fusion proteins can block IgG binding to a human FcRn expressing cell line.

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EXAMPLE 7: LINKER SEQUENCE BETWEEN TACI AND FC DOMAINS INCREASES PROTEIN STABILITY

To examine whether inclusion of certain amino acids or peptides can increase the isoelectric point of the TACI-Fc fusion proteins can increase protein stability, a high isoelectric point (pI) polypeptide linker was inserted between the TACI domain and the IgG Fc domain and compared to other variants without the linker. Among the TACI-Fc variants tested which are identified in Figures 16A-16B, TPP-5776 (SEQ ID NO: 28) and TPP-5780 (SEQ ID NO: 35) include the high pI linker GGRSSKARSSSRGGGRGG ("PI linker", SEQ ID NO: 8). TPP-5776 further includes the mutations M252Y, S254T, and T256E. TPP-5777 (SEQ ID NO: 71) and TPP-5779 (SEQ ID NO: 36) do not contain the PI linker, whereas TPP-5791 (SEQ ID NO: 34) contains the linker (AGGGG)3 (SEQ ID NO: 7).

Accelerated stability testing was performed on the foregoing TACI-Fc fusion proteins by incubating them at 37°C for a total of 3 weeks. Size exclusion chromatography (SEC) using a mobile phase buffer of 0.15 M sodium phosphate buffer pH 7.0 was performed on 20 µg on-column TACI-Fc fusions with a flow rate of 0.7 mL/min at a wavelength of 280nm. As shown in Figures 16A-16B, when incubated at 37°C for a total of 3 weeks, the TPP-5776 (SEQ ID NO: 28) and TPP-5780 (SEQ ID NO: 35) fusion proteins containing the high isoelectric point (PI) linker exhibited higher percent monomers and reduced aggregation over time compared to TPP-5777 (SEQ ID NO: 71) and TPP-5779 (SEQ ID NO: 36), which do not contain the PI linker. It was further observed that inclusion of the PI linker resulted in less aggregation during purification process.

EXAMPLE 8: ENHANCED BINDING TO FcRn WITH BLOCKADE OF BAFF AND APRIL ADDITIVELY REDUCE IMMUNOGLOBULIN LEVELS *IN-VITRO* AND *IN-VIVO*

TPP-6269 (SEQ ID NO: 25) was evaluated for its ability to reduce IgGs, IgMs, and mature B-cells following *in-vivo* treatment of mice with Keyhole Limpet Hemocyanin (KLH). Figure 17A shows the *in-vivo* study design of this experiment. On Day 0, mice were

subcutaneously immunized with 0.5 mg KLH in 0.1 mL of PBS. Control mice received PBS only. On days 1-7, the mice were intravenously administered TPP-6269 at daily doses of 5 mg/kg. Serum was collected after the day 7 dose to measure anti-KLH IgM and IgG, as well as total IgM and IgG levels. On day 8, 5 mice per group were euthanized and their spleens were collected and processed individually as single-cell suspensions. Absolute numbers of B220+ cells and CD4+ T cells from mouse splenocytes were counted by flow cytometry, the results of which are shown in Figure 17B. The resulting serum levels of anti-KLH IgM and IgG antibodies are shown in Figure 17C. The resulting serum levels of total IgM and total IgG antibodies are shown in Figure 17D.

The results in Figures 17B-17D show that TPP-6269 exhibits similar results as the individual protein components tested in the functional assays. Figure 17B shows that the mouse B220+ cell levels were significantly reduced in response to administration of 5mg/kg TPP-6269. In contrast, CD4+ T-cell levels were not significantly affected. Figures 17C and 17D show that TPP-6269-mediated blockade of FcRn activity resulted in significantly reduced anti-KLH IgM and anti-KLH IgG levels (Figure 17C), as well as significantly reduced total IgM and total IgG levels (Figure 17D).

EXAMPLE 9: EFFECTS OF THE ENHANCED Fc DOMAIN ON HUMAN IgG CLEARANCE *IN-VIVO* IN HUMAN FcRn MICE

With its enhanced Fc domain, TPP-6269 was further evaluated for its ability to promote clearance of human IgG (biotin-hIgG) antibodies in a muFcRn^{-/-}, huFcRn Tg32 transgenic mouse. Inasmuch as Tg32 mice suffer from hypogammaglobulinemia due to the low affinity of mouse IgGs for hFcRn, they provide a useful model for directly tracing IgG lowering *in vivo*.

Figure 18A shows the design of an experiment to evaluate IgG lowering by TPP-6269 *in vivo*. At day 0, Tg32 transgenic mice were administered a large bolus dose of biotin-hIgG (245 mg/kg) by intravenous injection (i.v.). TPP-6269 (20 mg/kg and 60mg/kg) or a vehicle control were administered by i.v. 49 hours post-IVIg dosing, while serum samples were serially collected at 48 hours, 72 hours, 96 hours, and 120 hours post-IVIg dosing.

Circulating levels of biotin-IgG remaining in the serum at the various points of collection were measured using the Gyrolab generic PK kit (Gyrolab, Catalog # P0020499). By running the serum as the first step over the streptavidin coated beads in the affinity capture column in accordance with the Gyrolab Bioaffy CD, the biotin-human IgG in circulation were directly

captured so that the IgGs could be detected with the included detection reagent in order to determine the % of total human IgGs in the mice.

As shown in Figure 18B, treatment of Tg32 mice with TPP-6269 (20 mg/kg and 60mg/kg) significantly reduced the plasma concentration of biotin-hIVIg compared with the vehicle-treated control group in a dose dependent manner.

EXAMPLE 10: ENHANCED FC DOMAIN PHARMACOKINETICS IN HUMAN FCRN MICE

To determine if an enhanced Fc domain provides improved pharmacokinetic (PK) properties, human FcRn Mice (mFcRn^{-/-}, hFcRn Tg32) were utilized and TPP-6269 was tested as an example. Following injection of TPP-6269 in the mice, the C_{max} was determined from blood collected 10 min after drug injection. Serial blood collections were obtained over a period of 21 days and circulating drug levels were directly measured using the Gyrolab generic PK kit (Cat# P0020499). Based on the results shown in Figure 19, the engineered Fc variant was found to exhibit a 53.7 hour $\frac{1}{2}$ -life calculated using a two phase decay non-linear regression in GraphPad Prism Version 9.3.1.

SEQUENCE SUMMARY

SEQ ID NO: 1

20 TACI_DR1/DR2-1

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRS

SEQ ID NO: 2

TACI_DR1/DR2-2

25 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCEN

SEQ ID NO: 3

EPKSS linker

EPKSS

30

SEQ ID NO: 4

EPKSS-(AGGGG)₂ linker

EPKSS-(AGGGG)₂

SEQ ID NO: 5

EPKSS-(AGGGG)3 linker

EPKSS-(AGGGG)3

5 SEQ ID NO: 6

(AGGGG)2 linker

(AGGGG)2

SEQ ID NO: 7

10 (AGGGG)3 linker

(AGGGG)3

SEQ ID NO: 8

PI linker

15 GGRSSKARSSSRGGGRGG

SEQ ID NO: 9

N-linked linker

GKTKPREEQYNSTYRVVSVLTG

20

SEQ ID NO: 10

A-(EAAAK)2-AGGGG linker

A-(EAAAK)2-AGGGG

25 SEQ ID NO: 11

A-EAAAK-AGGGG linker

A-EAAAK-AGGGG

SEQ ID NO: 12

30 SA-(EAAAK)2-AGGGG linker

SA-(EAAAK)2-AGGGG

SEQ ID NO: 13

SA-EAAAK-AGGGG linker

SA-EAAAK-AGGGG

SEQ ID NO: 14

GGNGSAEAAAKEAAAKANGTGG linker

5 GGNGSAEAAAKEAAAKANGTGG

SEQ ID NO: 15

GGNGSAEAAAKEAAAKANGSGG linker

GGNGSAEAAAKEAAAKANGSGG

10

SEQ ID NO: 16

IgG1/IgG4-Fc

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY

VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK

15 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

KTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK

SEQ ID NO: 17

IgG1/IgG4-Fc -- T307A, E380A, N434A

20 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY

VDGVEVHNAKTKPREEQFNSTYRVVSVLAVLHQDWLNGKEYKCKVSNKGLPSSIEK

TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVAWESNGQPENNY

KTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHAHYTQKSLSLGLGK

25 SEQ ID NO: 18

IgG1/IgG4-Fc -- Y436L

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY

VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK

TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

30 KTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHILTQKSLSLGLGK

SEQ ID NO: 19

IgG1/IgG4-Fc – L234F, L235E, M252Y, S254T, T256E, N434H

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALH~~HH~~HYTQKSLSLSLG

5

SEQ ID NO: 20

IgG1/IgG4-Fc -- L234F, L235E, M252Y, S254T, T256E

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
10 KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALH~~NH~~HYTQKSLSLSLG
K

SEQ ID NO: 21

15 IgG1/IgG4-Fc -- L234F, L235E, N434H

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALH~~HH~~HYTQKSLSLSLG
20 K

SEQ ID NO: 22

IgG1/IgG4-Fc -- M252Y, S254T, T256E, N434H

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWY
25 VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTTPVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALH~~HH~~HYTQKSLSLSLGK

SEQ ID NO: 23

30 IgG1/IgG4-Fc – L234F, L235E

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN

YKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 24

5 IgG1/IgG4-Fc – M252Y, S254T, T256E

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLGK

10

SEQ ID NO: 25

TPP-6269

TACI_DR1/DR2-1, EPKSS-(AGGGG)2 linker, L234F, L235E, M252Y, S254T, T256E,
N434H

15 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSEEPKSSAGGGGAGGG
GDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS
IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSL

20 G

SEQ ID NO: 26

TPP-6270

TACI_DR1/DR2-1, EPKSS-(AGGGG)3 linker, L234F, L235E, M252Y, S254T, T256E,
N434H

25 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSEEPKSSAGGGGAGGG
GAGGGGDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN
30 GQPENNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKS
LSLSLG

SEQ ID NO: 27

TPP-6267

TACI_DR1/DR2-1, no linker, L234F, L235E, M252Y, S254T, T256E, N434H

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSDKTHTCPPCPAPEFE
GGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV
5 YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLG

SEQ ID NO: 28

TPP-5776

10 TACI_DR1/DR2-2, PI linker, M252Y, S254T, T256E

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
15 TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 29

TPP-5954

TACI_DR1/DR2-2, PI linker, M252Y, S254T, T256E, N434H

20 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLGK

25

SEQ ID NO: 30

TPP-5957

TACI_DR1/DR2-2, PI linker, L234F, L235E, M252Y, S254T, T256E

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
30 KTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 31

TPP-5958

TACI_DR1/DR2-2, PI linker, L234F, L235E, M252Y, S254T, T256E, N434H

5 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
 KTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWY
 VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHHHYTQKSLSLSLGK

10

SEQ ID NO: 32

TPP-5975

TACI_DR1/DR2-2, PI linker, L234F, L235E

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
 15 KTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
 VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK

20 SEQ ID NO: 33

TPP-5977

TACI_DR1/DR2-2, PI linker, L234F, L235E, N434H

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
 KTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
 25 VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
 TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
 KTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHHHYTQKSLSLSLGK

SEQ ID NO: 34

30 **TPP-5791**

TACI_DR1/DR2-2, N-linked linker

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGKTKPREEQYNSTYRVVSVL
 TGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
 WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS

IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGL
 GK

5 SEQ ID NO: 35

TPP-5780

TACI_DR1/DR2-2, PI linker

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGGRSSKARSSSRGGGRGGD
 KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYV
 10 DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
 ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 36

15 **TPP-5779**

TACI_DR1/DR2-2, no linker

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
 FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP
 20 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 37

TPP-5792

25 TACI_DR1/DR2-2, N-linked linker, N434A

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENGKTKPREEQYNSTYRVVSVL
 TGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFN
 WYVDGVEVHNAKTKPREEQFNSTYRVVSVLAVLHQDWLNGKEYKCKVSNKGLPSS
 IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVAWESNGQPEN
 30 NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHAHYTQKSLSLGL
 GK

SEQ ID NO: 38

TPP-5298

TACI_DR1/DR2-2, no linker, Y436L

SLSCRKEQGKIFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
 FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP
 5 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHLTQKSLSLSLGK

SEQ ID NO: 39

TACI protein

10 MSGLGSRRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGTCSCKTICN
 HQSQRRTCAAFCSRSLSCRKEQGKIFYDHLLRDCISCASICGQHPKQCAYFCENKLRSPV
 NLPPELRRQRSGEVENNSDNSGRYQGLEHRGSEASPALPGLKLSADQVALVYSTLGL
 CLCAVLCCFLVAVACFLKKRGDPCSCQPRSRPRQSPAKSSQDHAMEAGSPVSTSPEP
 VETCSFCFPECRAPTQESAVTPGTPDPTCAGRWGCHTRTTVLQPCPHIPDSGLGIVCV
 15 PAQEGGPGA

SEQ ID NO: 40

TACI Extracellular Domain with signal peptide

MSGLGSRRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGTCSCKTICN
 20 HQSQRRTCAAFCSRSLSCRKEQGKIFYDHLLRDCISCASICGQHPKQCAYFCENKLRSPV
 NLPPELRRQRSGEVENNSDNSGRYQGLEHRGSEASPALPGLKLSADQVALVYS

SEQ ID NO: 41

TACI CRD1

25 CPEEQYWDPLLGTCSCKTICNHQSQRRTCAAFCSRSLSCRKEQGKIFYDHLLRDC

SEQ ID NO: 42

TACI cysteine-rich TNFR repeat (DR1)

CPEEQYWDPLLGTCSCKTICNHQSQRRTCAAF
 30

SEQ ID NO: 43

TACI_CRD2

CASICGQHPKQCAYFCENKLRSPVNLPELRRQRSGEVENNSDNSGRYQGLEHRGSE
 ASPALPGLKLSADQVALVYS

SEQ ID NO: 44

TACI_cysteine-rich TNFR repeat (DR2)

CRKEQGKGYDHLLRDCISCASICGQHPKQCAYFCEN

5

SEQ ID NO: 45

TPP-5274

TACI_DR1/DR2-2, no linker, M228L, N434S

SLSCRKEQGKGYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS

10 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSR
LTVDKSRWQEGNVFSCSVLHEALHSHYTQKSLSLSLGKHHHHHH

15 SEQ ID NO: 46

TPP-7165

TACI_DR1/DR2-1, A-(EAAAK)₂-AGGGG, L234F, L235E, M252Y, S254T, T256E,
N434H

SLSCRKEQGKGYDHLLRDCISCASICGQHPKQCAYFCENKLRS*AEAAAKEAAAKAGGG*

20 GDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS
IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHHYTQKSLSLSL
G

25

SEQ ID NO: 47

TPP-7166

TACI_DR1/DR2-2, SA-(EAAAK)₂-AGGGG linker, L234F, L235E, M252Y, S254T,
T256E, N434H

30 SLSCRKEQGKGYDHLLRDCISCASICGQHPKQCAYFCENS*AEAAAKEAAAKAGGGGDK*
THTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHHYTQKSLSLSLG

SEQ ID NO: 48

TPP-7167

TACI_DR1/DR2-2, SA-EAAAK-AGGGG linker, L234F, L235E, M252Y, S254T, T256E,
5 N434H

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENSAEAAAKAGGGGDKTHTCP
PCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
10 LDDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLG

SEQ ID NO: 49

TPP-5262

TACI_DR1/DR2-2, (AGGGG)₃ linker, M252Y, S254T, T256E, , 6xHis

15 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR
LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKHHHHHHH

20

SEQ ID NO: 50

TPP-5260

TACI_DR1/DR2-2, no linker, M252Y, S254T, T256E, 6xHis

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
25 VFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQ
FNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPPVLDSDGSFFLYSR
LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKHHHHHHH

30 SEQ ID NO: 51

TPP-5272

TACI_DR1/DR2-2, no linker, 6xHis

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQ

FNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKHHHHHH

5 SEQ ID NO: 52

TPP-5317

TACI_DR1/DR2-2, (AGGGG)3 no linker, M252Y, S254T, T256E, 6xHis

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLYITREPEVTCVVVDVSHEDPQVQFKWYVDGVEVHNAKTKLREEQ
 10 YNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKHHHHHH

SEQ ID NO: 53

15 **TPP-5273**

TACI_DR1/DR2-2, (AGGGG)3 linker, 6xHis

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPQVQFKWYVDGVEVHNAKTKLREEQ
 YNSTFRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 20 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYNTTPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKHHHHHH

