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(54) Title: CELLS FOR GLYCOENGINEERING AND METHODS OF USE

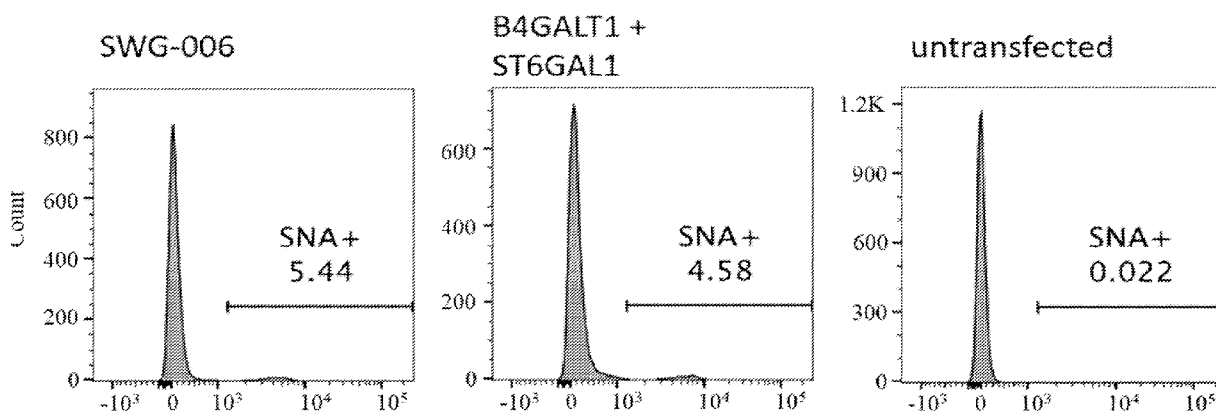


FIG. 1

(57) Abstract: The present disclosure relates to glycoengineering, including cells and methods for glycoengineering a recombinant glycoprotein, whereby the produced glycoproteins are conjugated with desired glycans.

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Cells for Glycoengineering and Methods of Use

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority of U.S. Provisional Patent Applications No. 63/639,286, filed on April 26, 2024. The entirety of the aforementioned application is incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which is submitted electronically in .xml format and is hereby incorporated by reference in its entirety. The .xml copy, created on June 17, 2024, is named "A1000-01700PCT_20240617_SeqListing.xml" and is 77 kilobytes in size.

FIELD

[0003] The present disclosure is related to compositions and methods for glycoprotein engineering and production of glycoengineered therapeutic antibodies.

BACKGROUND OF THE INVENTION

[0004] Over the past few years, protein therapeutics have gained increasing prominence in almost every field of medicine. Most of these protein therapeutics are glycoproteins, and the glycans on those glycoproteins have been proven to be essential and determinative of therapeutic efficacy. Therefore, post-translational modifications of these biologics, particularly towards their glycosylation states, have drawn increased attention in academic and industrial sectors. With the ongoing trend to produce protein therapeutics in mammalian expression platforms, there is a continuing need for platforms that offer a desired glycan profile that is favorable for improved efficacy.

BRIEF SUMMARY OF THE INVENTION

[0005] In one aspect, the present disclosure provides a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase

catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity.

[0006] In one aspect, the present disclosure provides a method for glycoengineering a recombinant glycoprotein comprising: delivering an expression vector into a cell according to an embodiment of the present disclosure, wherein the expression vector comprises a payload nucleic acid configured to encode the recombinant glycoprotein; and expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a sialylated glycan.

[0007] In one aspect, the present disclosure provides a plurality of enriched recombinant glycoproteins, wherein at least 50% of the plurality of recombinant glycoproteins is configured with a sialylated glycan.

[0008] In one aspect, the present disclosure provides a cell for expressing a GlcNAc glycoprotein, being deficient in N-acetylglucosaminyltransferase I (GnTI) activity and constitutively or controllably expressing an exogenous endoglycosidase.

[0009] In one aspect, the present disclosure provides a method for glycoengineering a recombinant glycoprotein, comprising: delivering an expression vector into a cell according to an embodiment of the present disclosure, wherein the expression vector comprises a payload nucleic acid configured to encode a recombinant glycoprotein; and expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a GlcNAc glycan.

[0010] In one aspect, the present disclosure provides a plurality of enriched recombinant glycoproteins, wherein at least 50% of the plurality of recombinant glycoproteins is configured with a GlcNAc glycan.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 is a graphic representation of a flow cytometry assay showing the exemplary results of an SNA staining assay conducted to examine the exemplary Adalimumab produced by using the exemplary CHO cell (SWG-006) of the present disclosure. A comparative cell

transfected with B4GALT1 and ST6GAL1 configured to be expressed in separate transcriptions, and an untransfected cell (i.e., a parental cell, as a negative control). In this particular exemplary embodiment, the Adalimumab antibodies produced by the exemplary cell (SWG-006) of the present disclosure had a 5.44% SNA-positive population. The Adalimumab antibodies produced by the comparative cell had a 4.58% SNA-positive population. The parental cell expressed 0.022% SNA-positive Adalimumab antibodies.

[0012] FIG. 2 is a graphic representation of a flow cytometry assay showing the results of an SNA staining assay conducted to examine the Adalimumab produced by using the exemplary SWG-006 CHO cell of the present disclosure, the exemplary SWG-015 cell of the present disclosure, and untransfected cells (i.e., a parental cell, as a negative control). The Adalimumab antibodies produced by the SWG-006 cells of the present disclosure had a 39.8% SNA-positive population. The Adalimumab antibodies produced by the SWG-015 cells of the present disclosure had a 28.5% SNA-positive population. The parental cell expressed 0.25% SNA-positive Adalimumab antibodies.

[0013] FIG. 3A is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the Adalimumab antibodies produced by an exemplary stabilized clone SAI-A3 of the SWG-006 CHO cell according to the present disclosure. The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0014] FIG. 3B is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the exemplary Adalimumab antibodies produced by an exemplary stabilized clone SAI-G1 of the SWG-006 CHO cell according to the present disclosure. The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0015] FIG. 3C is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the exemplary Adalimumab antibodies produced by an exemplary

stabilized clone SAI-D4 of the SWG-006 CHO cell according to the present disclosure. The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0016] FIG. 3D is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the exemplary Adalimumab antibodies produced by an exemplary stabilized clone SAI-F12 of the CHO SWG-006 cell according to the present disclosure. The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0017] FIG. 3E is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the exemplary Adalimumab antibodies produced by a parental CHO cell. No sialylated glycans were observed. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0018] FIG. 4A is a graphic representation of an LS/MS-MS spectrum conducted to investigate the glycoforms of a commercial product of Adalimumab (Humira[®]). No sialylated glycans were observed. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0019] FIG. 4B is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the Adalimumab antibodies produced by a parental CHO cell. No sialylated glycans were observed. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0020] FIG. 4C is a graphic representation of an LS/MS-MS spectrum conducted to demonstrate the glycoforms of the Adalimumab antibodies produced by an exemplary stabilized clone SAI-A3 of the SWG-006 CHO cell according to the present disclosure. The sialylated

glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0021] FIG. 5A is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the glycoforms of the chMC81370 antibodies produced by a parental HEK293T cell. No sialylated glycans were observed. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0022] FIG. 5B is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to demonstrate the glycoforms of the chMC81370 antibodies produced by an exemplary HEK293 cell according to the present disclosure. The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0023] FIG. 6 is a graphic representation of the data of a glycoform analysis conducted to investigate the chMC81370 antibodies produced by a wild-type cell (i.e. a parental cell) and an exemplary HEK293 cell according to the present disclosure (an “engineered” cell). Left: percentage of fucosylated glycan; Middle: percentage of galactosylated glycan; Right: percentage of sialylated glycan.

[0024] FIG. 7 is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to demonstrate the glycoforms of the chMC81370 antibodies produced by an exemplary HEK293 cell according to the present disclosure, untreated(top), treated with *Streptococcus pneumoniae* α 2-3 neuraminidase (middle), or treated with *Clostridium perfringens* neuraminidase (bottom). The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0025] FIG. 8 is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to demonstrate the glycoforms of the chMC81370 antibodies produced by a comparative cell transfected with a hB4GalT1 gene but not hST6GAL1 gene. The data shows that the antibodies had increased galactosylation generated from cells with knock-in of hB4GalT1 only but did not show terminal sialylation. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0026] FIG. 9A is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the changes in glycoforms of the chMC81370 antibodies produced by an exemplary HEK293 cell according to the present disclosure over five days. Top: Day 0 to Day 3 (samples collected on Day 3); Bottom: Day 4 to Day 5 (samples collected on Day 5). The sialylated glycans are labeled with solid arrows. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0027] FIG. 9B is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the changes in glycoforms of the chMC81370 antibodies produced by a comparative FUT8 knock-out cell over five days. Top: Day 0 to Day 3 (samples collected on Day 3); Bottom: Day 4 to Day 5 (samples collected on Day 5). The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0028] FIG. 10 is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the changes in glycoforms of the chMC81370 antibodies produced by an exemplary HEK293 cell according to the present disclosure over three days. Samples were collected on Day 1 (Top), Day 2 (Middle), and Day 3 (Bottom) respectively. The Diamond shape represents sialic acid; the open circle shape represents galactose; the square shape represents GlcNAc; the solid circle shape represents mannose; the triangle shape represents fucose.

[0029] FIG. 11 is a graphic representation of ELISA data showing the binding affinity of the antibodies produced by a wild-type cell (i.e. a parental cell) and an exemplary HEK293 cell according to the present disclosure (an “SCT-enriched” cell) to FcγIIA receptors (Left), FcγIIB receptors (Middle), and FcγIIIA receptors (Right). The binding of antibody was measured by anti-human IgG Fc antibody conjugated with HRP.

[0030] FIG. 12A presents a result of protein electrophoresis showing that the chMC18370 antibodies expressed by an exemplary HEK293 cell according to the present disclosure (Endo H KI “+”) and a parental GnTI-KO EXPI293 cell (Endo H LI “-“) are different in molecular weight as band shifting was observed.

[0031] FIG. 12B presents a result of a transglycosylation assay showing that the chMC18370 antibodies expressed by an exemplary Endo H knock-in cell, according to the present disclosure, had a high efficiency of transglycosylation. Transglycosylation was observed within 15 minutes.

[0032] FIG. 12C is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the glycoforms of the chMC81370 antibodies produced by a parental cell (GnTI-KO EXPI293 cell). The square shape represents GlcNAc, and the solid circle shape represents mannose.

[0033] FIG. 12D is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the glycoforms of the chMC81370 antibodies produced by an exemplary Endo H knock-in cell according to the present disclosure. The square shape represents GlcNAc, and the solid circle shape represents mannose.

[0034] FIG. 12E is a graphic representation of the data of a glycoform analysis conducted to investigate the chMC81370 antibodies produced by a GnTI-KO EXPI293 cell, an exemplary Endo H knock-in according to the present disclosure, and an exemplary Endo S2 knock-in cell according to the present disclosure. Left: percentage of mannose type glycan; Right: percentage of mono-GlcNAc glycan.

[0035] FIG. 13A presents a result of protein electrophoresis showing that the chMC18370 antibodies expressed by an exemplary HEK293 cell according to the present disclosure (Endo S2 KI “+”) and a parental GnTI-KO EXPI293 cell (Endo S2 KI “-“) are different in molecular weight as band shifting was observed.

[0036] FIG. 13B presents a result of a transglycosylation assay showing that the chMC18370 antibodies expressed by an exemplary Endo S2 knock-in cell, according to the present disclosure, had a high efficiency of transglycosylation. Transglycosylation was observed within 15 minutes.

[0037] FIG. 13C is a graphic representation of the data of an intact protein mass (IPM) analysis conducted to investigate the glycoforms of the chMC81370 antibodies produced by an exemplary Endo S2 knock-in cell according to the present disclosure. The square shape represents GlcNAc.

[0038] FIG. 14A is a graphic representation of ELISA data showing the binding affinity of the antibodies produced by an exemplary HEK293 cell according to the present disclosure (“SCT-enriched”), and an exemplary Endo H knock-in cell with a transglycosylation according to the present disclosure (“SCT homogeneous”).

[0039] FIG. 14B is a graphic representation of the data of an ADCC reporter assay showing the comparison between the antibodies produced by a wild-type cell (i.e. a parental cell), an exemplary HEK293 cell according to the present disclosure (“SCT-enriched”), and an exemplary Endo H knock-in cell with a transglycosylation according to the present disclosure (“SCT homogeneous”).