SEQ ID NO: 54

TPP-5275

25 TACI_DR1/DR2-2, no linker, 6xHis

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPQVKFNWYVDGVQVHNAKTKPREQQ
 YNSTYRVSVLTVLHQNWLDGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNDGEPENYKTTTPVLDSDGSFFLYSK
 30 LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGHHHHHH

SEQ ID NO: 55

TPP-6265

TACI_DR1/DR2-1, (AGGGG)3 linker, M252Y, S254T, T256E

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSAGGGGAGGGGAGGG
 GDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNW
 YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
 KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
 5 YKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHHHYTQKSLSLSLG

SEQ ID NO: 56

TPP-6458

TACI_DR1/DR2-1, L234F, L235E, M252Y, S254T, T256E, N434H

10 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSDKTHTCPPCPAPEFE
 GGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
 REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV
 YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFF
 LYSRLTVDKSRWQEGNVFSCSV MHEALHHHYTQKSLSLSLGK

15

SEQ ID NO: 57

TPP-4286

TACI_DR1/DR2-1, EPKSS linker, FLAG Tag

20 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSEPKSSDKTHTCPPCP
 APEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
 DGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGKGGSGGSGGD
 YKDDDDK

25

SEQ ID NO: 58

TPP-4291

TACI_DR1/DR2-1 (L82H), EPKSS linker, FLAG Tag

30 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSEPKSSDKTHTCPPCP
 APEAEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPSSIEKTISKAKGQ
 PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
 DGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGKGGSGGSGGD
 YKDDDDK

SEQ ID NO: 59

TPP-5726

TACI-DR1/DR2-2, no linker, M252H, N434H

5 SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLHISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
 SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
 TVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLGKHHHHHH

10

SEQ ID NO: 60

TPP-5721

TACI-DR1/DR2-2, no linker, N434H

SLSCRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENDKTHTCPPCPAPELLGGPS
 15 VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ
 FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHHHYTQKSLSLSLGKHHHHHH

20 SEQ ID NO: 61

EPKSS-(AGGGG)2 linker, L234F, L235E, M252Y, S254T, T256E, N434H

*EPKSSAGGGGAGGGG*DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVV
 VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
 25 IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
 HHHYTQKSLSLSLG

SEQ ID NO: 62

EPKSS-(AGGGG)3 linker, L234F, L235E, M252Y, S254T, T256E, N434H

30 *EPKSSAGGGGAGGGGAGGGG*DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPE
 VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD
 WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV
 KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
 VMHEALHHHYTQKSLSLSLG

SEQ ID NO: 63

PI linker, L234F, L235E, M252Y, S254T, T256E, N434H

GGRSSKARSSSRGGGRGGDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTC
 5 *VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN*
GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVM
HEALHHHYTQKSLSLSLGK

10 SEQ ID NO: 64

A-(EAAAK)2-AGGGG linker, L234F, L235E, M252Y, S254T, T256E, N434H

AEAAAKEAAAKAGGGGDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCV
VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP
 15 *SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHE*
ALHHHYTQKSLSLSLG

SEQ ID NO: 65

SA-(EAAAK)2-AGGGG linker, L234F, L235E, M252Y, S254T, T256E, N434H

20 *SAEAAAKEAAAKAGGGGDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCV*
VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP
SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHE
ALHHHYTQKSLSLSLG

25

SEQ ID NO: 66

SA-EAAAK-AGGGG, L234F, L235E, M252Y, S254T, T256E, N434H

SAEAAKAGGGGDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDV
SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
 30 *KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYP*
SDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHHH
YTQKSLSLSLG

SEQ ID NO: 67

TPP-6419

TACI_DR1/DR2-1, EPKSS-(AGGGG)2 linker, L234F, L235E, M252Y, S254T, T256E, N434H

5 SLSCRKEQGKIFYDHLLRDCISCASICGQHPKQCAAYFCENKLRSEPKSSAGGGGAGGG
GDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS
IEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMEALH~~HH~~HYTQKSLSLSL
10 GK

SEQ ID NO: 68

TPP-6777

TACI_DR1/DR2-2, no linker, M252Y, S254T, T256E, M247L, N253S

15 SLSCRKEQGKIFYDHLLRDCISCASICGQHPKQCAAYFCENDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRL
TVDKSRWQEGNVFSCSVLHEALHSHYTQKSLSLSLGK
20

SEQ ID NO: 69

TPP-6269

Nucleotide sequence

AGCCTGTCTTGTAGAAAAGAGCAGGGCAAGTTCTACGACCATCTGCTGAGAGAC
25 TGCATCAGCTGCGCCAGCATTGCGGACAGCACCTAAGCAGTGCGCCTACTTCT
GCGAGAACAAAGCTGAGAAGCGAGCCTAAATCTTCTGCCGGCGGAGGCGGAGCTG
GTGGTGGCGGAGATAAGACACACACCTGTCCTCCATGTCCTGCTCCAGAGTTTGA
AGGCGGCCCTAGCGTGTTCTGTTTCCTCCAAAGCCTAAGGACACCCTGTACATC
ACCCGCGAGCCTGAAGTGACATGTGTGGTGGTGGACGTGTCCCAAGAGGACCCC
30 GAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCTAGAGAGGAACAGTTCAACAGCACCTACAGAGTGGTGTCCGTGCTGACC
GTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTAGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCC
AAGGGAACCTCAGGTTTACACCCTGCCTCCAAGCCAAGAGGAAATGACCAAGAA

CCAGGTGTCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTG
GAATGGGAGAGCAATGGCCAGCCTGAGAACAACCTACAAGACCACACCTCCTGTG
CTGGACAGCGACGGCAGCTTCTTTCTGTACTCCCGGCTGACAGTGGACAAGAGC
AGATGGCAAGAGGGCAACGTGTTTCAGCTGCTCTGTGATGCACGAAGCCCTGCAC
5 CACCACTACACCCAGAAGTCTTTGAGCCTGAGCCTGGGC

SEQ ID NO: 70

TPP-6419

Nucleotide sequence

10 AGCCTGTCTTGTAGAAAAGAGCAGGGCAAGTTCTACGACCATCTGCTGAGAGAC
TGCATCAGCTGCGCCAGCATTGCGGACAGCACCTAAGCAGTGCGCCTACTTCT
GCGAGAACAAGCTGAGAAGCGAGCCTAAATCTTCTGCCGGCGGAGGCGGAGCTG
GTGGTGGCGGAGATAAGACACACACCTGTCCTCCATGTCCTGCTCCAGAGTTTGA
AGGCGGCCCTAGCGTGTTCTGTTTCCTCCAAAGCCTAAGGACACCCTGTACATC
15 ACCCGCGAGCCTGAAGTGACATGTGTGGTGGTGGACGTGTCCCAAGAGGACCCC
GAGGTGCAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCTAGAGAGGAACAGTTCAACAGCACCTACAGAGTGGTGTCCGTGCTGACC
GTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAAC
AAGGGCCTGCCTAGCAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCC
20 AAGGGAACCTCAGGTTTACACCCTGCCTCCAAGCCAAGAGGAAATGACCAAGAA
CCAGGTGTCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTG
GAATGGGAGAGCAATGGCCAGCCTGAGAACAACCTACAAGACCACACCTCCTGTG
CTGGACAGCGACGGCAGCTTCTTTCTGTACTCCCGGCTGACAGTGGACAAGAGC
AGATGGCAAGAGGGCAACGTGTTTCAGCTGCTCTGTGATGCACGAAGCCCTGCAC
25 CACCACTACACCCAGAAGTCTTTGAGCCTGAGCCTGGGCAAG

SEQ ID NO: 71

TPP-5777

TACI_DR1/DR2-2, no linker, M428L/N434S

30 SLSCRKEQGKFYDHLLRDCISCASICQHQPQCAYFCENDKTHTCPPELLGGPS
VFLFPPKPKDTLYITREPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVLHEALHSHYTQKSLSLGK

SEQ ID NO: 72

IgG1/IgG4-Fc – L234F, L235E, M252Y, S254T, T256E, N434H

DKTHTCPPCPAPEFEGGPSVFLFPPKPKDTL**Y**ITREPEVTCVVVDVSQEDPEVQFNW
5 YVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH**H**HYTQKSLSLSLG
K

Equivalents

- 10 Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A fusion protein comprising a TACI extracellular domain (ECD) portion and a hybrid IgG Fc domain,
5 wherein the Ig Fc domain comprises one or more mutations selected from the group consisting of N434H, Y436L, L234F/L235E, M252Y/S254T/T256E, L234F/L235E/N434H, L234F/L235E/N434H, M252Y/S254T/T256E/N434H, L234F/L235E/M252Y/S254T/T256E, L234F/L235E/M252Y/S254T/T256E/N434H, and T307A/E380A/N434, and combinations thereof.
10
2. The fusion protein of claim 1, wherein the TACI ECD portion comprises a TACI DR2 portion.
3. The fusion protein of claim 2, wherein the TACI ECD portion further comprises a
15 TACI DR1 portion.
4. The fusion protein of any of the preceding claims, wherein the TACI ECD portion comprises the amino acid sequence set forth in SEQ ID NO: 1 or 2.
- 20 5. The fusion protein of claim 4, wherein the TACI ECD portion consists of the amino acid sequence set forth in SEQ ID NO: 1 or 2.
6. The fusion protein of any of the preceding claims, wherein the TACI ECD portion comprises one or more mutations selected from the group consisting of L82H,
25 F78H/D80H/L82H, Y79H/D80H/L82H, D80H/L82H/L83H, Y79H/D80H/L83H, D80/L83H/I87H, and combinations thereof.
7. The fusion protein of any one of the preceding claims, wherein the mutant Ig Fc domain is derived from IgG1, IgG2, IgG3, IgG4, or a hybrid Fc domain thereof.
30
8. The fusion protein of claim 7, wherein the mutant Ig Fc domain comprises a hybrid IgG1-IgG4 Fc domain.

9. The fusion protein of any one of the preceding claims, wherein the mutant Ig Fc domain comprises an amino acid sequence at least 90%, 95% or 99% identical to an amino acid sequence set forth in any one of SEQ ID NOS: 16-24, and 72.
- 5 10. The fusion protein of claim 9, wherein the mutant Ig Fc domain comprises an amino acid sequence set forth in any one of SEQ ID NOS: 16-24, and 72.
11. The fusion protein of any one of the preceding claims, wherein the TACI ECD portion is coupled to the mutant Ig Fc domain by a polypeptide linker.
- 10 12. The fusion protein of claim 11, wherein the polypeptide linker comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3-15.
13. The fusion protein of any of the preceding claims, comprising an amino acid sequence at least 96%, 97%, 98%, or 99% identical to an amino acid sequence set forth in any one of SEQ ID NOS: 25-38, 46-48, 55, 56, and 67.
- 15 14. The fusion protein of claim 13, comprising an amino acid sequence set forth in any one of SEQ ID NOS: 25-38, 46-48, 55, 56, and 67.
- 20 15. The fusion protein of claim 14, comprising an amino acid sequence set forth in SEQ ID NO: 25 or 67.
16. The fusion protein of any of the preceding claims, wherein the fusion protein exhibits a binding affinity (K_D) for FcRn at pH 6.0 that is less than 50 nM, less than 20 nM, less than 12 nM, less than 6 nM, or less than 4 nM.
- 25 17. The fusion protein of any of the preceding claims, wherein the fusion protein exhibits an increase in binding affinity (K_D) for FcRn of greater than 50-fold, greater than 100-fold, or greater than 200-fold at pH 6.0 relative to pH 7.4
- 30 18. A pharmaceutical composition comprising the fusion protein of any one of the preceding claims and a pharmaceutically acceptable carrier.

19. A nucleic acid encoding the fusion protein of any of the preceding claims.
20. The nucleic acid of claim 19 encoding the fusion protein set forth in SEQ ID NO: 25 or 67.
- 5 21. The nucleic acid of claim 20, comprising a nucleotide sequence set forth in SEQ ID NO: 69 or 70.
- 10 22. The nucleic acid of any one of claims 19-21 encoding a codon-optimized fusion protein.
23. An expression vector comprising the nucleic acid of any one of claims 19-22.
24. The expression vector of claim 23, wherein the expression vector is a viral vector.
- 15 25. The expression vector of claim 24, wherein the viral vector is an AAV vector.
26. A pharmaceutical composition comprising the expression vector of any one of claims 23-25 and a pharmaceutically acceptable carrier.
- 20 27. A host cell comprising the nucleic acid of any one of claims 19-22.
28. A host cell comprising the expression vector of any one of claims 23-25.
- 25 29. A method of treating a malady in a patient comprising administering to the patient in need thereof an effective amount of the pharmaceutical composition of claim 18.
- 30 30. A method of treating a malady in a patient comprising administering to the patient in need thereof an effective amount of the pharmaceutical composition of claim 26.
31. The method of claim 29 or 30, wherein the malady is an autoimmune disorder.
32. The method of claim 31, wherein the autoimmune disorder is selected from the group consisting of Acute Antibody Mediated Rejection, Chronic Antibody Mediated Rejection

primary Sjogren's syndrome, Autoimmune Hemolytic Anemia, Antiphospholipid Syndrome (APS), Catastrophic APS, Refractory Rheumatoid Arthritis, Autoimmune Vasculitis, Cryoglobulinemia, Antineutrophil Cytoplasmic Autoantibodies (ANCA), IgA Vasculitis, Rheumatoid Vasculitis, Anti-Synthetase Syndrome, Primary Membranous Nephropathy, 5 Juvenile Idiopathic Arthritis (JIA), Systemic Sclerosis, Guillain-Barre Syndrome, Autoimmune Pulmonary Alveolar Proteinosis, Autoimmune Hemolytic Anemia (AIHA), Hemolytic Disease Of The Fetus And Newborn (HDFN), Acute Hemolytic Transfusion Reaction, Preventative Treatment with Adeno-Associated Virus (AAV), Thyroid Eye Disease (TED), Huntington's Disease, Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), 10 Pemphigus Vulgaris (PV), Bullous Pemphigoid (BP), Immune Thrombocytopenic Purpura (ITP), Myasthenia Gravis (MG), and Neuromyelitis Optica Spectrum Disorder (NMOSD).

33. A method of decreasing an immune response in a patient in need thereof comprising administering to the patient an effective amount of the pharmaceutical composition of claim 15 18 or 26.

34. A method of inhibiting the activity of one or more pro-inflammatory cytokines in a patient in need thereof comprising administering to the patient in need thereof an effective amount of the pharmaceutical composition of claim 18 or 26.

20

35. The method of claim 34, wherein the one or more pro-inflammatory cytokines comprise BAFF and/or APRIL.

36. A method of enhancing clearance of IgG immunoglobulins in a patient in need thereof 25 comprising administering to the patient an effective amount of the pharmaceutical composition of claim 18 or 26.

37. A method of reducing levels of mature B-cells, IgG, IgM, IgA, or a combination thereof in a patient in need thereof comprising administering to the patient an effective 30 amount of the pharmaceutical composition of claim 18 or 26.

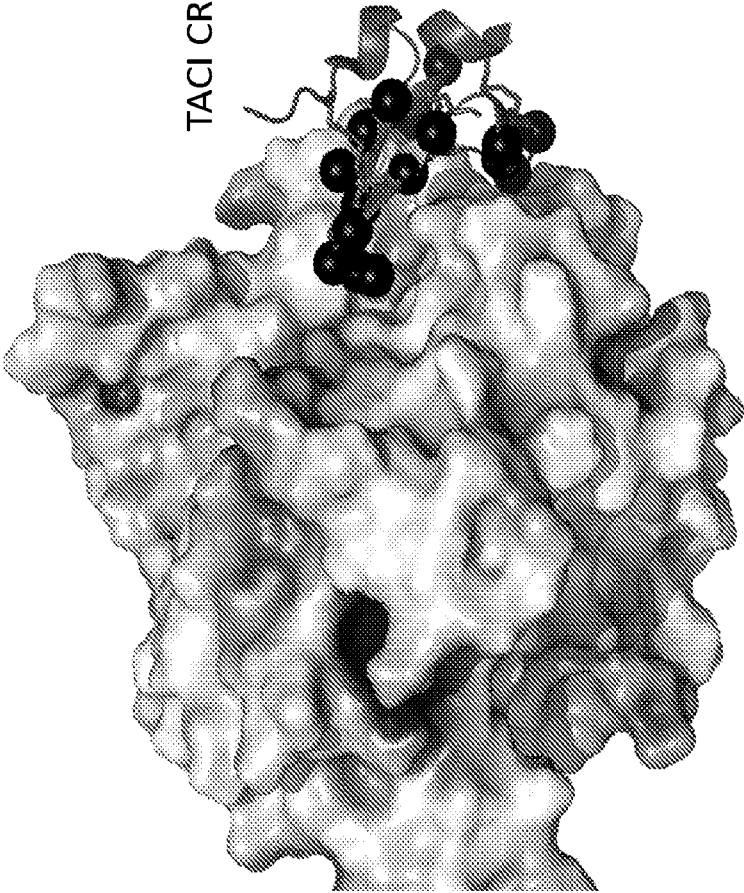
38. The method of claim 37, wherein administration of the pharmaceutical composition does not significantly lower levels of IgD or IgE.

39. A kit for treating one or more maladies in a patient, the kit comprising: at least one dose of the pharmaceutical composition of claim 18 or 26 and instructions for use.
40. A polypeptide linker comprising an amino acid sequence set forth in any one of SEQ
- 5 ID NOS: 4, 5, and 6.

FIG. 1

BAFF

TACI CRD2



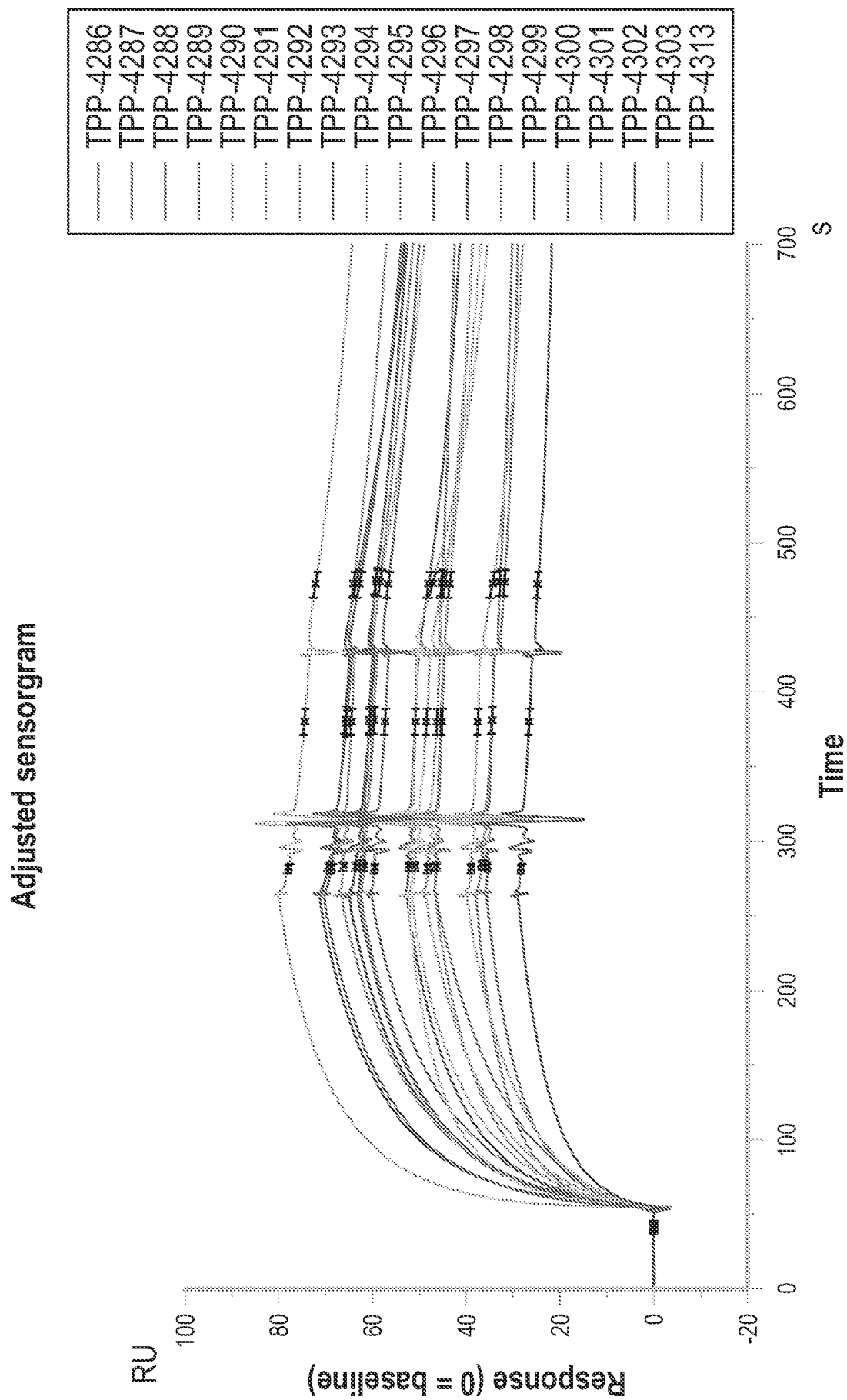