DETAILED DESCRIPTION OF THE INVENTION

[0040] Glycosylation is a common post- or co-translational modification found on most cell proteins, especially surface proteins. The importance of a glycan profile (i.e., glycoform) of a glycoprotein and the impact on therapeutic effects has been recognized. Taking therapeutic antibodies as an example, binding between an antibody and a target cell or a pathogen triggers a variety of downstream immune functions, including phagocytosis, cellular cytotoxicity, vaccinal

effect, complement activation, etc. These immune cell-based responses require the binding of the antibody Fc domain to specific Fc receptors on immune cells, in which the glycoform of the antibody is believed to be critical. For instance, it is believed that the IgG N-glycosylation at N297 on the constant region of the heavy chain is critical in the binding between the IgG and the FcγIIIA receptor on NK cells that results in the activation of antibody-dependent cellular cytotoxicity (ADCC). Therefore, it is important to engineer and obtain an optimized glycoform to improve the efficacy and safety of therapeutic glycoproteins.

[0041] Conventional mammalian cell lines used for glycoprotein production usually produce a mixture of glycoforms with core-fucosylated bi-antennary complex-type glycans, which are not optimal for binding an Fcγ receptor because of the inhibitory function of core-fucosylation or the off-target delivery caused by terminal galactosylation. In comparison, an α2-6 linked sialyl complex type (SCT) glycan provides enhanced binding to FcγIIIA receptors and FcγIIA receptors, which are associated with ADCC, antibody-dependent cellular phagocytosis (ADCP), and vaccinal effect. In addition, mono-GlcNAc (N-Acetylglucosamine) and GlcNAc-Fuc-bearing glycoforms are good acceptor substrates for endoglycosidase (glycosynthase)-mediated transglycosylation and are, therefore, beneficial for engineering a desired glycan chain. Accordingly, efforts must be employed to modify the glycoforms.

[0042] **CELL**

[0043] *Cells for expressing a sialylated glycoprotein*

[0044] One aspect of the present disclosure provides a cell for expressing a sialylated glycoprotein, which expresses constitutively and/or controllably an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity. As used herein, “**translated in close proximity**” describes the translation event of the exogenous sialyltransferase catalytic peptide and the translation event of the transcription of the exogenous galactosyltransferase catalytic peptide happen closely to each other both temporally and spatially.

[0045] In some embodiments, the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are expressed in a single transcript. As used herein, “**in a single transcript**” means the transcription of the exogenous sialyltransferase catalytic peptide and the transcription of the exogenous galactosyltransferase catalytic peptide are performed transcriptionally under the same promoter or the DNA encoding the exogenous sialyltransferase catalytic peptide and the DNA encoding the exogenous galactosyltransferase catalytic peptide are transcribed into a single mRNA molecule.

[0046] The present disclosure contemplates that translating the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide in proximity to each other or expressing the two peptides in a single transcription provides higher sialylation to the glycoprotein. Without wishing to be bound by any theories, since the catalytic reaction exerted by a galactosyltransferase generates a galactosylated glycan, which is the substrate of a sialyltransferase, translating the two enzymes in proximity or expressing them in a single transcription ensures the two catalytic reactions happen closely or nearly simultaneously, thereby higher sialylation efficiency.

[0047] *Ribosomal shifting approach.* In some embodiments, the cell of the present disclosure comprises a first nucleic acid configured to express the exogenous sialyltransferase catalytic peptide; and a second nucleic acid configured to express the exogenous galactosyltransferase catalytic peptide, wherein the first nucleic acid and the second nucleic acid are controlled under the same promoter. In some embodiments, the first nucleic acid and the second nucleic acid are connected to each other via a connecting nucleic acid, which is configured to encode a ribosomal shifting peptide. A ribosomal shifting peptide is configured to render ribosomal skipping, where the ribosomal shifting peptide prevents the ribosome from covalently linking a newly inserted amino acid while continuing the translation, resulting in co-translational cleavage of a polyprotein into separate peptides/proteins. In some embodiments, the ribosomal shifting peptide comprises an amino acid sequence of DxExNPGP, wherein x denotes any amino acid, D denotes aspartic acid, E denotes glutamic acid, N denotes asparagine, P denotes proline, and G denotes glycine. In certain embodiments, the ribosomal shifting peptide comprises an amino acid sequence as set forth in SEQ ID NO: 06, SEQ ID NO: 07, SEQ ID NO: 08, or SEQ

ID NO: 09. In certain embodiments, the connecting nucleic acid comprises a sequence as set forth in SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.

[0048] *Fusion protein approach.* Alternatively, the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are, in some embodiments, configured to be expressed into a fusion protein. In some embodiments, the fusion protein comprises a first portion having the sialyltransferase catalytic peptide and a second portion having the galactosyltransferase catalytic peptide, wherein the first portion and the second portion are connected to each other via a cleavable linker, and the cleavable linker is configured to be cleavable post-translation of the fusion protein, thereby upon cleavage releasing the sialyltransferase catalytic peptide and the galactosyltransferase catalytic peptide as separate proteins.

[0049] In some embodiments, the sialyltransferase catalytic peptide is an alpha-2,6-sialyltransferase. In some embodiments, the sialyltransferase is a beta-galactoside alpha-2,6-sialyltransferase 1. In certain embodiments, the sialyltransferase catalytic peptide comprises a product of an ST6Gal1 gene or a PspST gene. In certain embodiments, the sialyltransferase catalytic peptide comprises an amino acid sequence as the sequence set forth in SEQ ID NO: 01 or SEQ ID NO: 02. Yet in certain embodiments, the sialyltransferase catalytic peptide comprises an amino acid sequence as set forth in SEQ ID NO: 03 or SEQ ID NO: 04.

[0050] In some embodiments, the galactosyltransferase catalytic peptide is a beta-1,4-galactosyltransferase 1. In certain embodiments, the galactosyltransferase catalytic peptide comprises a product of a B4GALT1 gene. In certain embodiments, the galactosyltransferase catalytic peptide comprises an amino acid sequence as set forth in SEQ ID NO: 05.

[0051] In some embodiments, the cell comprises a first nucleic acid configured to express the exogenous sialyltransferase catalytic peptide, wherein the first nucleic acid is derived from an ST6Gal1 gene or a PspST gene. As used herein, “derived from” describes that the first nucleic acid comprises a nucleotide sequence exactly the same as a reference gene; for example, the first nucleic acid might comprise a nucleotide sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 11. In some other situations, the “derived from” describes that the first nucleic acid

comprises a nucleotide sequence modified from the reference gene, provided that the product of the modified nucleotide sequence exerts the same or similar catalytic functionality as the product of the reference gene. The modification might be made for accommodating codon usage or optimizing transcription/translation efficiency or accuracy in a host cell. In other examples, the modification adds a signal peptide directing the gene product to a certain place within or out of the cells. For example, the first nucleic acid can be derived from a PspST gene that the first nucleic acid comprises a nucleotide sequence encoding a product of the PspST gene and a nucleotide sequence encoding a signal peptide that directs the gene product to the Golgi body where glycosylation takes place. The signal peptide, in certain embodiments, can be the signal peptide of an ST6Gal1 gene or a B4GALT1 gene. In such embodiments, the first nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 12 or SEQ ID NO: 13.

[0052] In some embodiments, the cell comprises a second nucleic acid configured to express the exogenous galactosyltransferase catalytic peptide, wherein the second nucleic acid is derived from a B4GALT1 gene. In certain embodiments, the second nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 14.

[0053] In some embodiments, the first nucleic acid and the second nucleic acid are transcriptionally controlled under the same promoter, which can be a constitutive promoter or an activable promoter. The constitutive promoter can be but is not limited to a CMV promoter, a T7 promoter, a Human Elongation Factor 1 Alpha (EF1 α) promoter, a Chicken β -Actin (CAG) promoter, or an SV40 promoter. The activable promoter provides a controllable expression of the exogenous sialyltransferase and the exogenous galactosyltransferase. The activable promoter can be but is not limited to a Tetracycline-Inducible Promoter (activable by doxycycline) or a dihydrofolate reductase (DHFR) gene promoter (used to select and amplify gene expression).

[0054] *Parental cell.* In some embodiments, the cell of the present disclosure is derived from a parental cell (e.g., a wild-type cell), which can be a mammalian cell. In some embodiments, the parental cell does not have an endogenous sialyltransferase and/or an endogenous galactosyltransferase. In some other embodiments, the parental cell might have an endogenous sialyltransferase and/or an endogenous galactosyltransferase. Nevertheless, the present disclosure surprisingly discovers that overexpression of the endogenous sialyltransferase

and/or the endogenous galactosyltransferase does not increase the sialylation as expressing exogenous ones can achieve. In some embodiments, the parental cell and/or the cell of the present disclosure are deficient in fucosyltransferase 8 activity. In certain embodiments, the parental cell and/or the cell of the present disclosure are deficient in a FUT8 gene encoding a product of the fucosyltransferase activity. In some embodiments, the parental cell and/or the cell of the present disclosure are or are derived from a Chinese hamster ovary (CHO) cell or a HEK293 cell. The CHO cell can be but is not limited to ExpiCHO, CHO-K1, CHO-C, CHOZN, and CHOK1Q. In some embodiments, “cell” used herein is interchangeably with “cell line.”

[0055] *Cells for expressing a recombinant glycoprotein.* In some embodiments, the cell further comprises a payload nucleic acid configured to encode a recombinant glycoprotein, and the expression of the payload nucleic acid is transcriptionally controlled by a constitutive or an activable promoter. The constitutive promoter and the activable promoter are as described above and herein. In some embodiments, the recombinant glycoprotein expressed or produced by the cell is conjugated with a glycan. In certain embodiments, the glycan can be an N-linked glycan (i.e., N-glycan) or an O-linked glycan (i.e., O-glycan).

[0056] *Glycan and glycoprotein.* The cell of the present disclosure is capable of expressing a sialylated glycoprotein. In some embodiments, the sialylated glycoprotein comprises a sialyl complex type (SCT) glycan, which can be conjugated to the protein via a lysine residue (i.e., O-glycan) or an asparagine residue (i.e., N-glycan). In some embodiments, the cell of the present disclosure is configured to express an SCT-enriched protein. As used herein, “SCT-enriched” describes that the proteins expressed by the cell of the present disclosure have a higher SCT glycan percentage compared to the same proteins expressed by a parental cell used to produce the cells of the present disclosure. In some embodiments, the SCT glycan percentage is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500% higher than that of the proteins expressed by a parental cell, or any range defined by the foregoing endpoints, such as 10% to 500%, 10% to 400%, 10% to 300%, 10% to 200%, 10% to 100%, 10% to 90%, 10% to 80%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 30%, 10% to 20%, 30% to 500%, 30% to 400%, 30% to 300%, 30% to 200%, 30% to 100%, 30% to 90%, 30% to 80%, 30% to 70%, 30% to 60%, 30% to 50%, or 30% to 40%. In certain

embodiments, the SCT glycan percentage is enriched to 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 times the SCT glycan percentage of the proteins expressed by a parental cell, or any range defined by the foregoing endpoints, such as 1.2 to 10 times, 1.2 to 9 times, 1.2 to 8 times, 1.2 to 7 times, 1.2 to 6 times, 1.2 to 5 times, 1.2 to 4 times, 1.2 to 3 times, 1.2 to 2 times, 2 to 10 times, 2 to 9 times, 2 to 8 times, 2 to 7 times, 2 to 6 times, 2 to 5 times, 2 to 4 times, 2 to 3 times, 5 to 10 times, 5 to 9 times, 5 to 8 times, 5 to 7 times, or 5 to 6 times. In certain embodiments, the glycan is a mono-antennary or bi-antennary α 2-6 sialyl complex type (SCT) glycan. In some embodiments, the glycan is a galactose-rich SCT glycan with or without core-fucose. In some other embodiments, the glycan is a fully galactosylated SCT glycan with or without core-fucose.

[0057] In certain embodiments, the glycoprotein (e.g., a recombinant glycoprotein) is an antibody or an antigen-binding fragment thereof. A basic antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. Each L chain is linked to an H chain by at least one (and typically one) covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site.

[0058] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains (C_L). Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha (α), delta (δ), epsilon (ϵ), gamma (γ), and mu (μ), respectively. The γ and α classes are

further divided into subclasses on the basis of relatively minor differences in CH sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. It will be appreciated that mammals encoding multiple Ig isotypes will be able to undergo isotype class switching.

[0059] An “antibody fragment” or “antigen-binding fragment of an antibody” is a polypeptide comprising or consisting of a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include an F(ab')₂ fragment, an Fv fragment, a single-chain Fv (ScFv) antibody, a diabody, minibody, nanobody (V_HH), and a linear antibody (see U.S. Pat. No. 5,641,870; Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0060] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H) and the first constant domain of one heavy chain (C_{H1}). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')₂ fragment that roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Both the Fab and F(ab')₂ are examples of “antigen-binding fragments.” Fab' fragments differ from Fab fragments by having an additional few residue at the carboxy terminus of the C_{H1} domain, including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0061] The “Fc” fragment comprises the carboxy-terminal portions (i.e., the C_{H2} and C_{H3} domains of IgG) of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region. The Fc domain is the portion of the antibody recognized by cell receptors, such as the FcR, and to which the complement-activating

protein, C1q, binds. As discussed herein, modifications (e.g., amino acid substitutions) may be made to an Fc domain in order to modify (e.g., improve, reduce, or ablate) one or more functionality of an Fc-containing polypeptide (e.g., an antibody of the present disclosure).

[0062] “Fv” is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (three loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although typically at a lower affinity than the entire binding site.

[0063] “Single-chain Fv” also abbreviated as “sFv” or “scFv”, are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, *infra*.

[0064] The term “diabodies” refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the VH and VL domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, i.e., fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” sFv fragments in which the VH and VL domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993). Other antibody fragments and molecules comprising the same include, for example, linear antibodies, tandem scFv, scFv-Fc, tandem scFv-Fc, scFv dimer, scFv-zipper, diabody-Fc, diabody-CH₃, scDiabodies, scDiabody-Fc, scDiabody-CH₃, nanobodies, TandAbs, minibodies, miniantibodies, triabodies, tetrabodies, scFab, Fab-scFv, Fab-scFv-Fc, scFv-CH-CL-scFv, and F(ab')₂-scFv₂, all of which are also contemplated herein.

[0065] In the embodiments that the glycoprotein is an antibody or an antigen-binding fragment thereof, the glycan can be located on a heavy chain or an Fc region. It is important to note that the glycosylation site of an antibody that regulates the antibody functions can differ from subtype to subtype. For example, the preBCR assembly is important for B cell development and is critically regulated by the N-glycan at N46 on μ HC. The N-glycan at N402 on μ HC has been linked to antibody oligomerization and complement activation. Besides, IgG N-glycosylation at N297 on γ HC plays a critical role in complement activation and Fc γ receptor activation leading to various effector functions. Therefore, in some embodiments, the glycan can be located on N46, N402, and/or N297 of a heavy chain.

[0066] In certain embodiments, the glycoprotein is Adalimumab (Humira[®]), Adalimumab-atto (Amjevita[®]), Rituximab (Rituxan[®]), Rituximab -atto (Truxima[®]), Cetuximab (Erbix[®]), Bevacizumab (Avastin[®]), Infliximab (Remicade[®]), Trastuzumab (Herceptin[®]), Pembrolizumab (Keytruda[®]), Etanercept (Enbrel[®]), Ipilimumab (Yervoy[®]), Ofatumumab (Arzerra), Golimumab (Simponi[®]), Atezolizumab (Tecentriq[®]), Ocrelizumab (OCREVUS[®]), Durvalumab (Durvalumab[®]), Avelumab (Bavencio[®]), Nivolumab (Opdivo[®]), Pertuzumab (Perjeta[®]), Obinutuzumab (Gazyva[®]), Gazyvaro Infliximab (Remicade[®]), or Trastuzumab emtansine (Kadcyla[®]).

[0067] In certain embodiments, the glycoprotein is an immunogenic protein, such as a viral envelop protein, or a spike protein of a virus. For example, the immunogenic protein can be but is not limited to an influenza hemagglutinin or a SARS-CoV-2 spike protein.

[0068] ***Cells for expressing a GlcNAc glycoprotein***

[0069] One aspect of the present disclosure provides a cell for expressing a GlcNAc glycoprotein, wherein the cell is deficient in N-acetylglucosaminyltransferase I (GnTI) activity and constitutively or controllably expresses an exogenous endoglycosidase. As described herein, “GlcNAc glycoprotein” describes that the glycan conjugated on the glycoprotein is composed of mainly GlcNAc. In some embodiments, the GlcNAc glycan can be a mono-GlcNAc glycan or a GlcNAc-Fuc glycan. In some embodiments, the glycoprotein is as described above and herein.

[0070] The exogenous endoglycosidase can be but is not limited to an endoglycosidase H (Endo H) or an endoglycosidase S2 (Endo S2). In certain embodiments, the exogenous

endoglycosidase comprises an amino acid sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 16. In some certain embodiments, the cell comprises a nucleic acid configured to encode the exogenous endoglycosidase, wherein the nucleic acid might comprise a nucleotide sequence as set forth in SEQ ID NO: 17 or SEQ ID NO: 18. The nucleic acid can be controlled under a constitutive or an activable promoter. The constitutive promoter can be but is not limited to a CMV promoter, a T7 promoter, an EF1A promoter, a CAG promoter, or an SV40 promoter. The activable promoter provides a controllable expression of the exogenous sialyltransferase and the exogenous galactosyltransferase. The activable promoter can be but is not limited to a Tetracycline-Inducible Promoter (activable by doxycycline) or a dihydrofolate reductase (DHFR) gene promoter(used to select and amplify gene expression).

[0071] As described herein, “deficient in N-acetylglucosaminyltransferase I (GnTI) activity” means that the cell does not have a functioning N-acetylglucosaminyltransferase I, which can result from knocking out an endogenous gene encoding the N-acetylglucosaminyltransferase I or a mutation at the gene causing missense, nonsense, or frameshift silent of the gene. Without wishing to be bound by any theories, a cell deficient in GnTI activity produces a mannose-rich glycan, which is preferentially cleaved by an endoglycosidase, thereby generating a GlcNAc glycoprotein.

[0072] *Parent cell.* In some embodiments, the cell of the present disclosure is derived from a parent cell, which can be a mammalian cell. In some embodiments, the parent cell is deficient in N-acetylglucosaminyltransferase I (GnTI) activity. In some other embodiments, the parent cell is not deficient in acetylglucosaminyltransferase I (GnTI) activity, while the cell of the present disclosure is genetically engineered to be deficient in GnTI activity. In some embodiments, the parent cell and/or the cell of the present disclosure are deficient in fucosyltransferase 8 activity. In certain embodiments, the parent cell and/or the cell of the present disclosure are deficient in a FUT8 gene encoding a product of the fucosyltransferase activity. In some embodiments, the parent cell and/or the cell of the present disclosure are or are derived from a Chinese hamster ovary (CHO) cell or a HEK293 cell. The CHO cell can be but is not limited to ExpiCHO, CHO-K1, CHO-C, CHOZN, and CHOK1Q, and the HEK293 cell can be Expi293F™ GnTI KO.

[0073] *Cells for expressing a recombinant glycoprotein.* In some embodiments, the cell further comprises a payload nucleic acid configured to encode a recombinant glycoprotein, and the expression of the payload nucleic acid is controlled by a constitutive or an activable promoter. The constitutive promoter and the activable promoter are as described above and herein. In some embodiments, the recombinant glycoprotein expressed or produced by the cell is conjugated with a glycan. In certain embodiments, the glycan can be an N-linked glycan.

[0074] **PRODUCTION OF THE CELLS OF THE PRESENT DISCLOSURE**

[0075] The production of the cells according to an embodiment of the present disclosure entails engineering a parent cell so that the engineered cell expresses the exogenous enzymes. In some embodiments, the production of the cells entails engineering a parent cell to knock in and/or knock out a gene of interest. In some embodiments, the engineering can be performed using transfection approaches or gene editing approaches, which can be either stable or transient.

[0076] ***Transfection***

[0077] The transfection can be conducted using any conventional methodologies with the consideration of high transfection efficiency, minimal cell toxicity, low or no significant effects on normal physiology, and ease of operation.

[0078] In some embodiments, the transfection can be performed using virus-mediated methods. The virus-mediated methods use a viral vector to bring a nucleic acid configured to encode a desired gene product into a host cell. Some commonly used viral vectors include but are not limited to Adenovirus, Adeno-associated virus, retrovirus murine leukemia virus, Herpes simplex virus, Vaccinia virus, and Sindbis virus. Virus-mediated methods are highly effective, given the infectious nature of the viral particles. The downsides of these methods, however, lie in the concerns of immunogenicity and cytotoxicity. Nevertheless, many virus-mediated methods have been well-studied and developed to ease the concerns. Besides, in some embodiments, the cell of the present disclosure is used to produce a desired glycoprotein in which uses, the transfection is not performed on a patient or a human subject directly. Therefore, the immunogenicity and cytotoxicity concerns are less critical for the present disclosure.

[0079] In some embodiments, the transfection can be performed using chemical methods, such as using a cationic polymer, calcium phosphate, a cationic lipid, or a cationic amino acid. Specific examples of chemical methods include but are not limited to using DEAE-dextran, polyethyleneimine, dendrimer, polybrene, calcium phosphate, lipofectin, DOTAP, lipofectamine, CTAB/DOPE, or DOTMA. The underlying principle of those methods is using those positively charged chemicals to make nucleic acid/chemical complexes with negatively charged nucleic acids. The nucleic acid/chemical complexes are believed to be attracted to the negatively charged cell membrane and pass through it eventually via endocytosis, phagocytosis, or both. The efficiency of those chemical methods can be affected by several factors including the nucleic acid/chemical ratio, pH value of the environment, and cell membrane conditions.

[0080] In some embodiments, the transfection can be performed using physical methods, such as direct microinjection, biolistic particle delivery, electroporation, laser-based transfection, ultrasound transfection, and magnetofection. Those methods can be useful but usually require a higher skill level and are labor-intensive.

[0081] ***Genome editing***

[0082] In some embodiments, the engineering can be performed using genome editing. The genome editing methods can be non-targeted gene editing, such as Sleeping Beauty transposon or I-SceI meganuclease-mediated transposon, or targeted gene editing, such as using transcription activator-like effector nuclease (TALEN) or clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein. Gene editing provides integration of the exogenous nucleic acid into the genome of the host cell (parent cell).

[0083] The targeted gene editing methods induce DNA double-strand breaks (DSBs) at a targeted locus, which enable the opportunities to alter the function (inserting an exogenous gene or silencing an endogenous gene) of a gene of interest. The TALEN approach entails using a chimeric molecule consisting of a transcription activator-like effector (TALE) domain and a *FokI* nuclease catalytic domain. The TALE domain comprises a DNA-binding motif, which can be designed to target a specific locus of the host cell's genome. Normally, a pair of two chimeric molecules, each targeting the forward and reverse directions respectively, are required. Once the

TALE domains bind the targeted locus in the forward and reverse directions, the *FokI* nuclease catalytic domains of the pair of two chimeric molecules form a dimer, thereby activating the catalytic activity of the nuclease to cleave the DNA and generate a DSB. The CRISPR method comprises using a complex of Cas protein and a guide RNA. The guide RNA comprises a scaffold sequence for Cas-binding and a spacer (around 20 nucleotides) having a complementary sequence to a specific locus of the host cell's genome. Normally, the spacer must have a sequence that is unique and specific to the target locus to prevent off-target binding, and the target locus is followed by a protospacer adjacent motif (PAM) site, which is necessary for Cas protein recognition. Once the guide RNA/Cas protein complex binds to the target site, the Cas nuclease activity will be activated and cleave the DNA to generate a DCB.

[0084] The DSBs induced can be repaired by three different repair mechanisms, including homology-directed repair (HDR), non-homologous end joining (NHEJ), and micro-homology-mediated end joining (MMEJ). In the process of the repair mechanism, deletion or frameshift can result in gene silence, or, by using a donor vector comprising microhomology sequences homologous to a targeted locus, the repair mechanism can efficiently induce a targeted integration of exogenous genes into the genome.

[0085] **METHODS FOR GLYCOENGINEERING A RECOMBINANT GLYCOPROTEIN AND RECOMBINANT GLYCOPROTEINS OBTAINED THEREBY**

[0086] One aspect of the present disclosure provides a method for glycoengineering a recombinant glycoprotein. The method comprises delivering an expression vector into a cell according to an embodiment of the present disclosure, wherein the expression vector comprises a payload nucleic acid configured to encode a recombinant glycoprotein; and expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a desired glycan

[0087] The glycoprotein can be as those described herein. In certain embodiments, the glycoprotein is a therapeutic antibody or an antigen-binding fragment thereof. In the embodiments that the glycoprotein is an antibody or an antigen-binding fragment thereof, the glycan can be located on a heavy chain or an Fc region. In some embodiments, the glycan is

located at a glycosylation site of the antibody. In certain embodiments, the glycan can be located on N46, N402, and/or N297 of a heavy chain.

[0088] As used herein, “glycoengineering” refers to producing the recombinant glycoprotein using the cell whereby the recombinant glycoprotein carries or is conjugated with a desired glycoform or desired glycan. In some embodiments, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% of them carry the desired glycoform (e.g., a sialylated glycan or a GlcNAc glycan), or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 50 to 100%, 50 to 99%, 50 to 95%, 50 to 90%, 50 to 85%, 50 to 80%, 50 to 75%, 50 to 70%, 50 to 65%, 50 to 60%, 50 to 55%, 60 to 100%, 60 to 99%, 60 to 95%, 60 to 90%, 60 to 85%, 60 to 80%, 60 to 75%, 60 to 70%, 60 to 65%, 70 to 100%, 70 to 99%, 70 to 95%, 70 to 90%, 70 to 85%, 70 to 80%, 70 to 75%, 80 to 100%, 80 to 99%, 80 to 95%, 80 to 90%, 80 to 85%, 90 to 100%, 90 to 99%, or 90 to 95%.