FIG. 2A

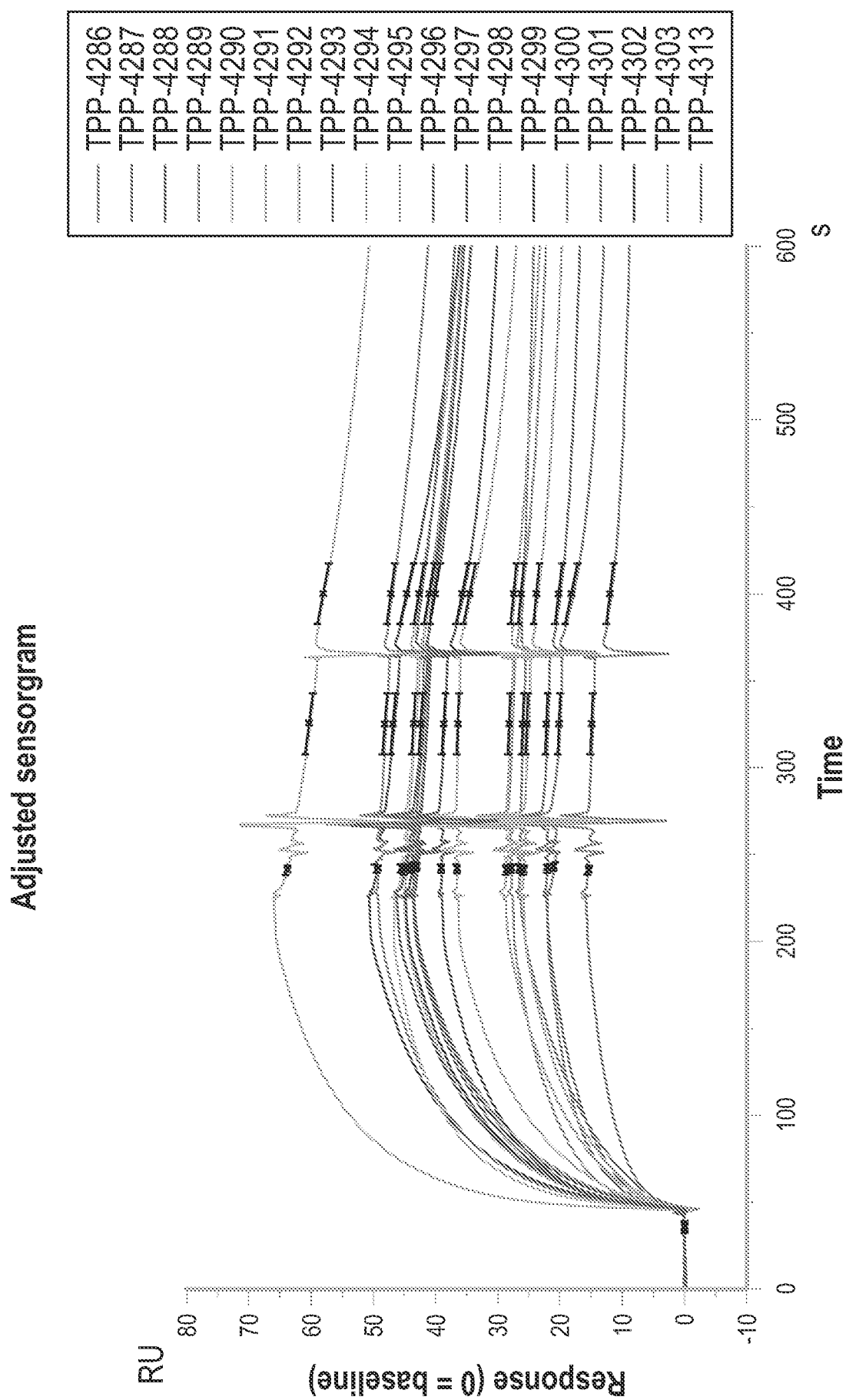


FIG. 2B

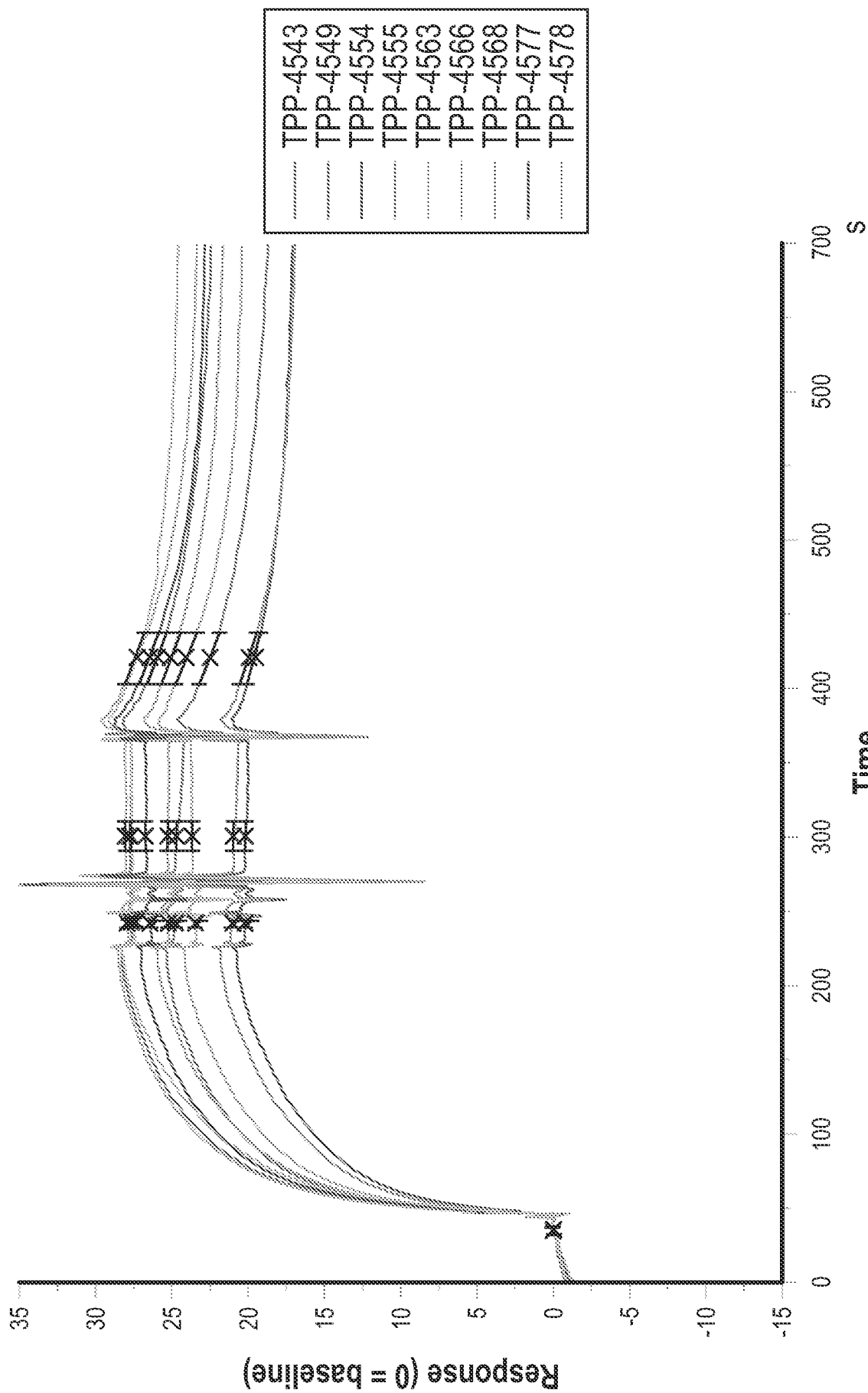


FIG. 3

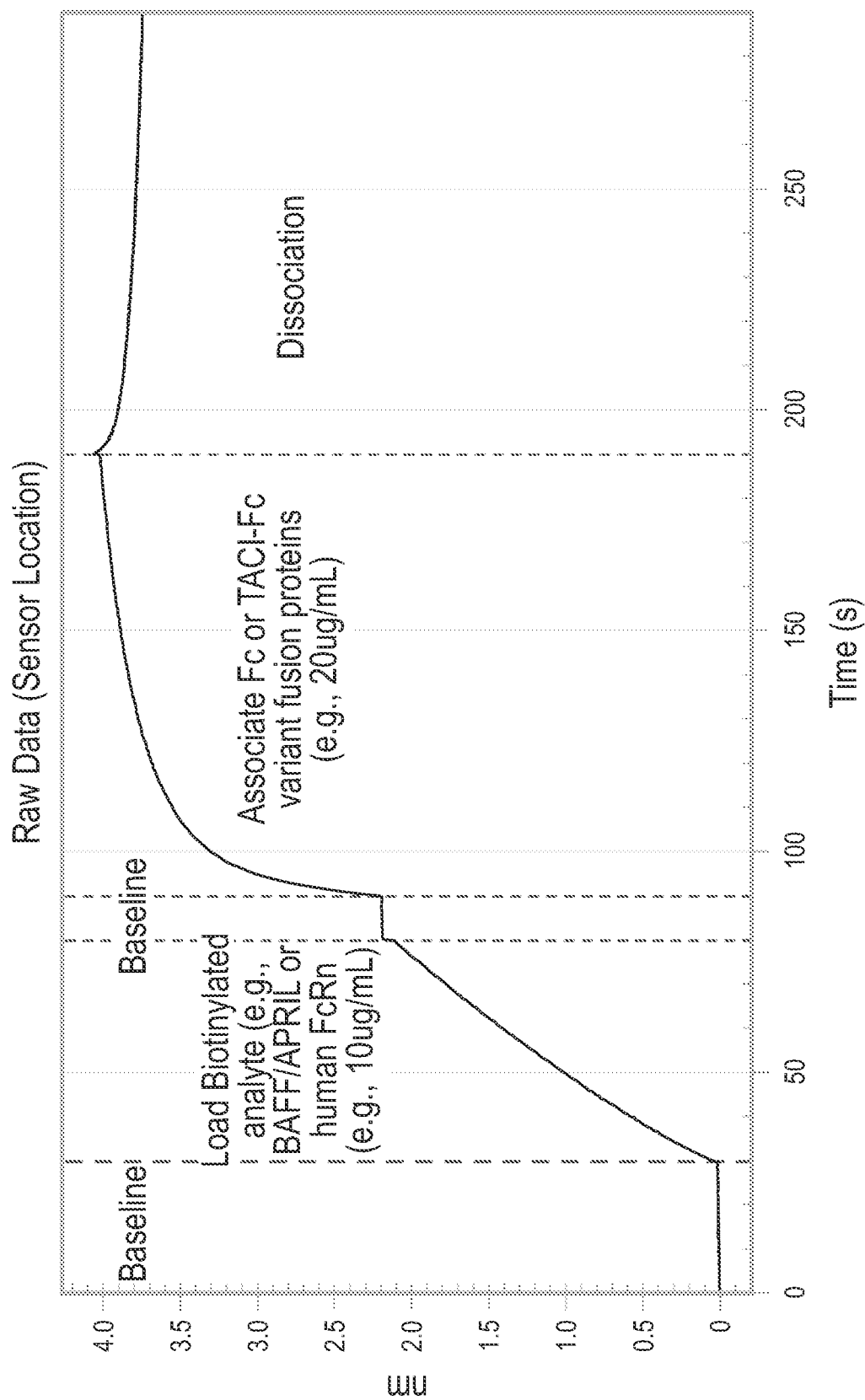


FIG. 4

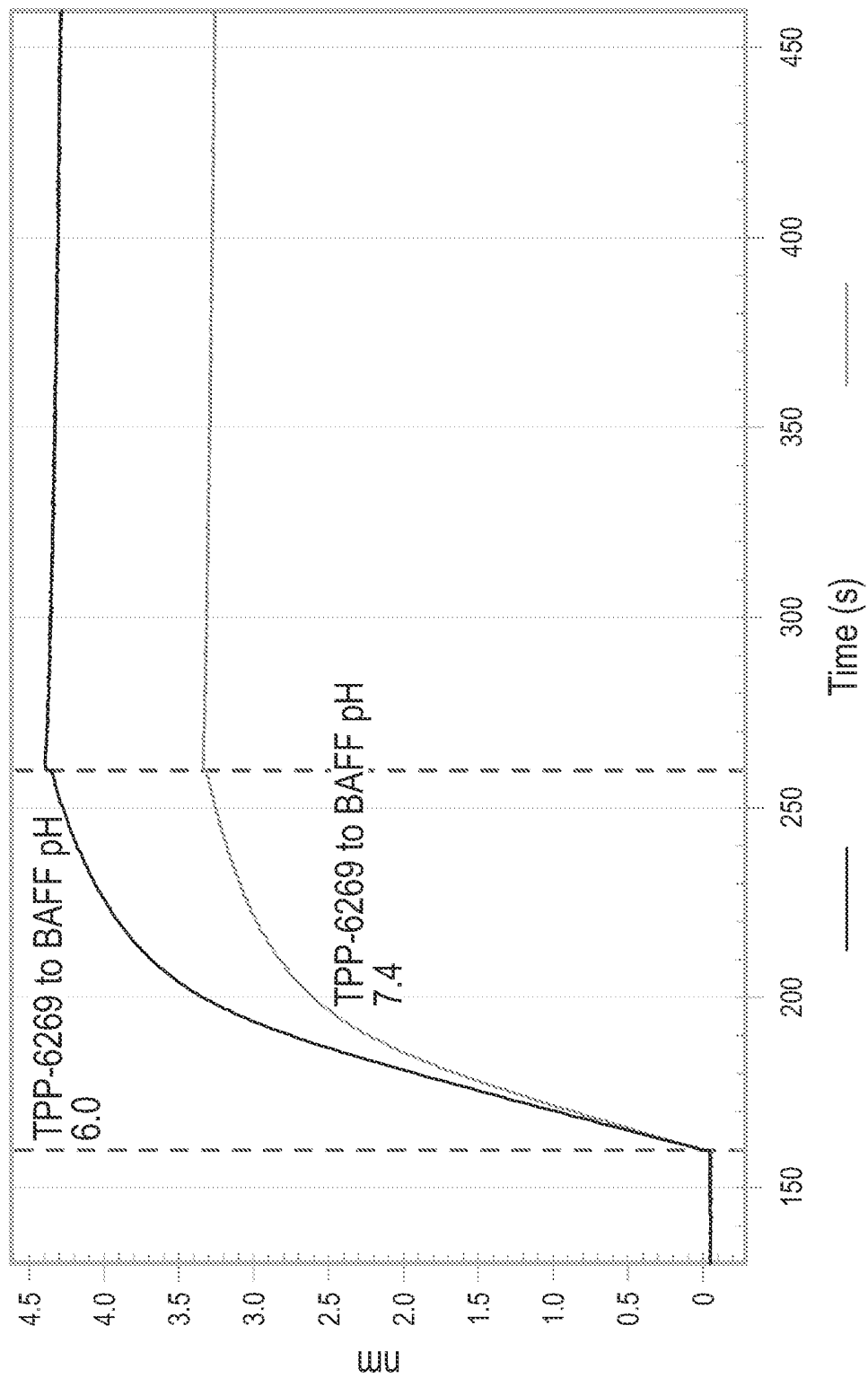


FIG. 5

FIG. 6

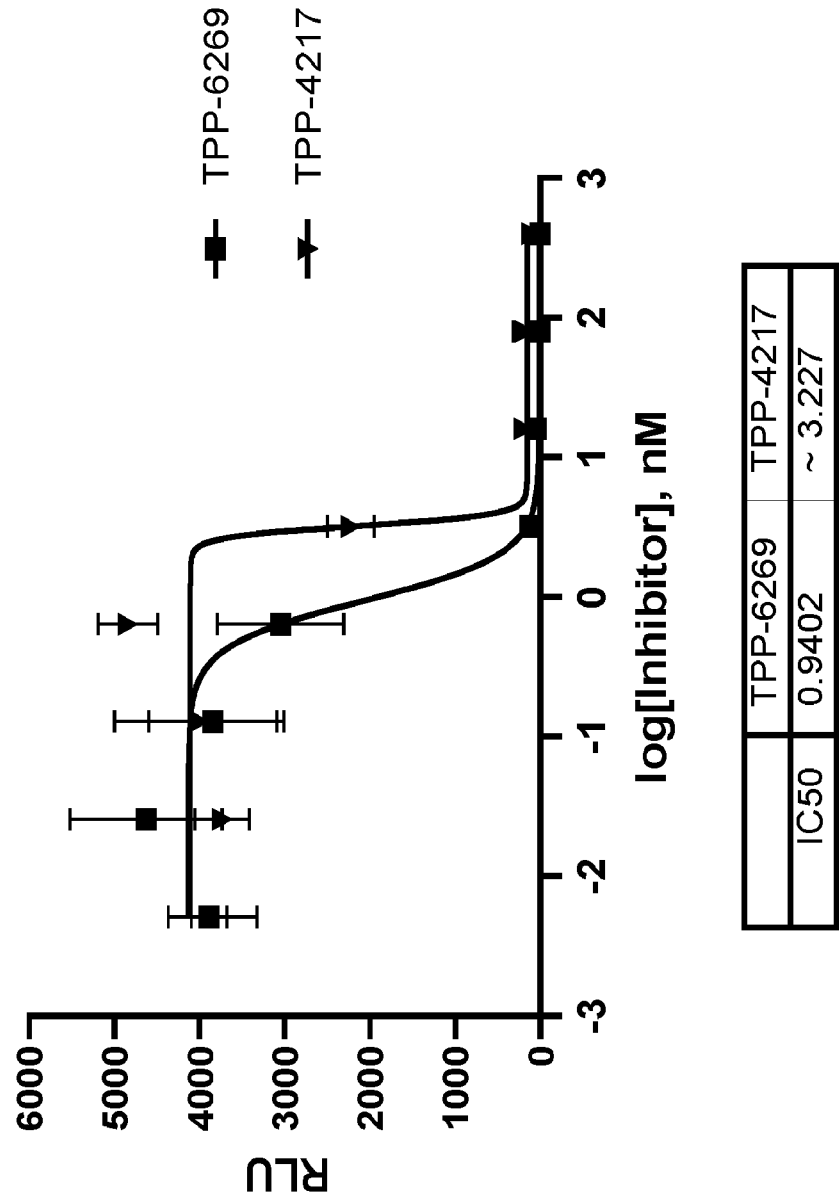
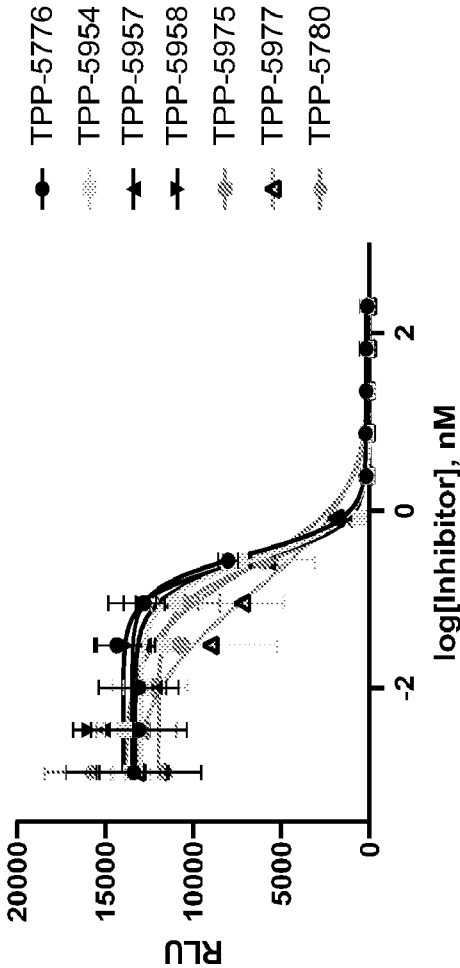
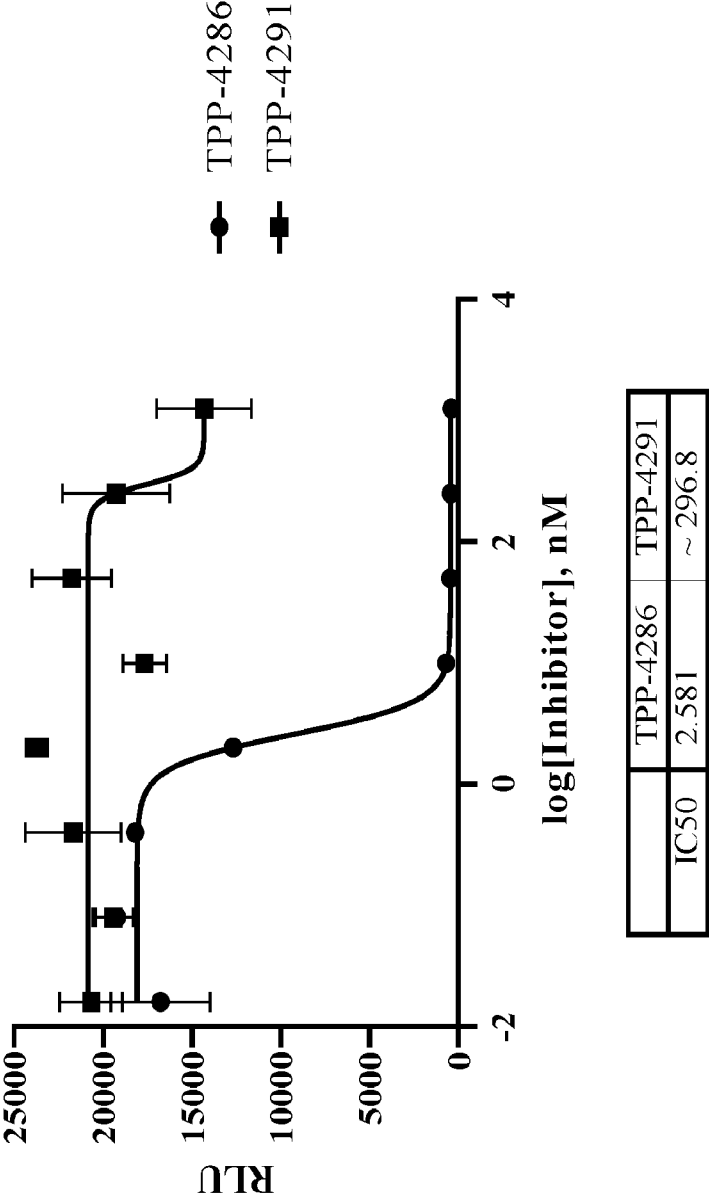