[0089] In some embodiments, the plurality of recombinant glycoproteins is conjugated with the desired glycoform (e.g., a sialylated glycan or a GlcNAc glycan) at homogeneity of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any range defined by the foregoing endpoints, such as 1% to 100%, 1% to 90%, 1% to 80%, 1% to 70%, 1% to 60%, 1% to 50%, 1% to 40%, 1% to 30%, 1% to 20%, 1% to 10%, 5% to 100%, 5% to 95%, 5% to 85%, 5% to 75%, 5% to 65%, 5% to 55%, 5% to 45%, 5% to 35%, 5% to 25%, 5% to 10%, 10% to 100%, 10% to 99%, 10% to 95%, 10% to 90%, 10% to 85%, 10% to 80%, 10% to 75%, 10% to 70%, 10% to 65%, 10% to 60%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 20% to 100%, 20% to 99%, 20% to 95%, 20% to 90%, 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 40% to 100%, 40% to 99%,

40% to 95%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 60% to 100%, 60% to 99%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 50% to 100%, 50% to 99%, 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 55% to 100%, 55% to 99%, 55% to 98%, 55% to 97%, 55% to 96%, 55% to 95%, 55% to 94%, 55% to 93%, 55% to 92%, 55% to 91%, 55% to 90%, 55% to 85%, 55% to 80%, 55% to 75%, 55% to 70%, 55% to 65%, 55% to 60%, 70% to 100%, 70% to 99%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 80% to 100%, 80% to 99%, 80% to 95%, 80% to 90%, 80% to 85%, 90% to 100%, 90% to 99%, or 90% to 95%, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein.

[0090] In some embodiments, the cell is according to the cell of the present disclosure described above for expressing a sialylated glycoprotein. In some embodiments, the sialylated glycoprotein comprises a sialyl complex type (SCT) glycan. In some embodiments, the method of the present disclosure is configured to express an SCT-enriched protein, which is defined as described herein. In some other embodiments, the cell is according to the cell of the present disclosure above for expressing a GlcNAc glycoprotein, which is defined as described herein.

[0091] In some embodiments, the method further comprises harvesting the recombinant glycoprotein within 200, 180, 150, 120, 100, 80, 50, 30, 20, 10, 5, or 1 hour from the expression of the payload nucleic acid, or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 1 to 200 hours, 1 to 180 hours, 1 to 150 hours, 1 to 120 hours, 1 to 100 hours, 1 to 80 hours, 1 to 50 hours, 1 to 30 hours, 1 to 20 hours, 1 to 10 hours, 1 to 5 hours, 5 to 200 hours, 5 to 180 hours, 5 to 150 hours, 5 to 120 hours, 5 to 100 hours, 5 to 80 hours, 5 to 50 hours, 5 to 30 hours, 5 to 20 hours, or 5 to 10 hours. In some embodiments, the recombinant glycoprotein is harvested within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 day from the expression of the payload nucleic acid, or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 1 to 10 days, 1 to 9 days, 1 to 8 days, 1 to 7 days, 1 to 6 days, 1 to 5 days, 1 to 4 days, 1 to 3 days, or 1 to 2 days. As described herein, “from the expression of the payload nucleic acid” means starting from the time point that the expression vector is delivered into the cell, or starting from the time point that the expression

vector is delivered into the cell and the promoter controlling the expression of the payload nucleic acid is activated.

[0092] In some embodiments, the expression of the payload nucleic acid is constitutive or controllable. In certain embodiments, the expression of the payload nucleic acid is controlled by a constitutive promoter or an activable promoter. The constitutive promoter can be but is not limited to a CMV promoter, a T7 promoter, an EF1A promoter, a CAG promoter, or an SV40 promoter. The activable promoter provides a controllable expression of the exogenous sialyltransferase and the exogenous galactosyltransferase. The activable promoter can be but is not limited to a Tetracycline-Inducible Promoter (activable by doxycycline) or a dihydrofolate reductase (DHFR) gene promoter (used to select and amplify gene expression).

[0093] In some embodiments, the expression vector can be delivered via gene engineering, which can be performed using transfection approaches or gene editing approaches, which can be either stable or transient. The transfection approaches and the gene editing approaches can be as those described herein for producing the cells of the present disclosure.

[0094] Another aspect of the present disclosure provides a plurality of recombinant glycoproteins, wherein at least 50% of the plurality of the recombinant glycoproteins is configured with a desired glycan, which can be a sialylated glycan or a GlcNAc glycan. The recombinant glycoprotein can be described herein. In certain embodiments, the plurality of recombinant glycoproteins is obtained by using the methods for glycoengineering a recombinant glycoprotein of the present disclosure.

[0095] In some embodiments, the plurality of recombinant glycoproteins is a plurality of enriched recombinant glycoprotein. The term “enriched” describes that the plurality of recombinant glycoproteins of the present disclosure have a higher or enriched desired glycan percentage compared to the same proteins expressed by a parental cell or a wild-type cell used to produce the cells of the present disclosure, or the plurality of recombinant glycoproteins of the present disclosure have a higher or enriched desired glycan percentage compared to the desired glycan percentage of a plurality of naturally-occurred proteins having the same or similar amino acid sequences. In some embodiments, the desired glycan percentage is enriched by at least 30%,

35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, or 500%, or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 10% to 500%, 10% to 400%, 10% to 300%, 10% to 200%, 10% to 100%, 10% to 90%, 10% to 80%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 30%, 10% to 20%, 30% to 500%, 30% to 400%, 30% to 300%, 30% to 200%, 30% to 100%, 30% to 90%, 30% to 80%, 30% to 70%, 30% to 60%, 30% to 50%, or 30% to 40%, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein. In certain embodiments, the desired glycan percentage is enriched to 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 times the SCT glycan percentage of the proteins expressed by a parental cell, or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 1.2 to 10 times, 1.2 to 9 times, 1.2 to 8 times, 1.2 to 7 times, 1.2 to 6 times, 1.2 to 5 times, 1.2 to 4 times, 1.2 to 3 times, 1.2 to 2 times, 2 to 10 times, 2 to 9 times, 2 to 8 times, 2 to 7 times, 2 to 6 times, 2 to 5 times, 2 to 4 times, 2 to 3 times, 5 to 10 times, 5 to 9 times, 5 to 8 times, 5 to 7 times, or 5 to 6 times, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein.

[0096] In some embodiments, the desired glycan percentage is enriched by at least 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95%, or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 51% to 95%, 51% to 94%, 51% to 93%, 51% to 92%, 51% to 91%, 51% to 90%, 51% to 85%, 51% to 80%, 51% to 75%, 51% to 70%, 51% to 65%, 51% to 60%, 55% to 95%, 55% to 90%, 55% to 85%, 55% to 80%, 55% to 75%, 55% to 70%, 55% to 65%, 55% to 60%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 60% to 70%, 60% to 65%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, or 70% to 75%, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein.

[0097] In some embodiments, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% of the plurality of the recombinant glycoprotein carry the desired glycoform

(e.g., a sialylated glycan or a GlcNAc glycan), or an integer ranging between any of the above two numbers, or any range defined by the foregoing endpoints, such as 50 to 100%, 50 to 99%, 50 to 95%, 50 to 90%, 50 to 85%, 50 to 80%, 50 to 75%, 50 to 70%, 50 to 65%, 50 to 60%, 50 to 55%, 60 to 100%, 60 to 99%, 60 to 95%, 60 to 90%, 60 to 85%, 60 to 80%, 60 to 75%, 60 to 70%, 60 to 65%, 70 to 100%, 70 to 99%, 70 to 95%, 70 to 90%, 70 to 85%, 70 to 80%, 70 to 75%, 80 to 100%, 80 to 99%, 80 to 95%, 80 to 90%, 80 to 85%, 90 to 100%, 90 to 99%, or 90 to 95%. All numbers can be modified by “about,” which is defined herein.

[0098] In some embodiments, the plurality of recombinant glycoproteins is conjugated with the sialylated glycan at homogeneity of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any range defined by the foregoing endpoints, such as 1% to 100%, 1% to 90%, 1% to 80%, 1% to 70%, 1% to 60%, 1% to 50%, 1% to 40%, 1% to 30%, 1% to 20%, 1% to 10%, 5% to 100%, 5% to 95%, 5% to 85%, 5% to 75%, 5% to 65%, 5% to 55%, 5% to 45%, 5% to 35%, 5% to 25%, 5% to 10%, 10% to 100%, 10% to 99%, 10% to 95%, 10% to 90%, 10% to 85%, 10% to 80%, 10% to 75%, 10% to 70%, 10% to 65%, 10% to 60%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 20% to 100%, 20% to 99%, 20% to 95%, 20% to 90%, 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 40% to 100%, 40% to 99%, 40% to 95%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 60% to 100%, 60% to 99%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 50% to 100%, 50% to 99%, 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 55% to 100%, 55% to 99%, 55% to 98%, 55% to 97%, 55% to 96%, 55% to 95%, 55% to 94%, 55% to 93%, 55% to 92%, 55% to 91%, 55% to 90%, 55% to 85%, 55% to 80%, 55% to 75%, 55% to 70%, 55% to 65%, 55% to 60%, 70% to 100%, 70% to

99%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 80% to 100%, 80% to 99%, 80% to 95%, 80% to 90%, 80% to 85%, 90% to 100%, 90% to 99%, or 90% to 95%, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein.

[0099] In the embodiments that the cell is according to the cell of the present disclosure above for expressing a GlcNAc glycoprotein, the method of the present disclosure can further comprise a transglycosylation step. The transglycosylation step can comprise collecting the plurality of recombinant glycoproteins expressed in the preceding steps of the method and incubating the plurality of recombinant glycoproteins with an endoglycosidase and a donor glycan, thereby conjugating the donor glycan onto at least one glycoprotein of the plurality of recombinant glycoproteins (i.e., a transglycosylation reaction).

[0100] In some embodiments, the donor glycan comprises an oxazoline moiety configured to react with the endoglycosidase thereby conjugating the donor glycan onto at least one glycoprotein of the plurality of recombinant glycoproteins. In certain embodiments, the donor glycan is a sialyl complex type (SCT) glycan. In some embodiments, the endoglycosidase is an endoglycosidase H (Endo H), an endoglycosidase S2 (Endo S2), or a derivative thereof. In some embodiments, the derivative of the Endo S2 comprises an Endo S2 D184 mutant, including but not limited to, a D184M mutant or a D184Q mutant.

[0101] In some embodiments, the transglycosylation step generates a plurality of recombinant glycoprotein conjugated with the donor glycan at a homogeneity of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any range defined by the foregoing endpoints, such as 1% to 100%, 1% to 90%, 1% to 80%, 1% to 70%, 1% to 60%, 1% to 50%, 1% to 40%, 1% to 30%, 1% to 20%, 1% to 10%, 5% to 100%, 5% to 95%, 5% to 85%, 5% to 75%, 5% to 65%, 5% to 55%, 5% to 45%, 5% to 35%,

5% to 25%, 5% to 10%, 10% to 100%, 10% to 99%, 10% to 95%, 10% to 90%, 10% to 85%, 10% to 80%, 10% to 75%, 10% to 70%, 10% to 65%, 10% to 60%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 20% to 100%, 20% to 99%, 20% to 95%, 20% to 90%, 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 40% to 100%, 40% to 99%, 40% to 95%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 60% to 100%, 60% to 99%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 50% to 100%, 50% to 99%, 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 55% to 100%, 55% to 99%, 55% to 98%, 55% to 97%, 55% to 96%, 55% to 95%, 55% to 94%, 55% to 93%, 55% to 92%, 55% to 91%, 55% to 90%, 55% to 85%, 55% to 80%, 55% to 75%, 55% to 70%, 55% to 65%, 55% to 60%, 70% to 100%, 70% to 99%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 80% to 100%, 80% to 99%, 80% to 95%, 80% to 90%, 80% to 85%, 90% to 100%, 90% to 99%, or 90% to 95%, including or excluding any foregoing numbers. All numbers can be modified by “about,” which is defined herein.

[0102] **DEFINITION**

[0103] Unless specifically defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of microbiology, tissue culture, molecular biology, chemistry, biochemistry, and recombinant DNA technology, which are within the skill of the art. The materials, methods, and examples are illustrative only and not limiting. The following is presented by way of illustration and is not intended to limit the scope of the disclosure.

[0104] Numbers expressing quantities of ingredients, properties such as homogeneity, enrich level, molecular weight, reaction conditions and results, and so forth, used to describe and claim certain embodiments of the present disclosure are to be understood as being modified in

some instances by the term "about." A skilled artisan in the field would understand the meaning of the term "about" in the context of the value that it qualifies. The numerical values presented in some embodiments of the present disclosure may contain certain errors resulting from the standard deviation in their respective testing measurements. For example, the term "about," as used herein, refers to a measurable value such as an amount, a temporal duration, and the like and is meant to encompass variations of $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate.

[0105] As used herein, "substantially" means sufficient to work for the intended purpose. The term "substantially" thus allows for minor, insignificant variations from an absolute or perfect state, dimension, measurement, result, or the like, such as expected by a person of ordinary skill in the field, but that does not appreciably affect overall performance. When used with respect to numerical values or parameters or characteristics expressed as numerical values, "substantially" means within ten percent.

[0106] As used herein, "treat," "treatment," and "treating" refer to an approach for obtaining beneficial or desired results, for example, clinical results. For this disclosure, beneficial or desired results may include inhibiting or suppressing the initiation or progression of an infection or a disease; ameliorating, or reducing the development of, symptoms of an infection or disease; or a combination thereof.

[0107] As used herein, "preventing" and "prevention" are used interchangeably with "prophylaxis" and can mean complete prevention of infection or prevention of the development of symptoms of that infection, a delay in the onset of a disease or its symptoms; or a decrease in the severity of a subsequently developed infection or its symptoms.

[0108] As used herein, "glycan" refers to a polysaccharide, oligosaccharide, or monosaccharide. Glycans can be monomers or polymers of sugar residues and can be linear or branched. A glycan may include natural sugar residues (e.g., glucose, N-acetylglucosamine, N-acetyl neuraminic acid, galactose, mannose, fucose, hexose, arabinose, ribose, xylose, etc.) and/or modified sugars (e.g., 2'-fluororibose, 2'-deoxyribose, phosphomannose, 6' sulfo N-acetylglucosamine, etc.).

[0109] As used herein, a “recombinant glycoprotein” or a “recombinant protein” is a protein produced by introducing an engineered nucleic acid into a host organism, like bacteria, yeast, or mammalian cells using laboratory or industrial processes, and in some situations, isolated or purified for clinical or industrial uses.

[0110] **EXAMPLE**

[0111] **EXAMPLE 1: Manufacturing Exemplary CHO Cells of The Present Disclosure for Expressing a Sialylated Glycoprotein**

[0112] This experiment manufactured an exemplary cell according to an embodiment of the present disclosure from a parental CHO cell. ExpiCHO cells (Thermo Fisher Scientific) were purchased and maintained ExpiCHO™ Expression Medium according to the product manual. Then, the cells were transferred with a SWG-006 plasmid (i.e. an expression vector) using an ExpiFectamine™ CHO Transfection Kit according to the manufacturer’s instructions. The SWG-006 plasmid (SEQ ID NO: 23) was made of pcDNA™3.1 plasmid and was constructed with a CMV enhancer, a CMV promoter, a ST6GAL1 gene (SEQ ID NO: 10), a P2A sequence (SEQ ID NO: 19), and a B4GALT1 gene (SEQ ID NO: 14). Alternatively, cells were co-transfected with a ST6GAL1 plasmid (SEQ ID NO: 25) and a B4GALT1 plasmid (SEQ ID NO: 26), each was constructed to express the ST6GAL1 gene and the B4GALT1 gene separately under a CMV promoter.

[0113] Then, the cells were transfected with an Adalimumab plasmid (pcDNA2 TADA, SEQ ID NO: 27) using the ExpiFectamine™ CHO Transfection Kit to express Adalimumab (Humira). Three days after the transfection, the transfected cells (a transient clone) were washed with staining buffer (EDTA 1 mM, NaN₃ 0.02%, BSA 1%, in PBS) and stained with biotinylated SNA (VectorLabs), which is designed to bind a sialic acid linked to a N-acetylgalactosamine or galactose. After being washed, the cells were stained with Streptoavidin-Alexa647 (BioLegend) and analyzed using flow cytometry (BD LSRFortessa).

[0114] The results (FIG. 1) showed transfection with the SWG-006 plasmid or co-transfected with the ST6GAL1 plasmid and the B4GALT1 plasmid were able to promote α 2,6-sialylation. In comparison, the parent CHO cell (ExpiCHO cells) does not have an endogenous

α 2,6-sialyltransferase, so no signal was detected from untransfected cells. Most importantly, the results also showed that transfection with the SWG-006 plasmid provided higher α 2,6-sialylation than co-transfection with the ST6GAL1 plasmid and the B4GALT1 plasmid.

[0115] Similarly, another exemplary cell according to an embodiment of the present disclosure was made by transfecting an ExpiCHO cell (Thermo Fisher Scientific) using the ExpiFectamine™ CHO Transfection Kit with a SWG-015 plasmid (SEQ ID NO: 24). The SWG-015 vector was made of pcDNA™3.1 plasmid and was constructed with a CMV enhancer, a CMV promoter, a modified PspST gene (SEQ ID NO: 12), a P2A sequence (SEQ ID NO: 19), and a B4GALT1 gene (SEQ ID NO: 14). The cell was also transfected with the Adalimumab plasmid (pcDNA2 TADA, SEQ ID NO: 27) to express Adalimumab (Humira). Then, the sialylation of Adalimumab produced by the cell, which is also a transient clone, was observed using SNA staining as described above. The cell transfected with the SWG-006 vector was also examined to compare with the cell transfected with the SWG-015 vector. The results showed that the cell transfected with the SWG-015 provided a similar level of sialylation as that of the cell transfected with the SWG-006 vector (FIG. 2).

[0116] **EXAMPLE 2: Stabilization of the exemplary cells of the present disclosure**

[0117] Plasmid SWG-006 was linearized by digestion with PvuI and then purification. The linearized SWG-006 was transfected into EXPICHO cells by using ExpiFectamine™ CHO Transfection Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. On day 2 post-transfection, G418 was added at 400 ug/ml for drug selection. After recovering to 90% survival, the cells were stained with biotinylated SNA and then streptavidin- BV421 wherein the SNA bound endogenous membrane proteins produced by the cells. The cells were then sorted for high SNA binding by using WOLF G2 Cell Sorter (Nanocollect Biomedical, Inc.) into single cells cultured in 96 well plates. After a period of time of culture, the single clone cells were stained with biotinylated SNA and then streptavidin-APC and analyzed by using flow cytometry to select clones with highly surface α 2,6-sialylation. The selected cells (>99% SNA positive) were then transfected with Adalimumab expression plasmid (SEQ ID NO: 27) to overexpress Adalimumab. The expressed Adalimumab was purified by using protein A beads and analyzed

by using an SNA hybridization blot to select clones that were able to express highly alpha2,6-sialylation Adalimumab. The glycoform of Adalimumab was further analyzed by LC/MS-MS.

[0118] The LC/MS-MS data shows that the four stabilized clones tested in this example, SAI-A3, SAI-GI, SAI-D4, and SAI-F12, all expressed a high percentage of sialylation of Adalimumab. The stabilized Clone SAI-A3 showed 70.9 % (about 70% to 72%) sialylation of the Adalimumab it expressed (FIG. 3A); the stabilized Clone SAI-GI showed 55.39 % (about 55% to 57%) sialylation of the Adalimumab it expressed (FIG. 3B); the stabilized Clone SAI-D4 showed 16.8 % (about 16% to 18%) sialylation of the Adalimumab it expressed (FIG. 3C); and the stabilized Clone SAI-F12 showed 36.14 % (about 36% to 38%) sialylation of the Adalimumab it expressed (FIG. 3D). In comparison, the Adalimumab expressed by the parental cell did not show significant sialylation (FIG. 3E).

[0119] **EXAMPLE 3: Glycoengineering Adalimumab using an exemplary stabilized cell according to the present disclosure**

[0120] In this experiment, a stabilized clone (SAI-A3) of the SWG-006 cells obtained as described in Example 2 above was transfected with an Adalimumab expression vector (SEQ ID NO: 27) using an ExpiFectamine™ CHO Transfection Kit (Thermo Fisher Scientific) according to the manufacturer's instructions to express Adalimumab, and the glycoform thereof was observed and compared with the same antibody expressed by a parent cell (ExpiCHO cell).

[0121] Briefly, cells were diluted to a final density of 5×10^6 viable cells/mL with fresh 15 mL ExpiCHO™ Expression Medium in a flask, pre-warmed to 37°C. The flask was then swirled gently. The cells were then maintained in a 37° C incubator with a humidified atmosphere of 8% CO₂ in the air on a 125 rpm orbital shaker before transfection. Plasmid DNA (i.e., the expression vector of SEQ ID NO: 27, 12 µg) was diluted with 0.6 mL of cold OptiPRO™ medium, and ExpiFectamine™ CHO Reagent (48 µL) was diluted with 552 µL of cold OptiPRO™ medium. The diluted DNA was then mixed with the diluted ExpiFectamine™ CHO Reagent to obtain ExpiFectamine™ CHO/plasmid DNA complexes. The ExpiFectamine™ CHO/plasmid DNA complexes were added to the cell culture in a 37° C incubator with a humidified atmosphere of 8% CO₂ in the air on a 125 rpm orbital shaker. After transfection, 90 µL ExpiFectamine™ CHO

Enhancer and 3.6 ml ExpiCHO™ Feed volumes were added to the cells. The cells in the flask were then maintained in the 37° C incubator with a humidified atmosphere of 8% CO₂ with shaking. After 8–10 days post-transfection, the supernatant of the cell culture was harvested for protein purification.

[0122] Then, the antibodies produced by the SAI-A3 cells and the parental EXPICHO cells were isolated respectively using protein A beads (GE HealthCare). Briefly, 15 mL supernatant was passed through the 200 uL protein A column. The column was washed with 10-column volume (CV) PBS. The bound antibody was eluted with 15 CV 0.1 M glycine pH=3. The yielding antibodies were dialysis against 10 mM ammonium acetate. The antibodies were then reduced using 25 mM DTT and analyzed by LC/MS-MS. A commercial product of Adalimumab (Humira®) was purchased and analyzed using LC/MS-MS as a control.

[0123] The LC/MS-MS data shows that the Adalimumab produced by the exemplary cells of the present disclosure had a variety of sialylated glycans (See FIG. 4C), the sialylated glycans are labeled with solid arrows), including G2S2F, G2S1F, G1S1F, N3H6F1S1, N3H4S1, N3H5F1S1, N4H6F1S1, N4H6F1S2. The sialylation percentage of the total glycans was 70.9%, and the sialylation percentage of the bi-antennary complex type glycan was 79.8%. In comparison, although the commercial products (FIG. 4A) and the Adalimumab produced by parental ExpiCHO cells (FIG. 4B) also carried glycans, no sialylation was observed.

[0124] **EXAMPLE 4: Manufacturing Exemplary HEK293 Cells of The Present Disclosure for Expressing a Sialylated Glycoprotein**

[0125] This experiment manufactured an exemplary cell according to an embodiment of the present disclosure from a parental HEK293T cell using CRISPR technologies. The results were very similar to the results of using the ExpiFectamine™ CHO Transfection Kit on CHO cells, as described in Example 1.

[0126] *Cells, Plasmids, and Transfection.* The parental HEK293T cells (ATCC) were cultured in the DMEM (Thermo Fisher Scientific) supplied with 10% FBS (Thermo Fisher Scientific) at 37°C with 5% CO₂ in the cell incubator. The plasmids for CRISPR/Cas9 expression were constructed as suggested by the kit manual (Thermo Fisher Scientific). Briefly,

the validated sgRNA sequence targeting glycosyltransferase was synthesized (Integrated DNA Technologies) and cloned into the vector for Cas9 and sgRNA expression. The synthetic codon-optimized gene insert (Integrated DNA Technologies), flanked by the homologous arm of the target gene at the sgRNA target site, was cloned into an empty vector as the donor plasmid. Transfection of 293T cells was mediated by TransIT-293 (Mirus Bio) following the reagent manual. A plasmid for expressing antibody chMC81370 (a humanized antibody targeting specific stage embryonic antigen 4 (SSEA4)) was transfected into cells followed by incubation. At the time of harvest, the culture medium was collected and then subjected to protein A sepharose beads (GE HealthCare) column to purify the antibodies.