FIG. 7



	TPP-5776	TPP-5954	TPP-5957	TPP-5975	TPP-5977	TPP-5780
IC50	0.3241	0.2831	0.2769	0.2629	0.1155	0.2259

FIG. 8



TPP-5260 binding to FcRn at pH 6.0 and pH 7.4

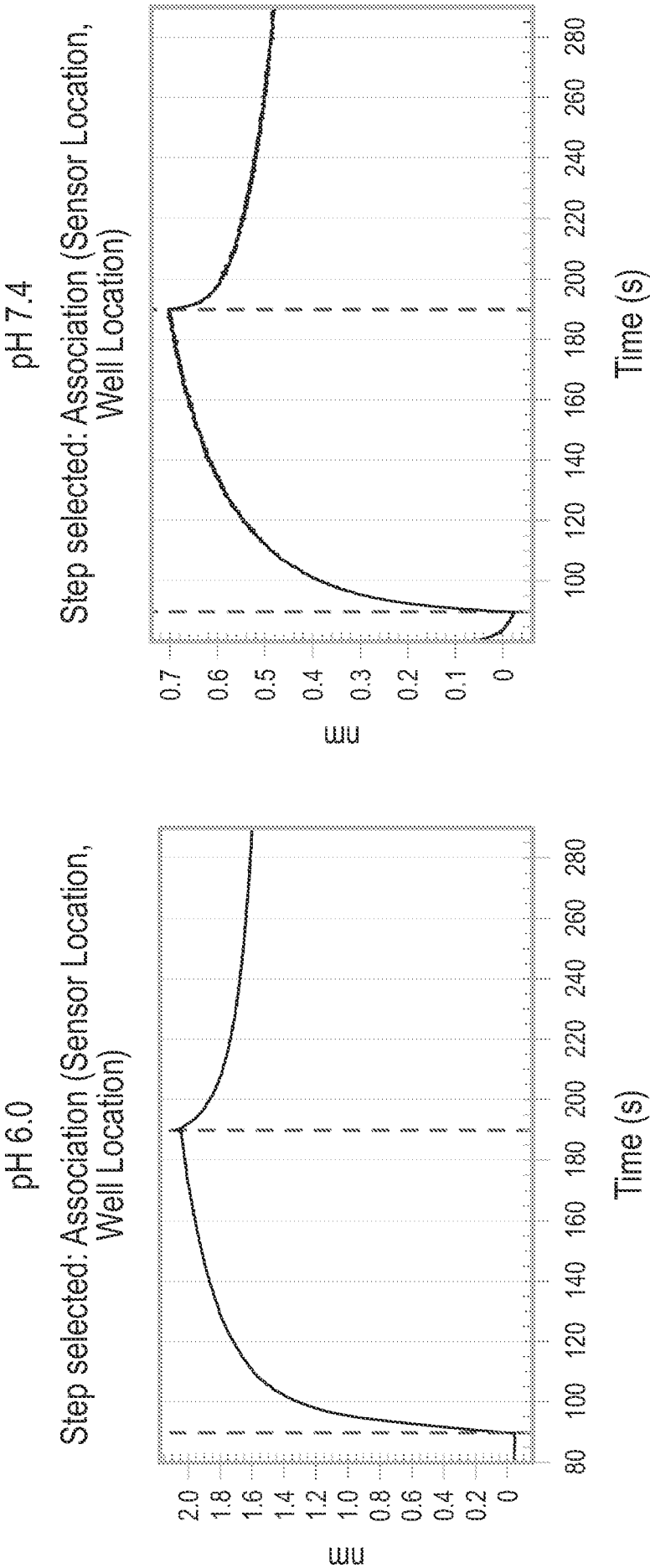


FIG. 9A

TPP-5262 binding to FcRn at pH 6.0 and pH 7.4

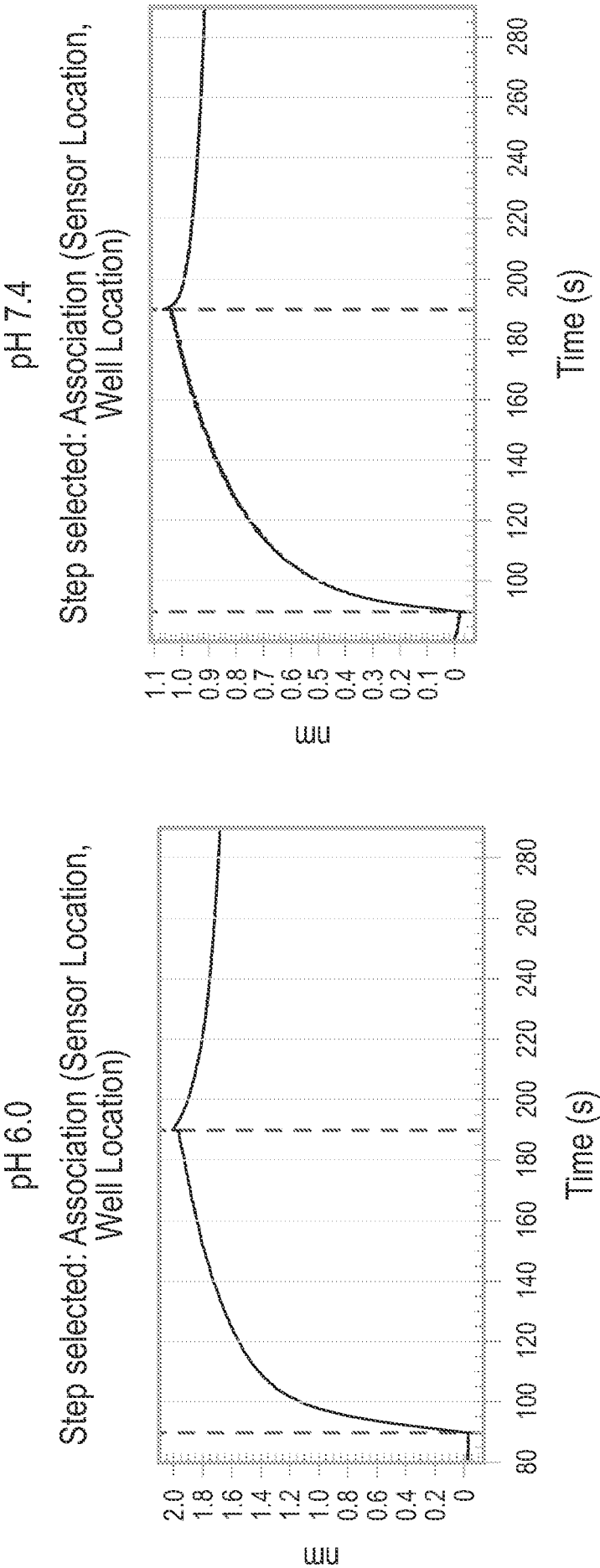


FIG. 9B

TPP-5317 binding to FcRn at pH 6.0 and pH 7.4

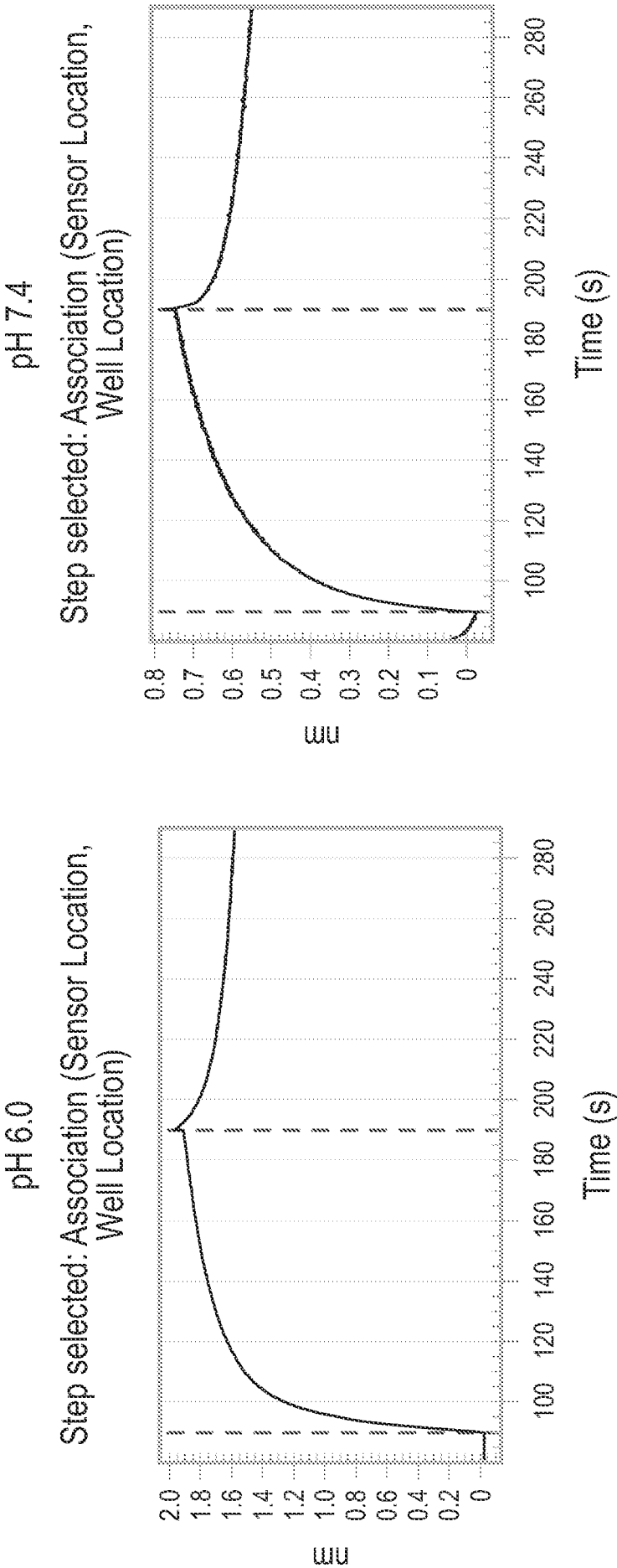


FIG. 9C

TPP-5272 binding to FcRn at pH 6.0 and pH 7.4

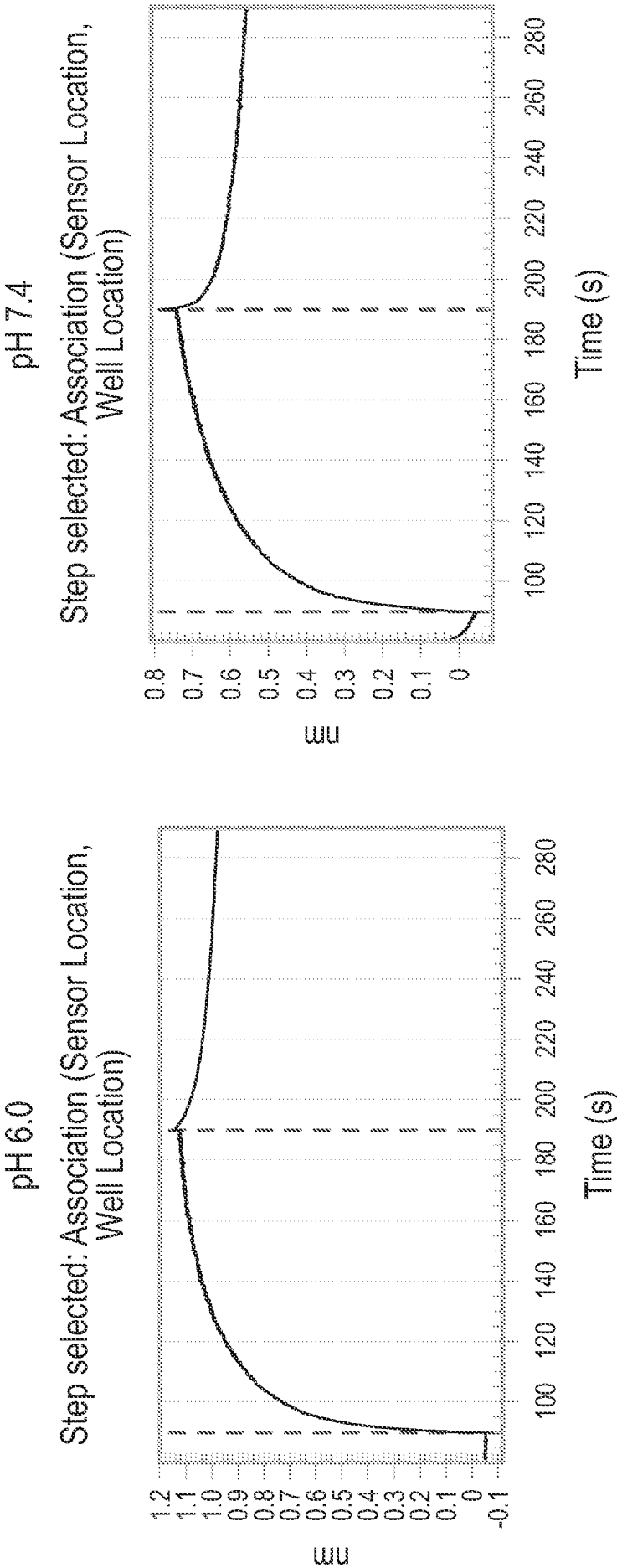


FIG. 9D

TPP-5273 binding to FcRn at pH 6.0 and pH 7.4

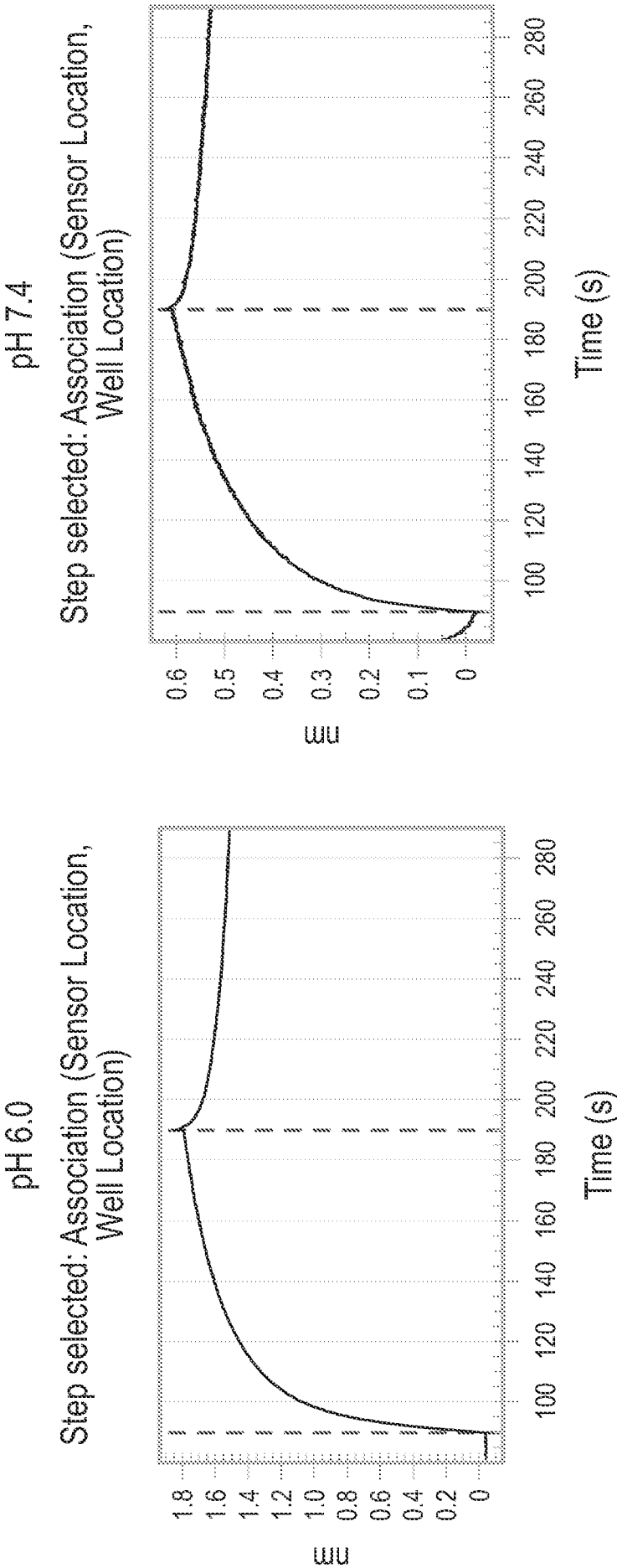


FIG. 9E

TPP-5274 binding to FcRn at pH 6.0 and pH 7.4

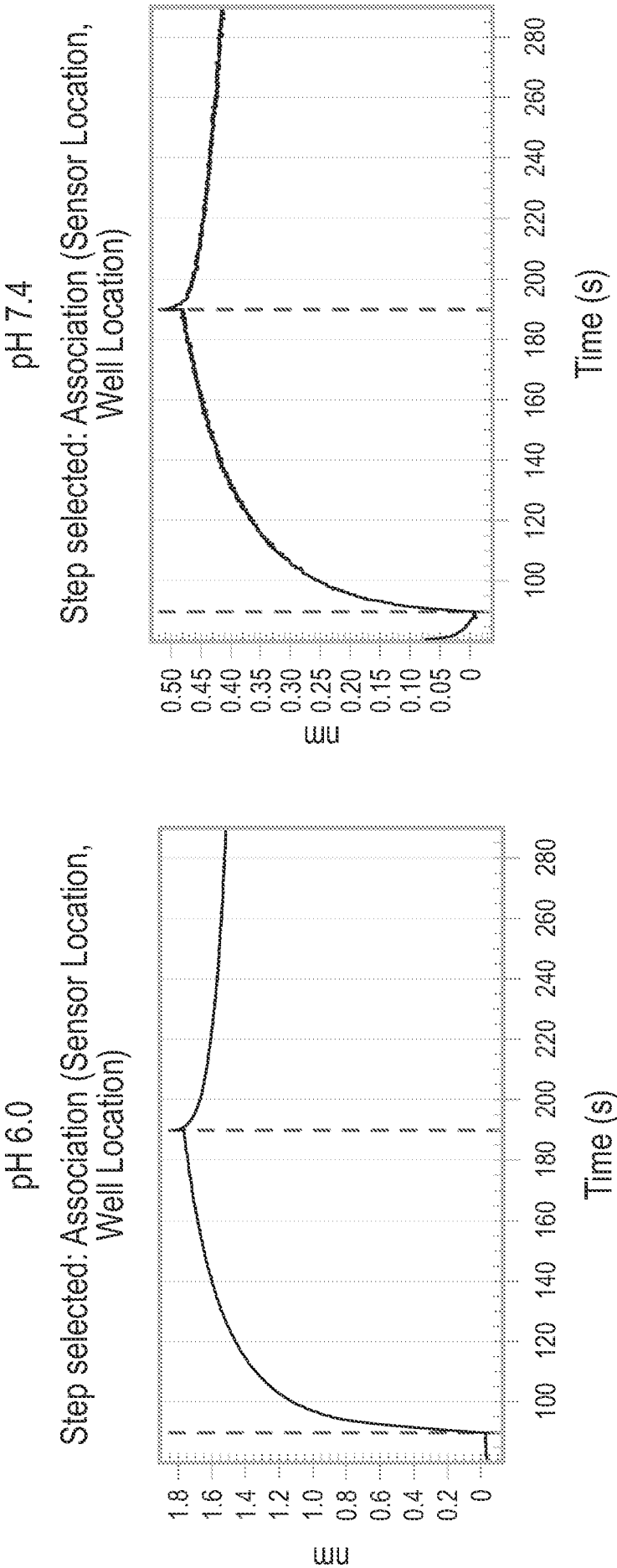