[0127] *Analysis.* The purified antibodies were analyzed using intact protein mass (IPM) analysis. Briefly, the samples were diluted with LCMS grade water at a concentration between 5-10 μ M and analyzed by 6230 TOF LC/MS with a Dual AJS ESI ion source (Agilent Technologies) with PLRP-S 1000Å 5 μ m column (Agilent Technologies). Solvent A was 0.1% Formic Acid in H₂O and Solvent B was 0.1% Formic Acid in CAN. The results show a significant increase in galactosylated and sialylated antibody glycoforms (FIG. 5B) compared to the antibodies generated by the parental HEK293T cells (FIG. 5A). The results of the IPM Glycoform analysis were presented in bar charts, which revealed a significant increase in antibody galactosylation and sialylation, with a lack of core-fucosylation (FIG. 6).

[0128] *Characterizing the sialylation.* To confirm the sialylation on the antibody was mediated by hST6Gal1, the antibody was treated with two different sialidases, *Streptococcus pneumonia* α 2-3 neuraminidase and *Clostridium perfringens* neuraminidase at 37°C overnight respectively followed by purification or gel electrophoresis. The intact protein analysis did not show any obvious difference in the distribution of asialylated and sialylated antibodies between the untreated and α 2-3 neuraminidase-treated group (FIG. 7 upper and middle; sialylated glycoforms are labeled with the solid arrows), indicating that the sialylation on antibody was not due to the endogenous α 2-3 sialyltransferases. On the other hand, treatment with *Clostridium perfringens* neuraminidase, which could remove all linkages of sialylation, largely eliminated the terminal sialic acids (FIG. 7 bottom). In addition, the antibodies with increased galactosylation generated from cells with knock-in of hB4GalT1 only did not show terminal sialylation (FIG. 8).

These results confirmed that sialylation on the antibody was introduced by hST6Gal1 and the increase of hB4Gal1 expression indeed elevated the galactosylation of antibody (FIG. 5).

[0129] The sialyltransferase hST6Gal1 is known as the only human glycosyltransferase that mediates α 2-6 sialylation and preferentially uses the α 1-3 branch arm of the biantennary glycan as substrates. Some *in vitro* studies show glycoengineering on antibody glycans by hST6Gal1 takes a long time and is difficult to yield fully sialylated antibody glycans. Furthermore, this also could explain why only the antibody with at least one fully galactosylated glycan was sialylated. If the galactose on G1 glycan was not on the α 1-3 branch arm, the hST6Gal1 may not sialylate it. Therefore, the expression level of glycosyltransferases may not be the bottleneck in this situation. Rather, substrate preference and enzyme activity are the major factors. Using α 2-6 sialyltransferases from other organisms may have similar or better activity than hST6Gal1, which is evident by the other examples described herein that used a PspST gene.

[0130] **EXAMPLE 5: Time Factor in Glycoengineering Using an Exemplary Cell of The Present Disclosure**

[0131] This experiment tested whether the amount and the time of antibody expression using an exemplary cell of the present disclosure affect glycosylation. The culture time was extended to 5 days, and the medium of the culture was collected on Day 3 and Day 5, respectively. The produced antibodies were examined using intact protein mass (IPM) analysis. The results show a relatively strong sialylation in the first collection (first three days) (FIG. 9A, D0-3), and then sialylation was decreased in the second collection (last two days), accompanied by an increase of terminal galactosylation (FIG. 9A, D4-5), suggesting that the proteins involved in glycosylation may not be sufficient to effectively produce Fc-SCT antibody at a later stage.

[0132] Because the hST6Gal1 and hB4GalT1 involved in the FC-SCT antibody production were almost artificially and constitutively expressed in the cells, we also examined if endogenous glycosyltransferases faced the same problem of reduced glycosylation. By expressing antibodies in FUT8 KO cells, the antibody was almost terminally glycosylated with at least one or more galactoses in the first collection (FIG. 9B, D0-3), but the proportion of galactosylated antibody decreased, and even the antibody glycans without any galactoses became the dominant

glycoforms in the last two days ((Fig. 9B, D4-5). The result showed that the dynamic glycosylation level after the antibody expression can be observed on endogenous glycosylation products as well.

[0133] The aforementioned finding suggests that antibody production at different time points may impact glycosylation differently. Therefore, in the next experiment, antibodies were purified every day after plasmid transfection and analyzed the dynamic change of antibody glycans from multiple engineered cells. The antibody with full sialylation could be detected by intact protein mass analysis on the first day and then gradually decreased over time (FIG. 10). Regarding galactosylation, the antibody glycans were capped by at least two galactoses (2~4 galactoses in total) on the first day, but started to decline on the second day, as evidenced by the appearance of one galactose on antibody glycans (FIG. 10). These observations suggest that antibody glycosylation was almost complete at the beginning of the protein expression and gradually decreased over time.

[0134] **EXAMPLE 6: Binding between Sialylated Antibodies and Fc γ Receptors**

[0135] Fc γ RIIA (Fc gamma RIIA) and Fc γ RIIB (Fc gamma RIIB) are usually expressed simultaneously by the antigen-presenting cells, such as dendritic cells and macrophages, and these two receptors work together to modulate immune responses. Fc γ RIIA is critical for NK cells to mediate ADCC3. This experiment tested whether the Fc-SCT-enriched antibodies produced using the exemplary cells of the present disclosure show better binding affinity than wide-type antibodies (i.e., antibodies produced by parental cells). The Fc-SCT-enriched antibodies were produced and purified as described in Example 4. The binding affinity was examined using an ELISA assay.

[0136] *ELISA.* The recombinant soluble Fc γ IIA, Fc γ IIB, and Fc γ RIIA (Fc gamma RIIA, R&D) was coated with 50 ng/well in bicarbonate/carbonate coating buffer (50 mM, pH 10) at 4°C overnight before blocking the well with 5% BSA in TPBS (0.05% Tween 20 in PBS) at 4°C overnight. The antibody was added into wells with final concentration started at 100 μ g/ml with 5x series dilution and incubated at room temperature for 1 hour followed by incubation with HRP-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch) for another 1 hour at

room temperature. Finally, the TMB substrate (Bethyl Laboratories) was added to react with HRP at RT prior to stopping the reaction by H₂SO₄. The absorbance at 450 nm was detected by SpectraMax M5 spectrum reader (Molecular Device). The wells were washed by TPBS 3-5 times between each step.

[0137] The results show that the binding of Fc-SCT-enriched antibodies to FcRs was increased in three tested Fc receptors (FIG. 11). Among these receptors, the increased binding of FcγRIIA and FcγRIIA was consistent with previous studies. Interestingly, the WT antibody showed very weak binding to the inhibitory receptor FcγRIIB. Despite the improvement being less significant than that of FcγRIIA and FcγRIIA, the glycoengineering by the cells of the present disclosure was able to improve the antibody's binding to receptor FcγRIIB. Although the increased binding to the two opposing functional receptors, FcγRIIA and FcγRIIB, which are typically expressed on the antigen-presenting cells (APCs) simultaneously, appears to be conflicting, the eventual immune response was determined by the expression level and signaling strength of FcγRIIA and FcγRIIB on effector cells. The Fc-SCT-enriched antibody also exhibited stronger FcγRIIA binding ability than the antibody produced from wild-type cells (FIG. 11). Without wishing to be bound by theories, this enhancement was also contributed by the removal of core-fucose11 and the addition of galactose.

[0138] **EXAMPLE 7: Manufacturing Exemplary HEK293 Cells of The Present Disclosure for Expressing GlcNAc glycoproteins**

[0139] This experiment manufactured an exemplary cell according to an embodiment of the present disclosure from a parental GnT1 KO ExpiHEK293F cell using CRISPR technologies. The parental cells were prepared as described in Example 4. The *N*-glycan expressed by this cell line was a high mannose type which was preferentially cleaved by Endo H to generate Fc-GlcNAc antibody. Therefore, this experiment aims to knock in Endo H (UniProtKB/Swiss-Prot: P04067.1, See SEQ ID NO: 15 and SEQ ID NO: 17) in this cell line by CRISPR-Cas9. The plasmid for the CRISPR technology was prepared as described below.

[0140] *Plasmids and Transfection.* The plasmids for CRISPR/Cas9 expression were constructed as suggested by the kit manual (Thermo Fisher Scientific). Briefly, the validated

sgRNA sequence targeting glycosyltransferase was synthesized (Integrated DNA Technologies) and cloned into the vector for Cas9 and sgRNA expression. The synthetic codon-optimized gene insert (Integrated DNA Technologies), flanked by the homologous arm of the target gene at the sgRNA target site, was cloned into an empty vector as the donor plasmid. Transfection of 293T cells was mediated by using an Expi293Fectamine™ Transfection Kit (Thermo Fisher Scientific) following the reagent manual. A plasmid for expressing antibody chMC81370 was transfected into cells followed by incubation. At the time of harvest, the culture medium was collected and then subjected to protein A sepharose beads (GE HealthCare) column to purify the antibody.

[0141] *Analysis.* The antibodies expressed by the cells were analyzed by electrophoresis. The protein was heated at 95°C for 5 mins in the LDS sample buffer supplied with 2-Mercaptoethanol and then subjected to gel electrophoresis with 12% SDS-PAGE. The protein on the gel was stained by Coomassie blue buffer (ApexBio). The result showed that the antibody heavy chain (AbHC) expressed by these cells had faster mobility than AbHC from its parental cells (GnT1 KO), and most AbHCs were downshifted (FIG. 12A).

[0142] This antibody was also subjected to a transglycosylation assay. The antibody was incubated with SCT glycan-oxazoline and Endo S2 mutant in Tris buffer at 37°C for the time indicated before harvest for purification or gel electrophoresis. The transglycosylation generated antibodies with homogenous glycan (in this experiment, homogenous SCT glycan). The efficiency of the transglycosylation was pretty good (FIG. 12B). The glycans on these antibodies were then by intact protein mass (IPM) analysis. The results showed that most antibodies (over 95%) contain only GlcNAc on each HC (FIG. 12C, FIG. 12D, and FIG. 12E).

[0143] Alternatively, instead of knocking in Endo H, endoglycosidase S2 (Endo S2; UniProtKB/Swiss-Prot: T1WGN1.1, See SEQ ID NO: 16 and SEQ ID NO: 18) from *Streptococcus pyogenes* was knocked in because this enzyme can hydrolyze both high mannose and complex type *N*-glycans to Fc-GlcNAc. The donor vector and CRISPR-Cas9 plasmids were delivered to GnT1 KO cells. Then the antibody glycans generated by these cells were examined as previously described. The protein gel showed an obvious downshifted band of AbHC compared with its parental cells (GNT1 KO) (FIG. 13A). Besides, the glycans also can be added onto AbHC through enzymatic transglycosylation (FIG. 13B) and intact protein mass analysis

showed a clear signal of Fc-GlcNAc antibody (FIG. 13C), most antibodies (over 90%) contain only GlcNAc on each HC (FIG. 12E).

[0144] Based on these results, it is concluded that both Endo H and Endo S2 can be introduced to cells by CRISPR-Cas9 to process the *N*-glycans to Fc-GlcNAc anti-body for *in vitro* transglycosylation. In addition, this cell-based method can be used for other glycoproteins, such as influenza hemagglutinin and SARS-CoV-2 spike protein to generate the mono-GlcNAc decorated glycoforms as vaccines to elicit broadly protective immune responses

[0145] Next, since the binding of Fc-SCT enriched antibodies (i.e., the antibody obtained in Example 4) to FcγIIIA receptor was elevated, the homogeneous Fc-SCT antibodies obtained above through transglycosylation were compared with the Fc-SCT enriched antibodies for their binding affinity to FcγIIIA receptors. The result showed that Fc-SCT enriched antibodies exhibited similar binding avidity to homogenous SCT antibodies (FIG. 14A).

[0146] Furthermore, an ADCC reporter assay was conducted. Briefly, the assay was performed according to the kit manual. The SSEA4 expressing target cells SK-OV3 were seeded into a 96-well plate, followed by the addition of an antibody with the final concentration started at 1g/ml with 5x series dilution. Then, the FcγRIIIA expressing effector cells (effector: target cell ratio, 6:1) were added and incubated with target cells in the incubator at 37 °C for 6 hrs. The plate was placed at room temperature for 15 min prior to adding the luciferase substrate. After 5 min incubation, the luminescence was measured by SpectraMax M5 spectrum reader. The induction fold was calculated by RLU (induced-background) /RLU (no antibody control-background). It was observed that at the lowest antibody concentration used for induction, WT antibody did not induce any obvious cell activation, but the homogenous Fc-SCT and Fc-SCT enriched antibodies can trigger cell activation and rapidly reached a maximum at next concentration (FIG. 14B). Even at the higher concentration, the WT antibody can reach a maximum induction, but the activity was still much lower than that of homogenous Fc-SCT or Fc-SCT enriched antibody (FIG. 14B).

[0147] **EXEMPLARY EMBODIMENTS**

[0148] Embodiment 1: A cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity.

[0149] Embodiment 2: The cell of Embodiment 1, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are expressed in a single transcript.