FIG. 9F

TPP-5275 binding to FcRn at pH 6.0 and pH 7.4

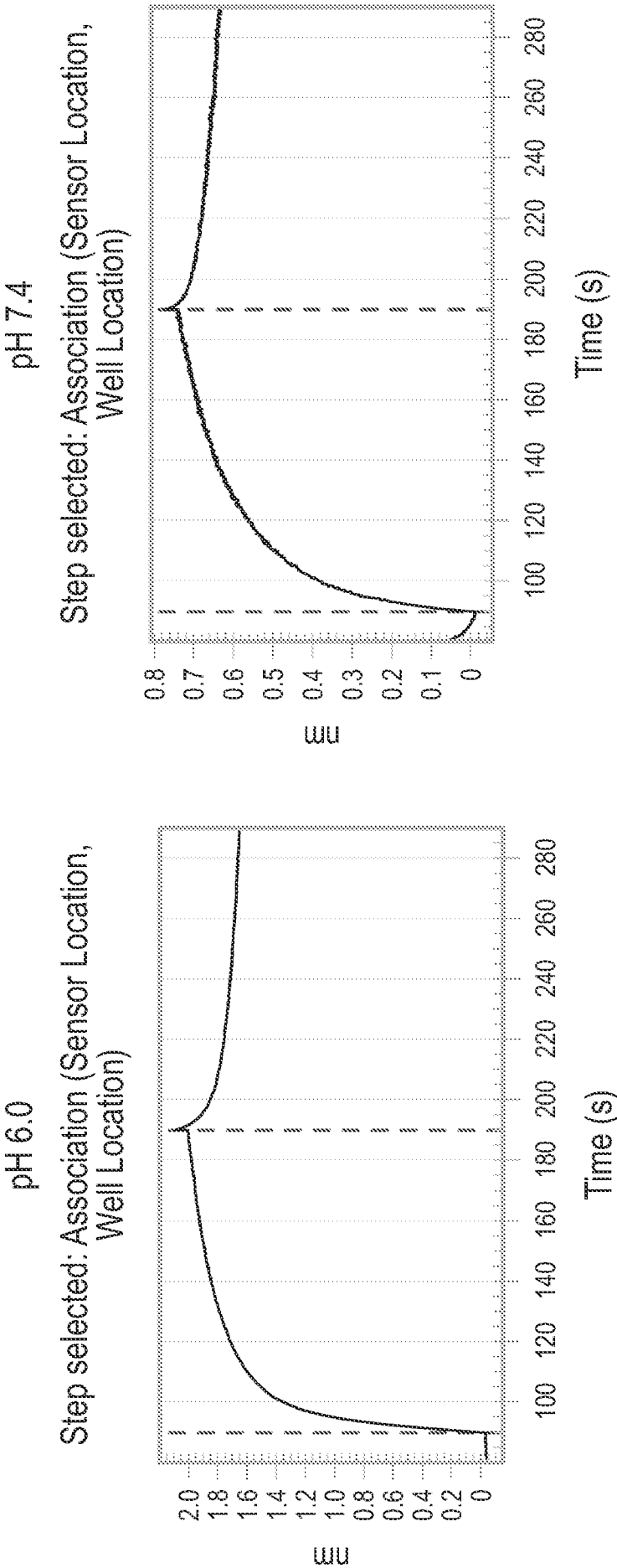


FIG. 9G

TPP-6269 binding to FcRn at pH 6.0 and pH 7.4

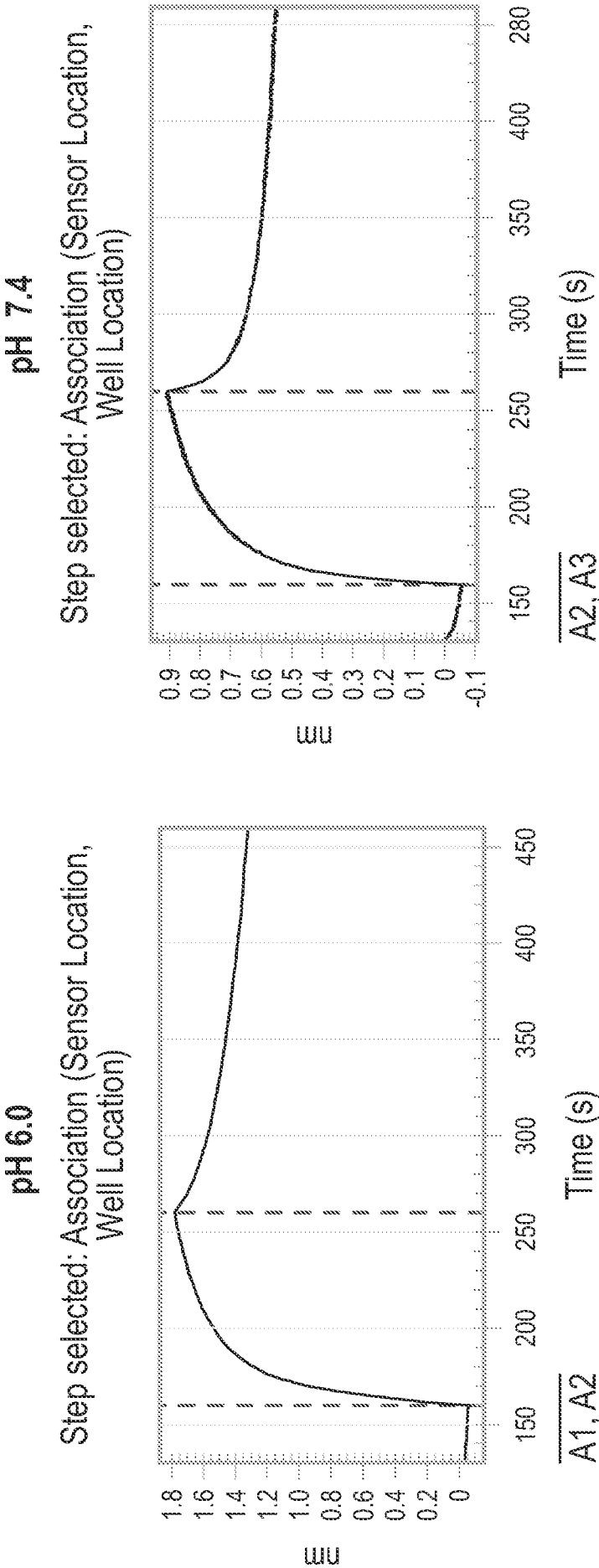


FIG. 9H

FIG. 10



Octet traces at pH 7.4 comparing single-histidine Fc variants to "WT" Fc

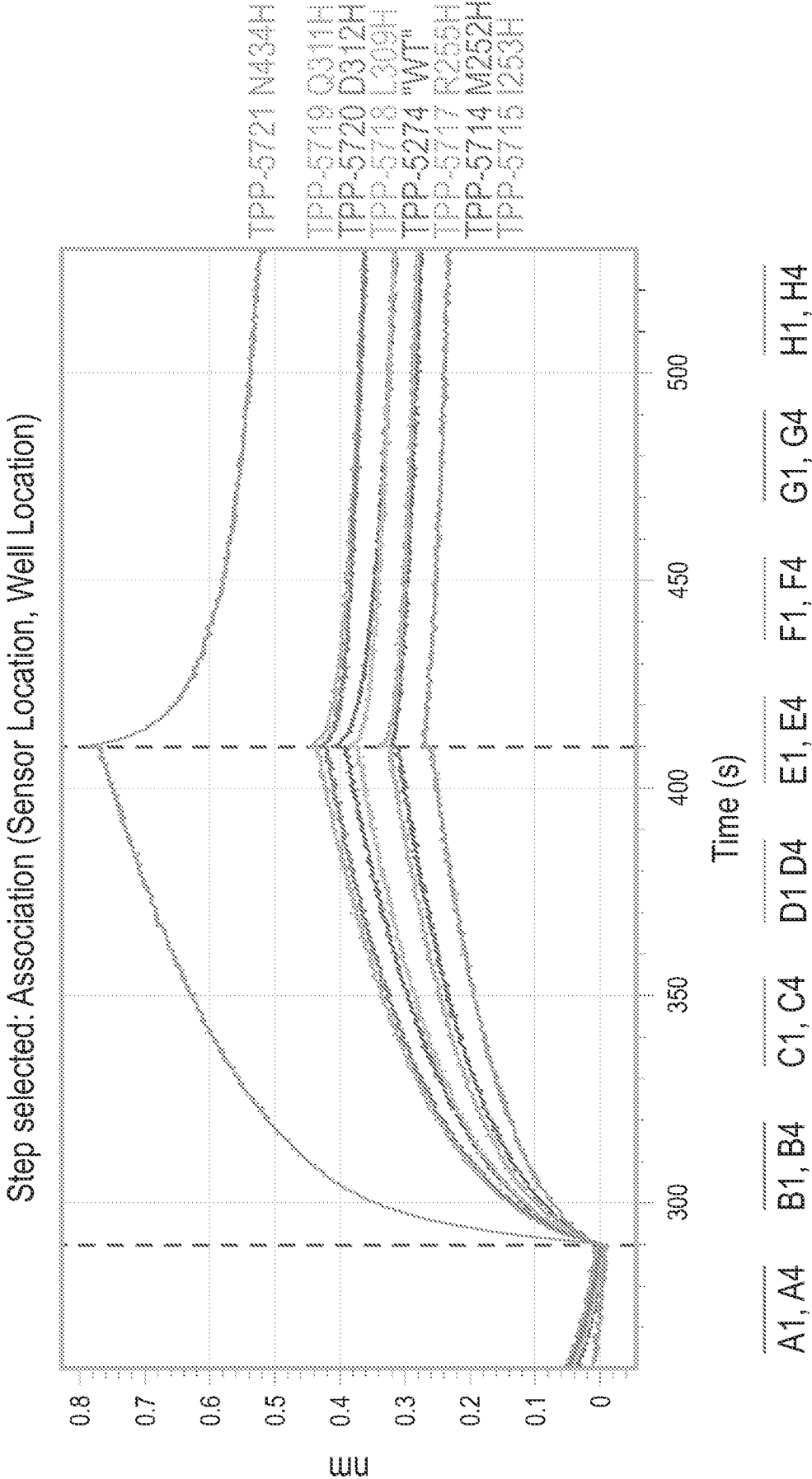


FIG. 11A

Octet traces at pH 7.4 comparing single- and double-added histidine Fc variants to "WT" Fc

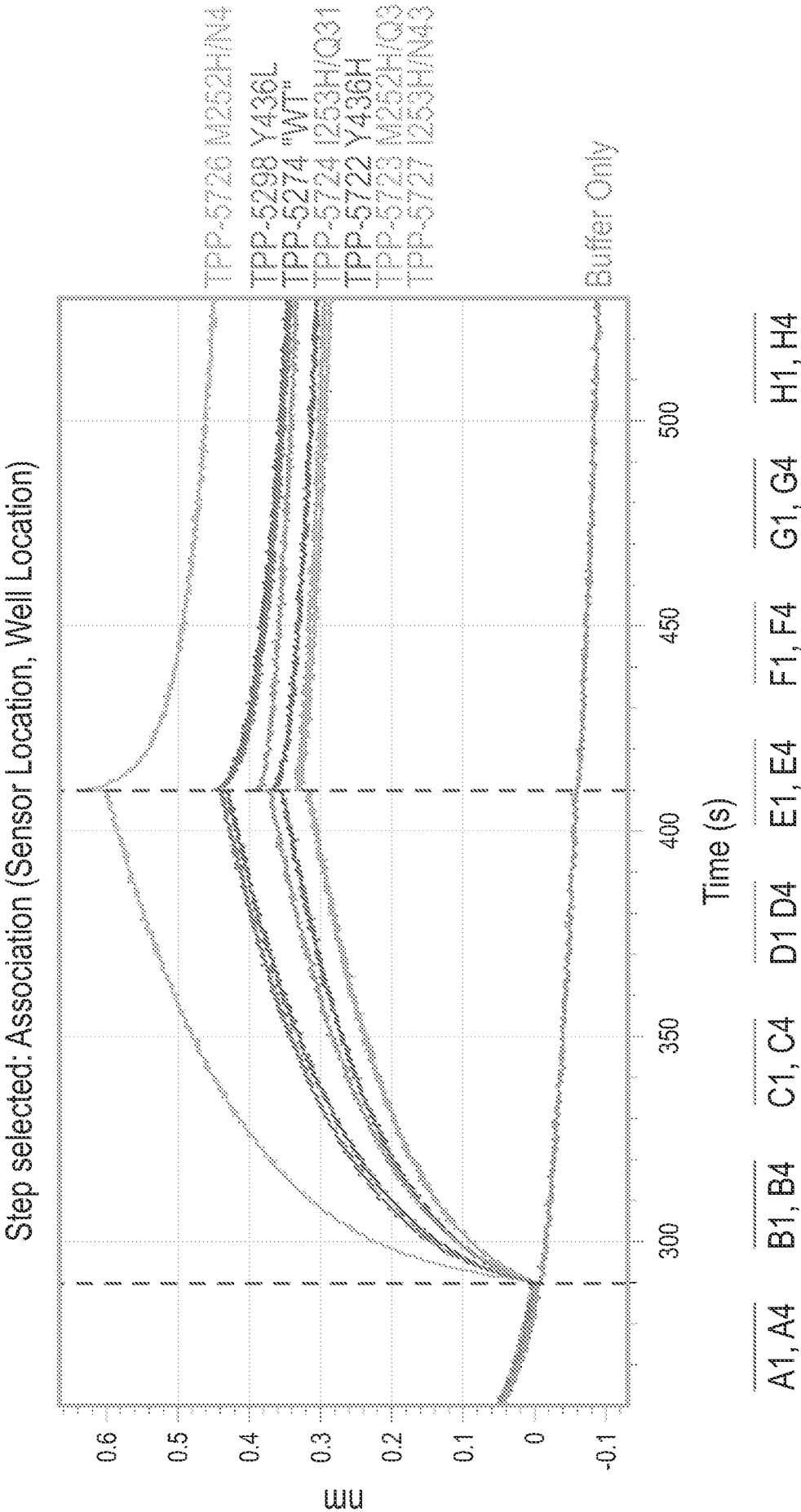


FIG. 11B

Octet traces at pH 6.0 comparing single-histidine Fc variants to "WT" Fc

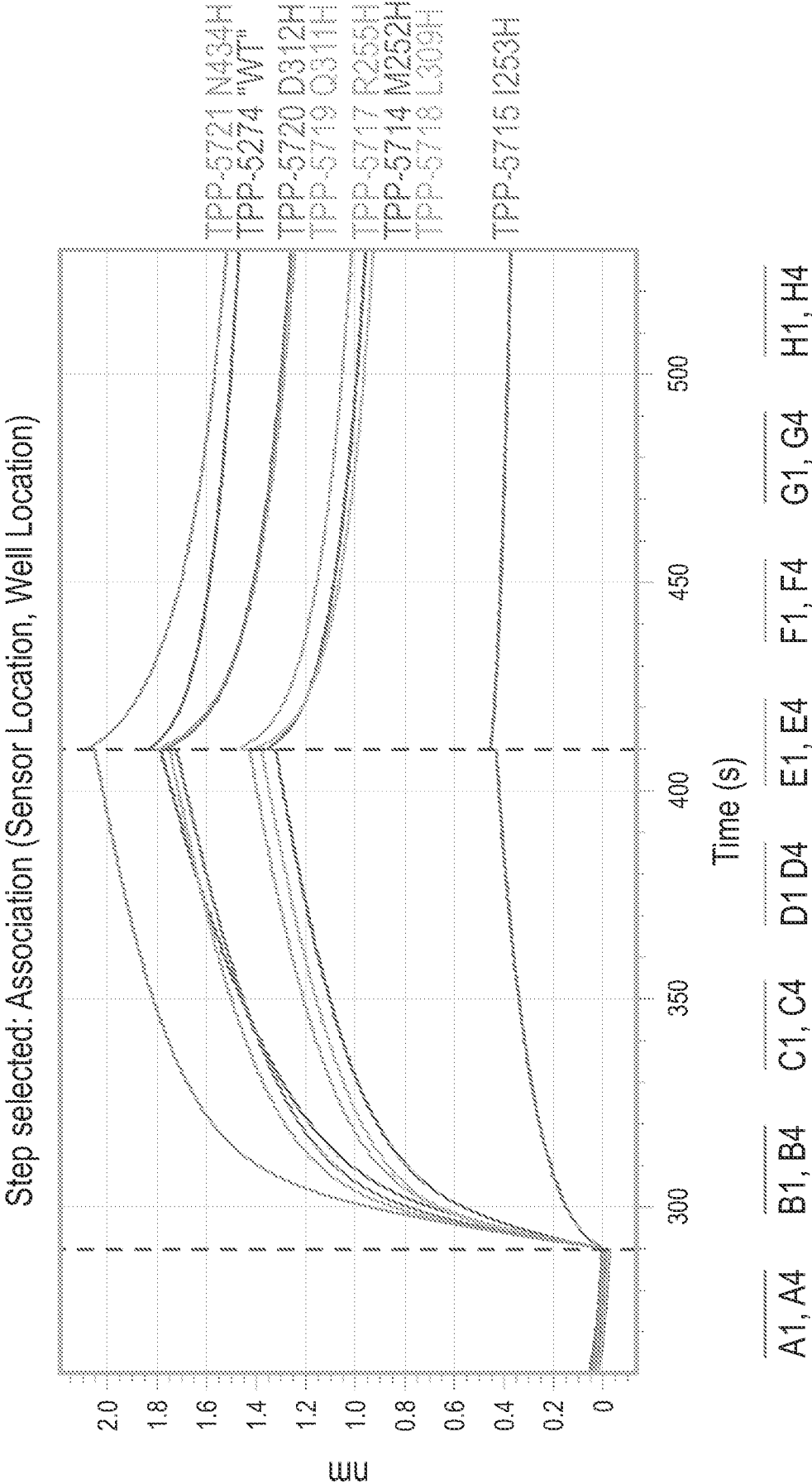


FIG. 11C

Octet traces at pH 6.0 comparing single- and double-added histidine Fc variants to "WT" Fc