[0150] Embodiment 3: The cell of Embodiment 1 or Embodiment 2, comprising a first nucleic acid configured to express the exogenous sialyltransferase catalytic peptide and a second nucleic acid configured to express the exogenous galactosyltransferase catalytic peptide, wherein the first nucleic acid and the second nucleic acid are transcriptionally controlled by the same promoter.

[0151] Embodiment 4: The cell of Embodiment 3, wherein the first nucleic acid and the second nucleic acid are connected to each other via a connecting nucleic acid, which is configured to encode a ribosomal shifting peptide.

[0152] Embodiment 5: The cell of Embodiment 4, wherein the ribosomal shifting peptide comprises an amino acid sequence of DxExNPGP, wherein x denotes any amino acid, D denotes aspartic acid, E denotes glutamic acid, N denotes asparagine, P denotes proline, and G denotes glycine.

[0153] Embodiment 6: The cell of Embodiment 4 or Embodiment 5, wherein the ribosomal shifting peptide comprises an amino acid sequence as set forth in SEQ ID NO: 06, SEQ ID NO: 07, SEQ ID NO: 08, or SEQ ID NO: 09.

[0154] Embodiment 7: The cell of Embodiment 3, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are co-configured to be expressed as a fusion protein.

[0155] Embodiment 8: The cell of Embodiment 7, wherein the fusion protein comprises a first portion having the sialyltransferase catalytic peptide domain and a second portion having the galactosyltransferase catalytic peptide domain, wherein the first portion and the second portion are connected to each other via a cleavable linker, further wherein the cleavable linker is configured to be cleavable post-translation of the fusion protein, thereby upon cleavage releasing the sialyltransferase catalytic peptide and the galactosyltransferase catalytic peptide as separate proteins.

[0156] Embodiment 9: The cell of any one of Embodiments 3 to 8, wherein the first nucleic acid is derived from an ST6Gal1 gene or a PspST gene.

[0157] Embodiment 10: The cell of any one of Embodiments 3 to 9, wherein the first nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 10 or SEQ ID NO: 11.

[0158] Embodiment 11: The cell of Embodiment 10, wherein the first nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 12 or SEQ ID NO: 13.

[0159] Embodiment 12: The cell of any one of Embodiments 3 to 11, wherein the second nucleic acid is derived from an B4GALT1 gene.

[0160] Embodiment 13: The cell of any one of Embodiments 3 to 12, wherein the second nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 14.

[0161] Embodiment 14: The cell of any one of Embodiments 3 to 13, wherein the promoter is a constitutive promoter or an activable promoter.

[0162] Embodiment 15: The cell of Embodiment 14, wherein the constitutive promoter is a CMV promoter, T7 promoter, a Human Elongation Factor 1 Alpha (EF1 α) promoter, a Chicken β -Actin (CAG) promoter, or an SV40 promoter.

[0163] Embodiment 16: The cell of Embodiment 14, wherein the activable promoter is a Tetracycline-Inducible Promoter (activable by doxycycline) or a dihydrofolate reductase (DHFR) gene promoter.

[0164] Embodiment 17: The cell of any one of Embodiments 1 to 16, wherein the sialyltransferase catalytic peptide is an alpha-2,6-sialyltransferase.

[0165] Embodiment 18: The cell of Embodiment 17, wherein the sialyltransferase is a beta-galactoside alpha-2,6-sialyltransferase 1.

[0166] Embodiment 19: The cell of any one of Embodiments 1 to 18, wherein the sialyltransferase catalytic peptide comprises an amino acid sequence as set forth in SEQ ID NO: 01 or SEQ ID NO: 02.

[0167] Embodiment 20: The cell of Embodiment 19, wherein the sialyltransferase catalytic peptide comprises an amino acid sequence as set forth in SEQ ID NO: 03 or SEQ ID NO: 04.

[0168] Embodiment 21: The cell of any one of Embodiments 1 to 20, wherein the galactosyltransferase catalytic peptide is a beta-1,4-galactosyltransferase 1.

[0169] Embodiment 22: The cell of Embodiment 21, wherein the galactosyltransferase catalytic peptide comprises an amino acid sequence as set forth in SEQ ID NO: 05.

[0170] Embodiment 23: The cell of any one of Embodiments 1 to 22, wherein the cell is deficient in fucosyltransferase activity.

[0171] Embodiment 24: The cell of Embodiment 23, wherein the cell is deficient in fucosyltransferase 8 activity.

[0172] Embodiment 25: The cell of Embodiment 23 or Embodiment 24, wherein the cell is deficient in a FUT8 gene encoding a product of the fucosyltransferase activity.

[0173] Embodiment 26: The cell of any one of Embodiments 1 to 25, wherein the cell is a mammalian cell.

[0174] Embodiment 27: The cell of Embodiment 26, wherein the cell is derived from a Chinese hamster ovary cell or a HEK293 cell.

[0175] Embodiment 28: The cell of any one of Embodiments 1 to 27, wherein the cell further comprises a payload nucleic acid configured to encode a recombinant glycoprotein, and the expression of the payload nucleic acid is transcriptionally controlled by a constitutive or an activable promoter.

[0176] Embodiment 29: The cell of Embodiment 28, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0177] Embodiment 30: The cell of Embodiment 29, wherein the antibody is a therapeutic antibody.

[0178] Embodiment 31: A method for glycoengineering a recombinant glycoprotein comprising: delivering an expression vector into a cell according to any one of Embodiments 1 to 27, wherein the expression vector comprises a payload nucleic acid configured to encode the recombinant glycoprotein; and expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a sialylated glycan.

[0179] Embodiment 32: The method of Embodiment 31, wherein the sialylated glycan is an α 2-6 sialyl complex type (SCT) glycan.

[0180] Embodiment 33: The method of Embodiment 32, wherein the α 2-6 sialyl complex type (SCT) glycan is mono-antennary or bi-antennary.

[0181] Embodiment 34: The method of Embodiment 31 or Embodiment 32, wherein the sialylated glycan lacks a core-fucose.

[0182] Embodiment 35: The method of any one of Embodiments 31 to 34, wherein the expression of the payload nucleic acid is constitutive or controllable.

[0183] Embodiment 36: The method of any one of Embodiments 31 to 35, wherein at least 50% of the plurality of the recombinant glycoproteins is conjugated with the sialylated glycan.

[0184] Embodiment 37: The method of any one of Embodiments 31 to 36, wherein the plurality of the recombinant glycoproteins is conjugated with the sialylated glycan at an enriched homogeneity of at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 94%, 95%, 99%, 100% or an integer ranging between any of the above two numbers.

[0185] Embodiment 38: The method of any one of Embodiments 31 to 37, further comprising harvesting the plurality of recombinant glycoproteins within 200 hours from the expression of the payload nucleic acid in the cell.

[0186] Embodiment 39: The method of Embodiment 38, wherein the plurality of recombinant glycoproteins was harvested within 100 hours from the expression of the payload nucleic acid in the cell.

[0187] Embodiment 40: The method of any one of Embodiments 31 to 39, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0188] Embodiment 41: The method of Embodiment 40, wherein the glycan is on a constant region of the antibody.

[0189] Embodiment 42: The method of Embodiment 41, wherein the glycan is on a heavy chain of the antibody.

[0190] Embodiment 43: The method of any one of Embodiments 40 to 42, wherein the glycan is on a glycosylation site of the antibody.

[0191] Embodiment 44: The method of Embodiment 43, wherein the glycan is on an N46, N402, and/or N297 site of the antibody.

[0192] Embodiment 45: The method of any one of Embodiments 40 to 44, wherein the antibody is a therapeutic antibody.

[0193] Embodiment 46: The method of Embodiment 45, wherein the therapeutic antibody is Adalimumab (Humira®), Adalimumab-atto (Amjevita®), Rituximab (Rituxan®), Rituximab -

atto (Truxima®), Cetuximab (Erbix®), Bevacizumab (Avastin®), Infliximab (Remicade®), Trastuzumab (Herceptin®), Pembrolizumab (Keytruda®), Etanercept (Enbrel®), Ipilimumab (Yervoy®), Ofatumumab (Arzerra), Golimumab (Simponi®), Atezolizumab (Tecentriq®), Ocrelizumab (OCREVUS®), Durvalumab (Durvalumab®), Avelumab (Bavencio®), Nivolumab (Opdivo®), Pertuzumab (Perjeta®), Obinutuzumab (Gazyva®), Gazyvaro Infliximab (Remicade®), or Trastuzumab emtansine (Kadcyla®).

[0194] Embodiment 47: A plurality of enriched recombinant glycoproteins, wherein at least 50% of the plurality of recombinant glycoproteins is configured with a sialylated glycan.

[0195] Embodiment 48: The plurality of enriched recombinant glycoproteins of Embodiment 47, wherein at least 70% of the plurality of enriched recombinant glycoproteins is configured with the sialylated glycan.

[0196] Embodiment 49: The plurality of enriched recombinant glycoproteins of Embodiment 47 or Embodiment 48, wherein the plurality of enriched recombinant glycoproteins is conjugated with the sialylated glycan at homogeneity of at least about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

[0197] Embodiment 50: The plurality of enriched recombinant glycoproteins of Embodiment 47 or Embodiment 48, wherein the plurality of the enriched recombinant glycoproteins is conjugated with the sialylated glycan at homogeneity of 1% to 100%, 1% to 90%, 1% to 80%, 1% to 70%, 1% to 60%, 1% to 50%, 1% to 40%, 1% to 30%, 1% to 20%, 1% to 10%, 5% to 100%, 5% to 95%, 5% to 85%, 5% to 75%, 5% to 65%, 5% to 55%, 5% to 45%, 5% to 35%, 5% to 25%, 5% to 10%, 10% to 100%, 10% to 99%, 10% to 95%, 10% to 90%, 10% to 85%, 10% to 80%, 10% to 75%, 10% to 70%, 10% to 65%, 10% to 60%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 20% to 100%, 20% to 99%, 20% to 95%, 20% to 90%, 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 40% to 100%, 40% to 99%, 40% to 95%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 60% to 100%, 60% to 99%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 50% to 100%, 50% to 99%, 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 70% to 100%, 70% to 99%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 80% to 100%, 80% to 99%, 80% to 95%, 80% to 90%, 80% to 85%, 90% to 100%, 90% to 99%, or 90% to 95%.

[0198] Embodiment 51: The plurality of enriched recombinant glycoproteins of any one of Embodiments 47 to 50, wherein the sialylated glycan is an α 2-6 sialyl complex type (SCT) glycan.

[0199] Embodiment 52: The plurality of enriched recombinant glycoproteins of Embodiment 51, wherein the α 2-6 sialyl complex type (SCT) glycan is mono-antennary or bi-antennary.

[0200] Embodiment 53: The plurality of enriched recombinant glycoproteins of Embodiment 51 or Embodiment 52, wherein the sialylated glycan lacks a core-fucose.

[0201] Embodiment 54: The plurality of enriched recombinant glycoproteins of any one of Embodiments 47 to 53, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0202] Embodiment 55: The plurality of enriched recombinant glycoproteins of Embodiment 54, wherein the glycan is on a constant region of the antibody.

[0203] Embodiment 56: The plurality of enriched recombinant glycoproteins of Embodiment 55, wherein the glycan is on a heavy chain of the antibody.

[0204] Embodiment 57: The plurality of enriched recombinant glycoproteins of any one of Embodiments 54 to 56, wherein the glycan is on a glycosylation site of the antibody.

[0205] Embodiment 58: The plurality of enriched recombinant glycoproteins of Embodiment 57, wherein the glycan is on an N46, N402, and/or N297 site of the antibody.

[0206] Embodiment 59: The plurality of enriched recombinant glycoproteins of any one of Embodiments 54 to 58, wherein the antibody is a therapeutic antibody.

[0207] Embodiment 60: The plurality of enriched recombinant glycoproteins of any one of Embodiments 47 to 59, being obtained by using the method for glycoengineering a recombinant glycoprotein of any one of Embodiments 31 to 46.

[0208] Embodiment 61: A cell for expressing a GlcNAc glycoprotein, being deficient in N-acetylglucosaminyltransferase I (GnTI) activity and constitutively or controllably expressing an exogenous endoglycosidase.

[0209] Embodiment 62: The cell of Embodiment 61, wherein the exogenous endoglycosidase is an endoglycosidase H (Endo H) or an endoglycosidase S2 (Endo S2).

[0210] Embodiment 63: The cell of Embodiment 62, wherein the exogenous endoglycosidase comprises an amino acid sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 16.

[0211] Embodiment 64: The cell of any one of Embodiments 61 to 63, wherein the cell comprises a nucleic acid configured to encode the exogenous endoglycosidase.

[0212] Embodiment 65: The cell of Embodiment 64, wherein the nucleic acid comprises a nucleotide sequence as set forth in SEQ ID NO: 17 or SEQ ID NO: 18.