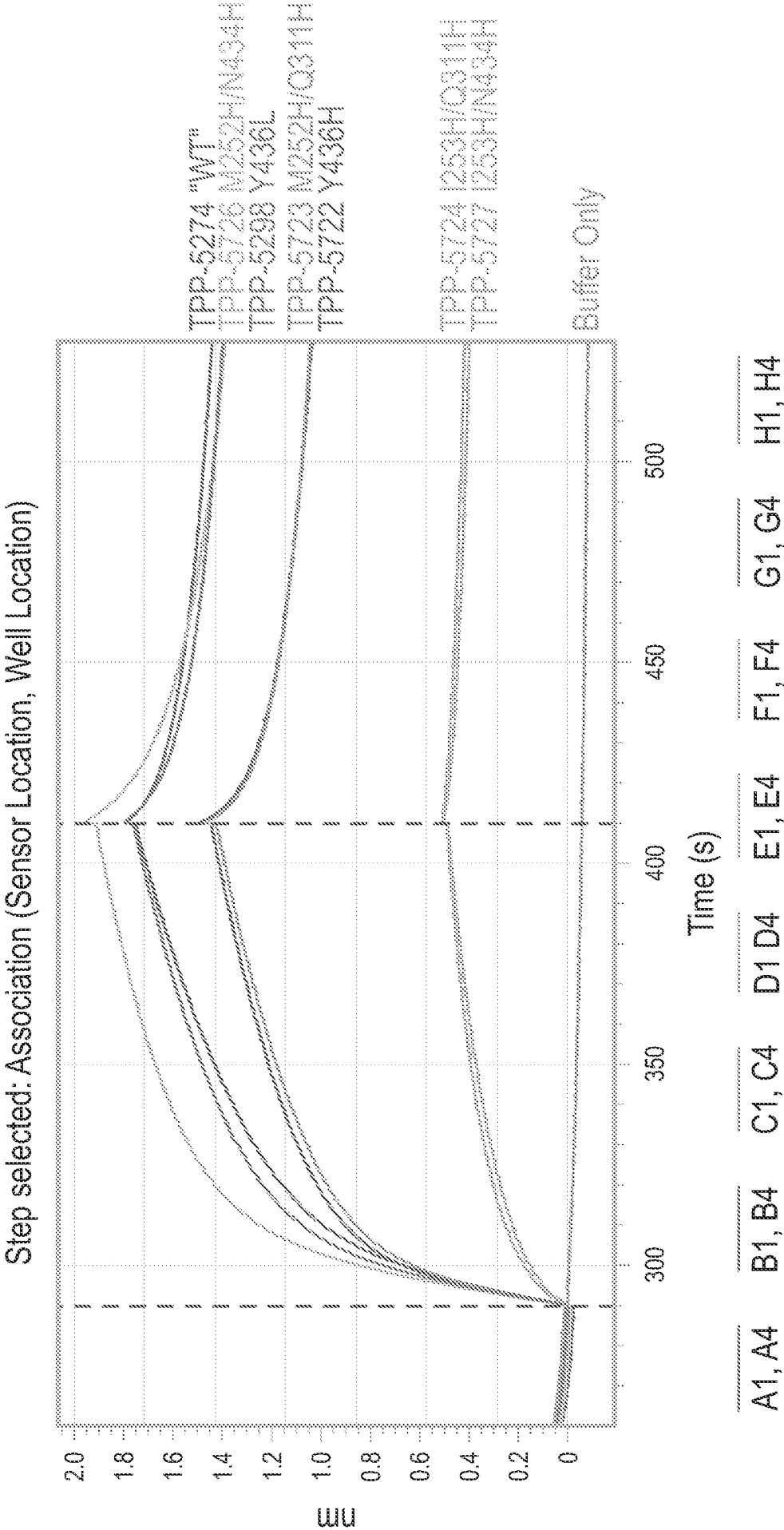


FIG. 11D

Comparative binding of TPP-5958 (L234F, L235E, M252Y, S254T, T256E, N434H) and TPP-5957 (L234F, L235E, M252Y, S254T, T256E) to FcRn at 6.0

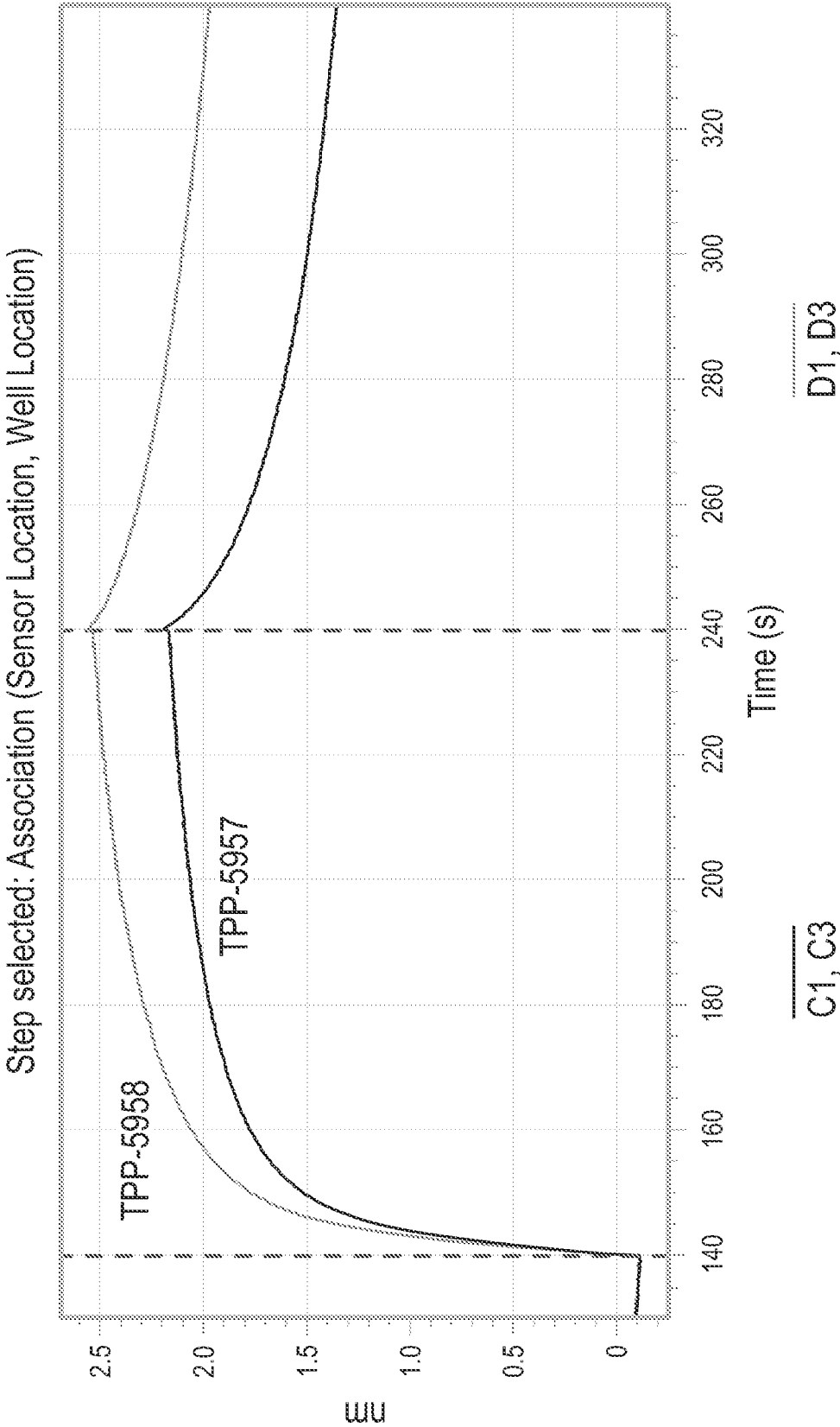


FIG. 12A

Comparative binding of TPP-5958 (L234F, L235E, M252Y, S254T, T256E, N434H) and TPP-5957 (L234F, L235E, M252Y, S254T, T256E) to FcRn at 7.4

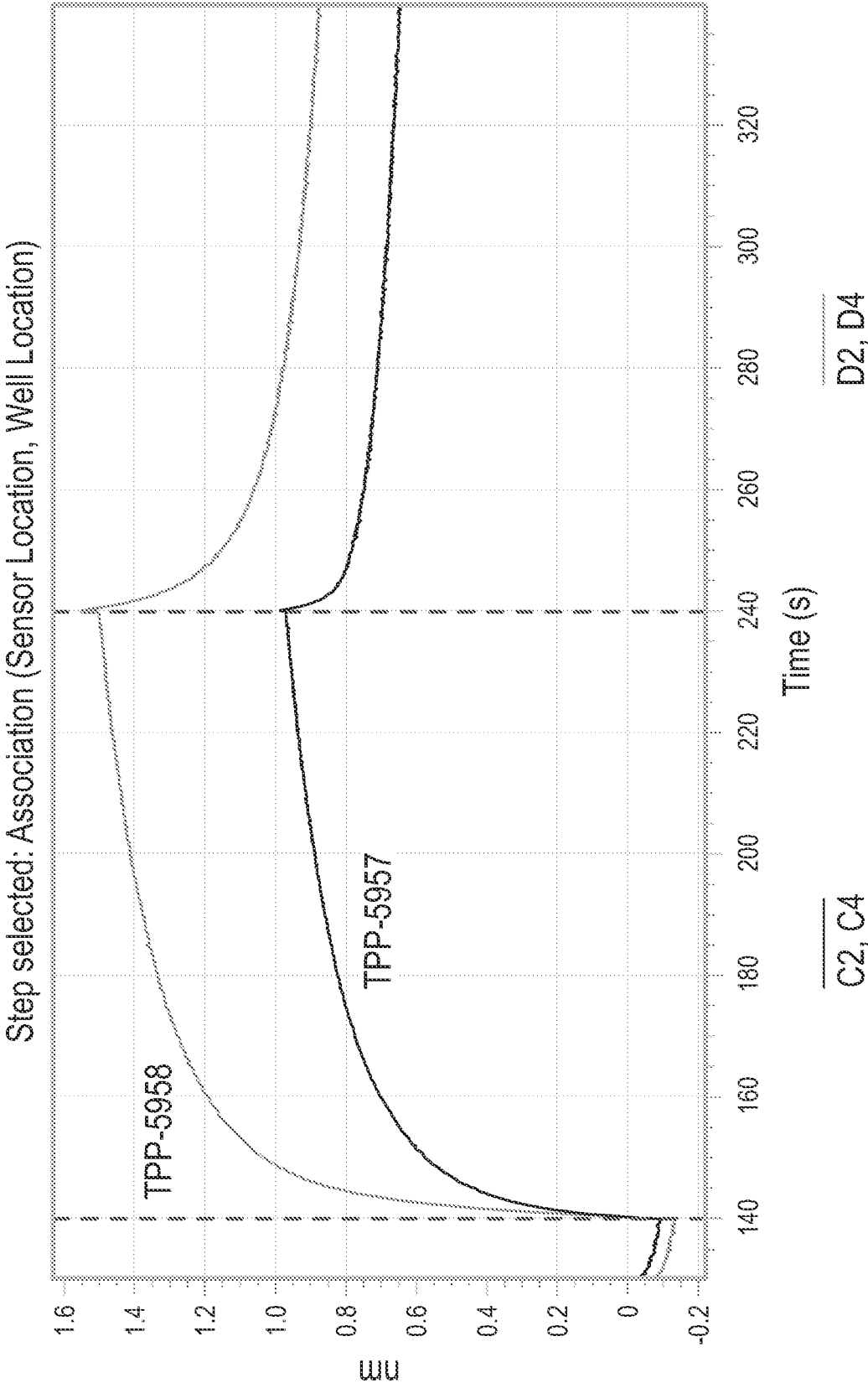


FIG. 12B

FIG. 13

FcRn binding kinetics to TPP-6269 at pH 6.0 and 7.4

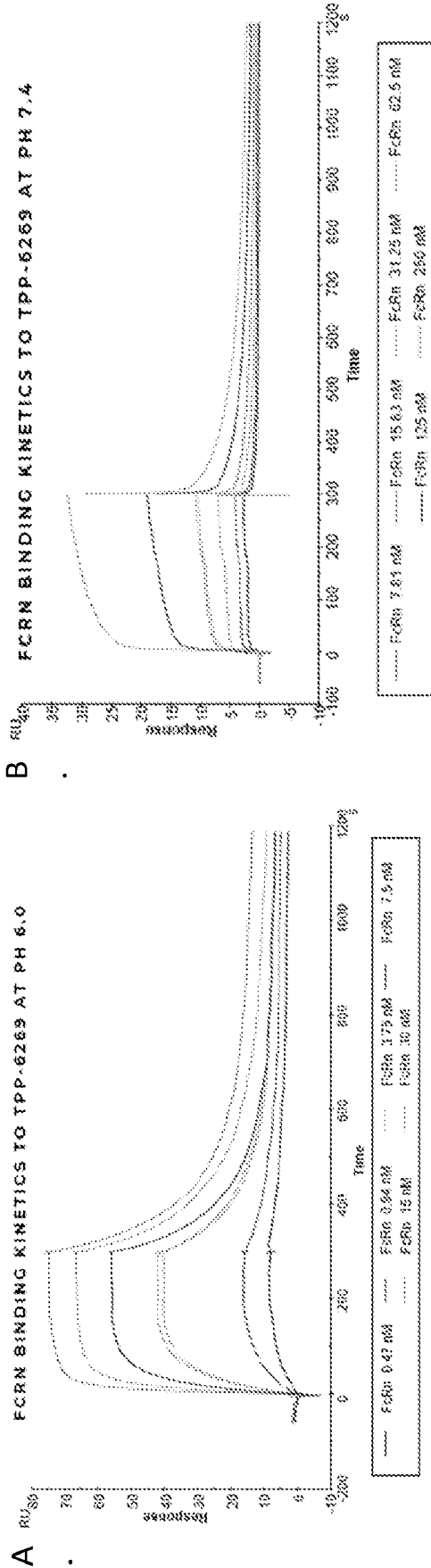


FIG. 14

Simultaneous binding of FeRn and BAFF by TACI_d2 Fe variants to CHO-hFeRn cells at pH 6.0

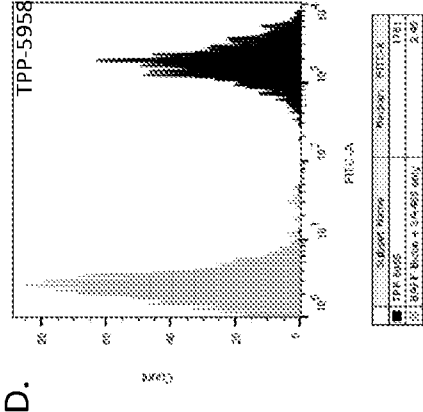
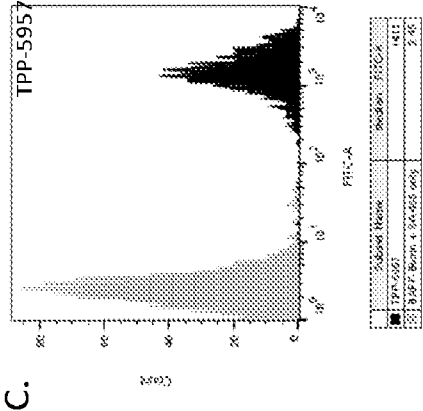
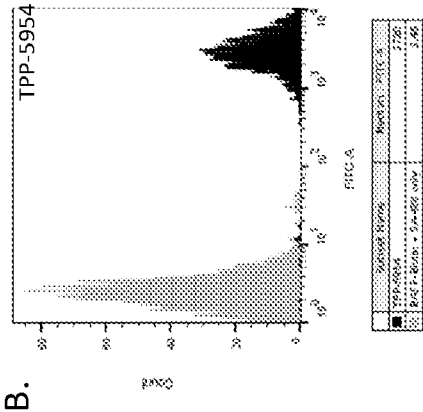
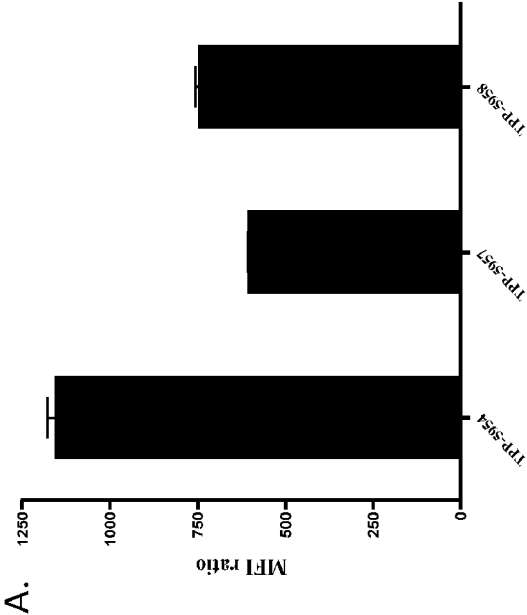


FIG. 15

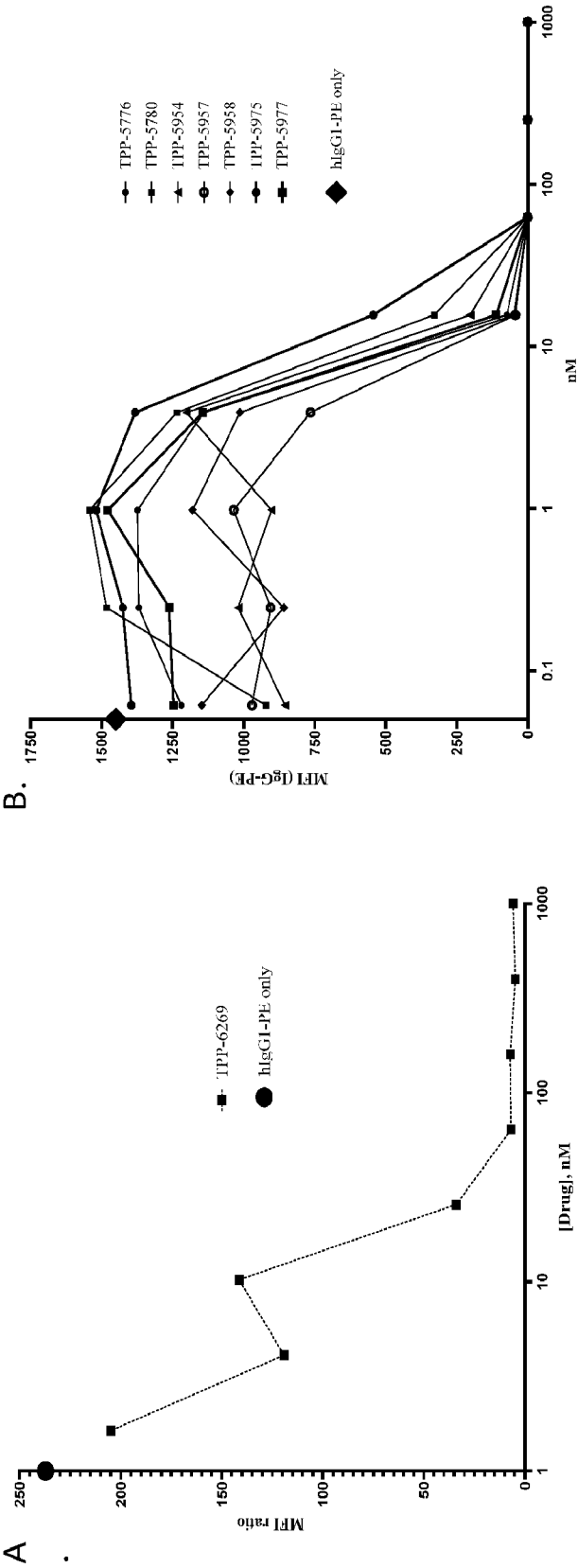
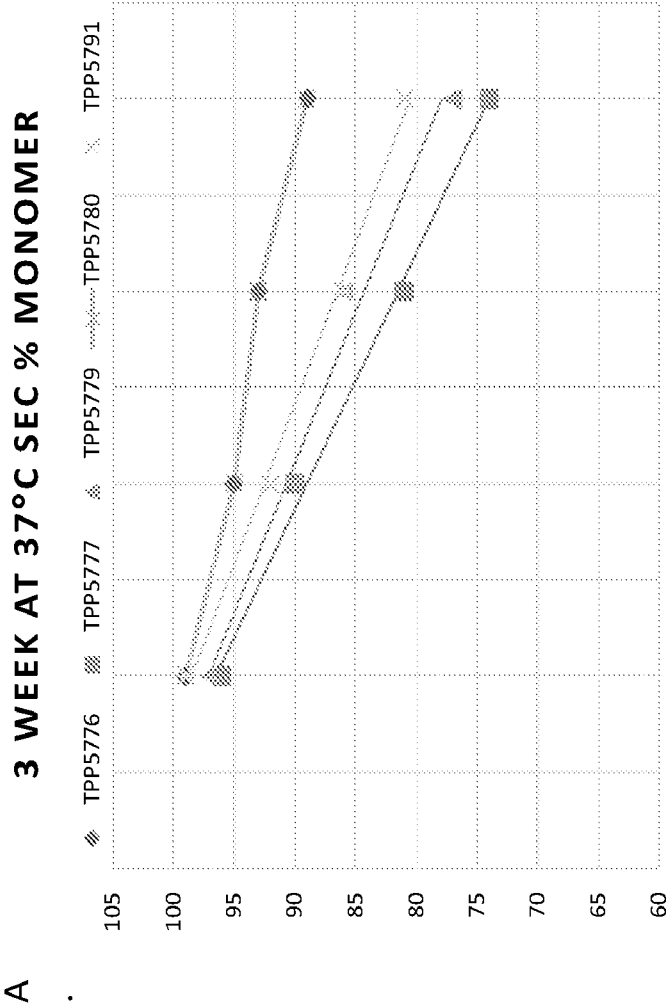


FIG. 16



B.

Variant	T0	Week 1	Week 2	Week 3
TPP-5776	99	95	93	89
TPP-5777	96	90	81	74
TPP-5779	97	90	86	77
TPP-5780	99	95	93	89
TPP-5791	99	92	86	81

FIG. 17

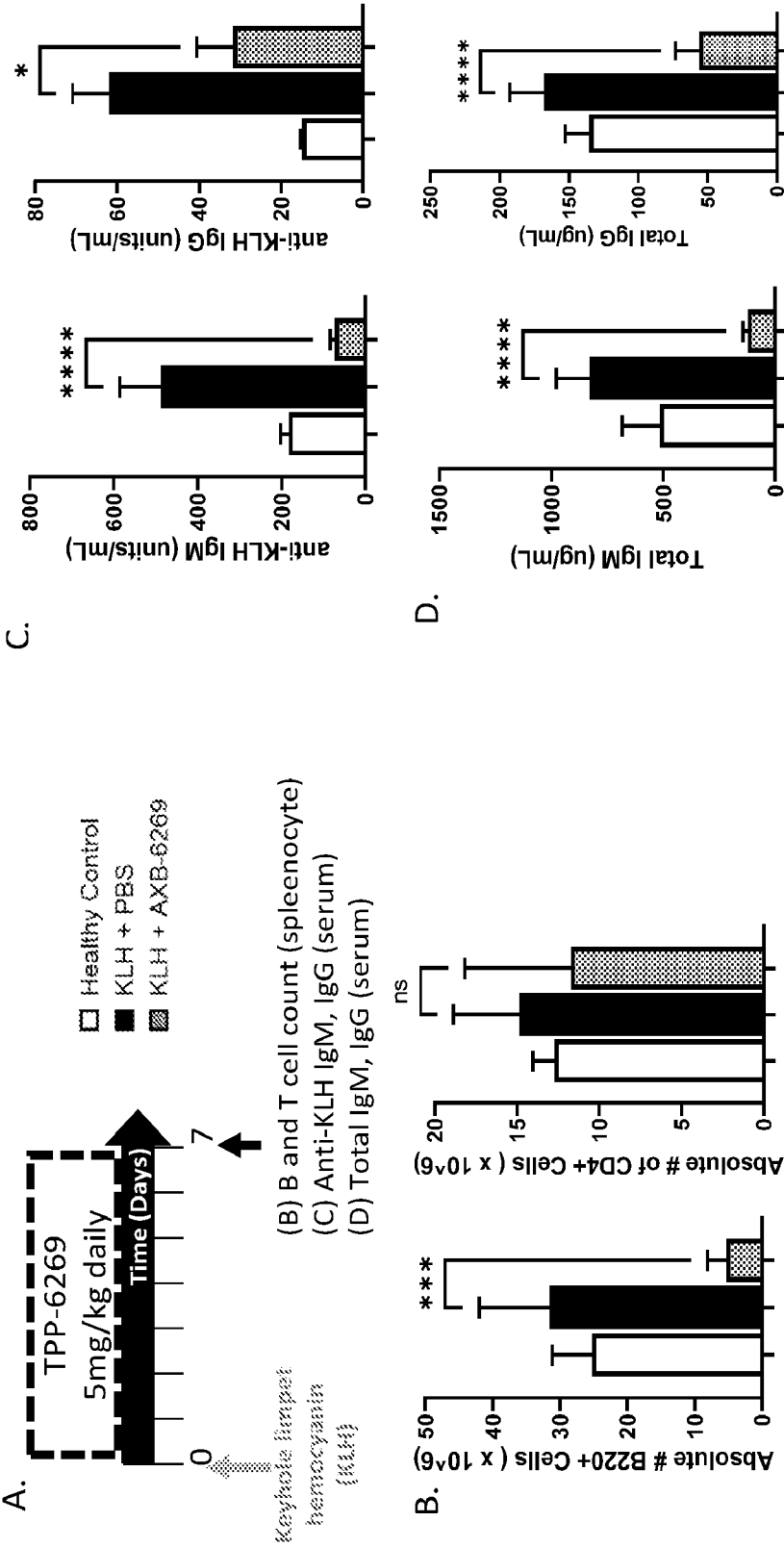


FIG. 18

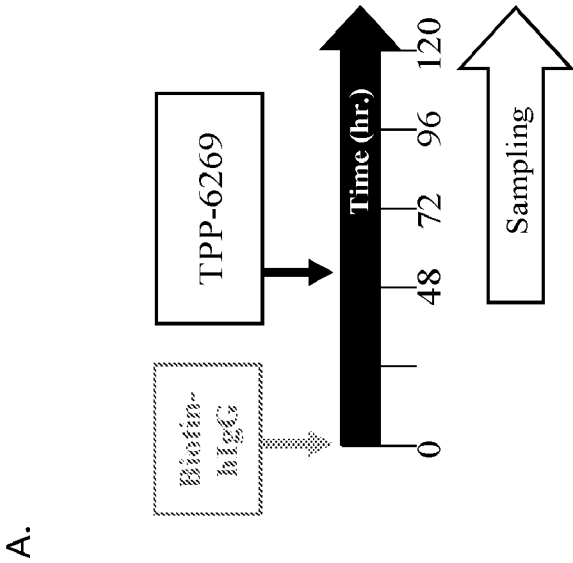
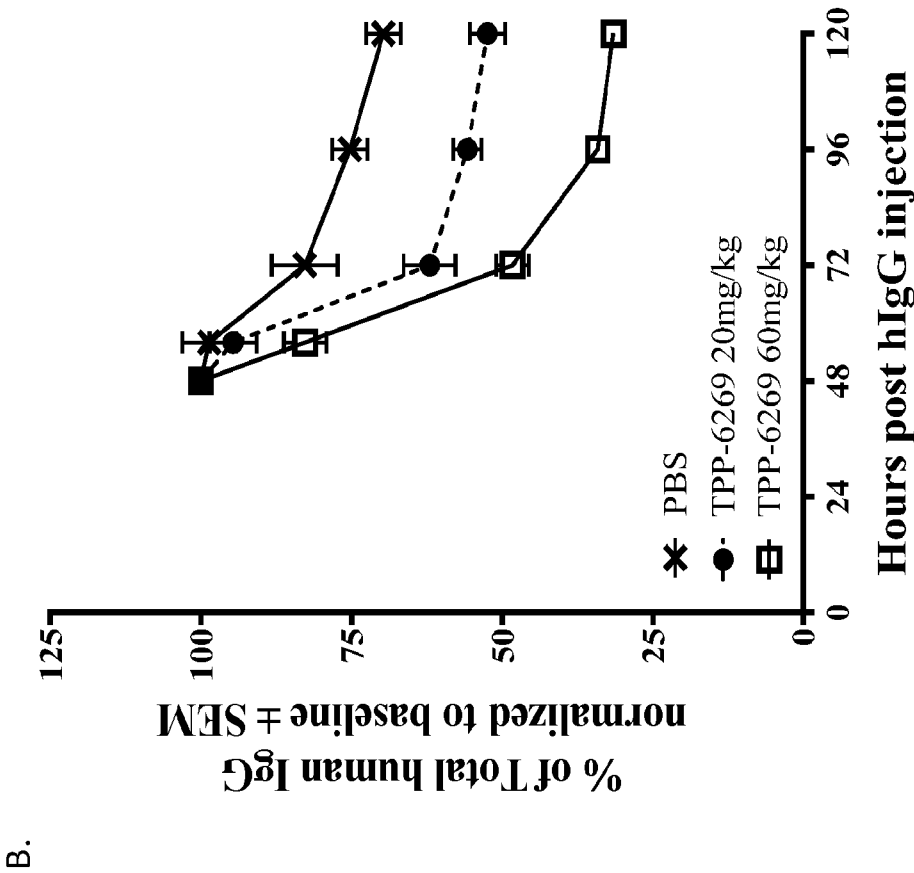


FIG. 19

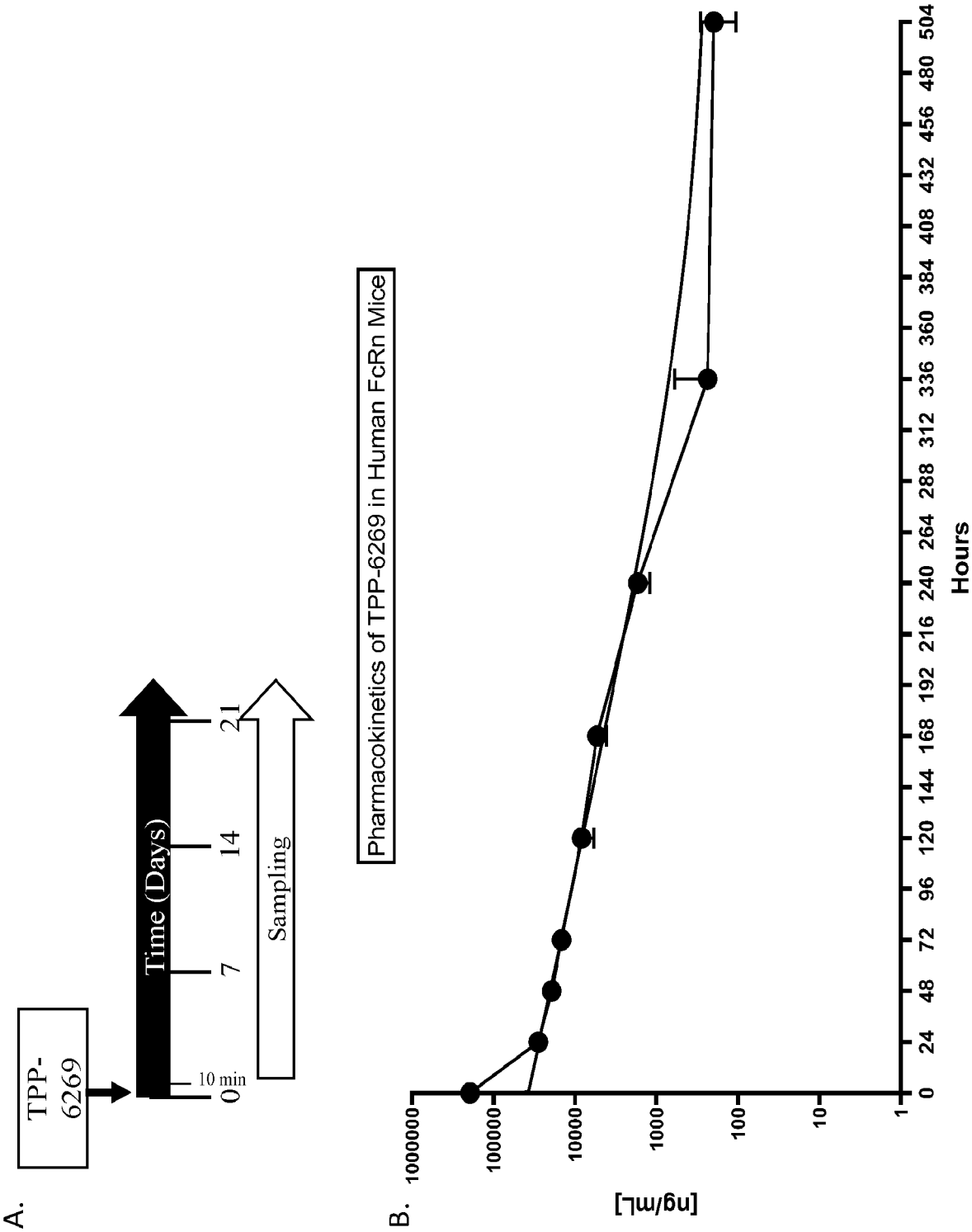


FIG. 20