[0213] Embodiment 66: The cell of any one of Embodiments 61 to 65, wherein the cell is a mammalian cell.

[0214] Embodiment 67: The cell of Embodiment 66, wherein the cell is derived from a Chinese hamster ovary cell or a HEK293 cell.

[0215] Embodiment 68: The cell of any one of Embodiments 61 to 67, wherein the cell is derived from an Expi293F™ GnTI KO Cell.

[0216] Embodiment 69: The cell of any one of Embodiments 61 to 68, wherein the cell further comprises a payload nucleic acid configured to encode a recombinant glycoprotein, and the expression of the payload nucleic acid is controlled by a constitutive or an activable promoter.

[0217] Embodiment 70: The cell of Embodiment 69, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0218] Embodiment 71: The cell of Embodiment 70, wherein the antibody is a therapeutic antibody.

[0219] Embodiment 72: A method for glycoengineering a recombinant glycoprotein, comprising: delivering an expression vector into a cell according to any one of Embodiments 61 to 68, wherein the expression vector comprises a payload nucleic acid configured to encode a recombinant glycoprotein; and expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a GlcNAc glycan.

[0220] Embodiment 73: The method of Embodiment 72, wherein the GlcNAc glycan is a mono-GlcNAc glycan or a GlcNAc-Fuc glycan.

[0221] Embodiment 74: The method of Embodiment 72 or Embodiment 73, wherein the expression of the payload nucleic acid is constitutive or controllable.

[0222] Embodiment 75: The method of any one of Embodiments 72 to 74, wherein at least 50% of the plurality of recombinant glycoproteins is conjugated with the GlcNAc glycan.

[0223] Embodiment 76: The method of any one of Embodiments 72 to 75, wherein the plurality of recombinant glycoproteins is conjugated with the GlcNAc glycan at homogeneity of at least about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%.

[0224] Embodiment 77: The method of any one of Embodiments 72 to 76, further comprising harvesting the plurality of recombinant glycoproteins within 200 hours from the expression of the payload nucleic acid in the cell.

[0225] Embodiment 78: The method of Embodiment 77, wherein the plurality of recombinant glycoproteins was harvested within 100 hours from the expression of the payload nucleic acid in the cell.

[0226] Embodiment 79: The method of any one of Embodiments 72 to 78, further comprising: collecting the plurality of recombinant glycoproteins; and incubating the recombinant glycoproteins with an endoglycosidase and a donor glycan.

[0227] Embodiment 80: The method of Embodiment 79, wherein the donor glycan comprises an oxazoline moiety.

[0228] Embodiment 81: The method of Embodiment 79 or Embodiment 80, wherein the donor glycan is a sialyl complex type (SCT) glycan.

[0229] Embodiment 82: The method of any one of Embodiments 79 to 81, wherein the endoglycosidase is an endoglycosidase H (Endo H) or an endoglycosidase S2 (Endo S2).

[0230] Embodiment 83: The method of any one of Embodiments 72 to 82, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0231] Embodiment 84: The method of Embodiment 83, wherein the glycan is on a constant region of the antibody.

[0232] Embodiment 85: The method of Embodiment 84, wherein the glycan is on a heavy chain of the antibody.

[0233] Embodiment 86: The method of any one of Embodiments 83 to 85, wherein the glycan is on a glycosylation site of the antibody.

[0234] Embodiment 87: The method of Embodiment 86, wherein the glycan is located at an N46, N402, and/or N297 site of the antibody.

[0235] Embodiment 88: The method of any one of Embodiments 83 to 87, wherein the antibody is a therapeutic antibody.

[0236] Embodiment 89: The method of Embodiment 88, wherein the therapeutic antibody is Adalimumab (Humira®), Adalimumab-atto (Amjevita®), Rituximab (Rituxan®), Rituximab - atto (Truxima®), Cetuximab (Erbitux®), Bevacizumab (Avastin®), Infliximab (Remicade®), Trastuzumab (Herceptin®), Pembrolizumab (Keytruda®), Etanercept (Enbrel®), Ipilimumab (Yervoy®), Ofatumumab (Arzerra), Golimumab (Simponi®), Atezolizumab (Tecentriq®), Ocrelizumab (OCREVUS®), Durvalumab (Durvalumab®), Avelumab (Bavencio®), Nivolumab (Opdivo®), Pertuzumab (Perjeta®), Obinutuzumab (Gazyva®), Gazyvaro Infliximab (Remicade®), or Trastuzumab emtansine (Kadcyla®).

[0237] Embodiment 90: A plurality of enriched recombinant glycoproteins, wherein at least 50% of the plurality of recombinant glycoproteins is configured with a GlcNAc glycan.

[0238] Embodiment 91: The plurality of enriched recombinant glycoproteins of Embodiment 90, wherein at least 70% of the plurality of recombinant glycoproteins is configured with the GlcNAc glycan.

[0239] Embodiment 92: The plurality of enriched recombinant glycoproteins of Embodiment 90 or Embodiment 91, wherein plurality of the recombinant glycoproteins is conjugated with the GlcNAc glycan at homogeneity of at least about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%, about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%

[0240] Embodiment 93: The plurality of enriched recombinant glycoproteins of Embodiment 90 or Embodiment 91, wherein plurality of the recombinant glycoproteins is conjugated with the GlcNAc glycan at homogeneity of 1% to 100%, 1% to 90%, 1% to 80%, 1% to 70%, 1% to 60%, 1% to 50%, 1 % to 40%, 1% to 30%, 1% to 20%, 1% to 10%, 5% to 100%, 5% to 95%, 5% to 85%, 5% to 75%, 5% to 65%, 5% to 55%, 5% to 45%, 5% to 35%, 5% to 25%, 5% to 10%, 10% to 100%, 10% to 99%, 10% to 95%, 10% to 90%, 10% to 85%, 10% to 80%, 10% to 75%, 10% to 70%, 10% to 65%, 10% to 60%, 10% to 55%, 10% to 50%, 10% to 45%, 10% to 40%, 10% to 35%, 10% to 30%, 10% to 25%, 10% to 20%, 10% to 15%, 20% to

100%, 20% to 99%, 20% to 95%, 20% to 90%, 20% to 85%, 20% to 80%, 20% to 75%, 20% to 70%, 20% to 65%, 20% to 60%, 20% to 55%, 20% to 50%, 20% to 45%, 20% to 40%, 20% to 35%, 20% to 30%, 20% to 25%, 40% to 100%, 40% to 99%, 40% to 95%, 40% to 90%, 40% to 85%, 40% to 80%, 40% to 75%, 40% to 70%, 40% to 65%, 40% to 60%, 40% to 55%, 40% to 50%, 60% to 100%, 60% to 99%, 60% to 95%, 60% to 90%, 60% to 85%, 60% to 80%, 60% to 75%, 50% to 100%, 50% to 99%, 50% to 95%, 50% to 90%, 50% to 85%, 50% to 80%, 50% to 75%, 50% to 70%, 50% to 65%, 50% to 60%, 70% to 100%, 70% to 99%, 70% to 95%, 70% to 90%, 70% to 85%, 70% to 80%, 70% to 75%, 80% to 100%, 80% to 99%, 80% to 95%, 80% to 90%, 80% to 85%, 90% to 100%, 90% to 99%, or 90% to 95%.

[0241] Embodiment 94: The plurality of enriched recombinant glycoproteins of any one of Embodiments 90 to 93, wherein the GlcNAc glycan is a mono-GlcNAc glycan or a GlcNAc-Fuc glycan.

[0242] Embodiment 95: The plurality of enriched recombinant glycoproteins of any one of Embodiments 90 to 94, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.

[0243] Embodiment 96: The plurality of enriched recombinant glycoproteins of Embodiment 95, wherein the glycan is on a constant region of the antibody.

[0244] Embodiment 97: The plurality of enriched recombinant glycoproteins of Embodiment 96, wherein the glycan is on a heavy chain of the antibody.

[0245] Embodiment 98: The plurality of enriched recombinant glycoproteins of any one of Embodiments 95 to 97, wherein the glycan is on a glycosylation site of the antibody.

[0246] Embodiment 99: The plurality of enriched recombinant glycoproteins of Embodiment 98, wherein the glycan is on an N46, N402, and/or N297 site of the antibody.

[0247] Embodiment 100: The plurality of enriched recombinant glycoproteins of any one of Embodiments 95 to 99, wherein the antibody is a therapeutic antibody.

[0248] Embodiment 101: The plurality of enriched recombinant glycoproteins of any one of Embodiments 90 to 100, being obtained by using the method for glycoengineering a recombinant glycoprotein of any one of Embodiments 72 to 89.

[0249] **SEQUENCES**

SEQ ID NO: 01 ST6GAL1, aa	MIHTNLKKKFSCCVLVFLLFAVICVWKEKKKGSYYDSFKLQTKFQVLKSLGKLAM GSDSQSVSSSSTQDPHRGRQTLGSLRGLAKAKPEASFQVWNKDSSSKNLIPRLQKIW KNYLSMNKYKVSYKGP GP GIKFSAEALRCHLRDHVNVSMVEVTDFFPNTSEWEGYL PKESIRTKAGPWGRC AVVSSAGSLKSSQLGREIDDHDAVLRFN GAPTANFQQDVGTK TTIRLMNSQLVTTEKRFLKDSLYNEGILIVWDPSVYHSDIPK WYQNP DYFFNNYKT YRKLHPNQPFYILKPMPWELWDILQEISPEEIQNPSSGMLGIIMMTLCDQVDIYE FLPSKRKTDVCYYYQKFSDACTMGAYHPLLYEKNLVKHLNQGTDEDIYLLGKATLP GFRTIHC
SEQ ID NO: 02 PspST only, aa	NVVAPSLEVYVDHASLPTLQQLMDIIKSEENPTAQRYIAWGRIVPTDEQMKELNITS FALINNHTPADLVQEIVKQAQTKHRLNVKLSSNTAHSFDNLVPILKELNSFNNTVTNI DLYDDGSMEYVNLYNWRDTLNKTDNLKIGKDYLEDVINGINEDTSNTGTSSVYNWQ KLYPANYHFLRKDYLTLEPSLHEL RDYIGDSLKQMQWDGFKKFNSKQQLFLSIVNF DKQKLQNEYNSSNLPNFVFTGTTVWGGNHEREYYAKQQINVINNAINESSPHYLGNS YDLFFKGHPGGGIINTLIMQNYPSMVDIPSKISFEVLMMTDMLPDAVAGIASSLYFTIP AEKIKFIVFTSTETITDRETALRSPLVQVMIKLGIVKEENVLFWADLPNCETGVC
SEQ ID NO: 03 Modified PspST (ST6GAL1 signal peptide), aa	MIHTNLKKKFSCCVLVFLLFAVICVWKEKKKGSYYDSFKLQTKFQVLKSLGKLAM GSDSQSVSSSSTQDPHRGRQTLGSLRGLAKAKPGGGSGGGGSGGGGSNVVAPSLE VYVDHASLPTLQQLMDIIKSEENPTAQRYIAWGRIVPTDEQMKELNITSFALINNHTP ADLVQEIVKQAQTKHRLNVKLSSNTAHSFDNLVPILKELNSFNNTVTNIDLYDDGS MEYVNLYNWRDTLNKTDNLKIGKDYLEDVINGINEDTSNTGTSSVYNWQKLYPANY HFLRKDYLTLEPSLHEL RDYIGDSLKQMQWDGFKKFNSKQQLFLSIVNFDKQKLQNE EYNSSNLPNFVFTGTTVWGGNHEREYYAKQQINVINNAINESSPHYLGNSYDLFFKG HPGGGIINTLIMQNYPSMVDIPSKISFEVLMMTDMLPDAVAGIASSLYFTIPAEKIKFIV FTSTETITDRETALRSPLVQVMIKLGIVKEENVLFWADLPNCETGVC GSGATNFSLLK QAGDVEENPGPMRLREPLLSGSAAMP GASLQACRLLVAVCALHLGVTLVYYLAGR DL S RLPQLVG VSTPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGGDSSPV VDSGPGPASNLTSVPVPHTTALSLPACPEESPLLVGPMLEFNMPVDLELVAKQNPVVK MGGRYAPRDCVSPHKVAIIPFRNRQEHLKYWLYYLHPVLQRQQLDYGIYVINQAGD TIFNRAKLLNVGFQEALKDYDYTCFVFSVDLIPMNDHNAYRCFSQPRHISVAMDKF GFSLPYVQYFGGV SALS KQQLTINGFPNNYWG WGGEDDDIFNRLVFRGMSISRPNA VVGRCRMIRHSRDKKNEPNPQRFDRIAHTKETMLSDGLNSLTYQVLDVQRYPLYTQI TVDIGTPS
SEQ ID NO: 04 Modified PspST (B4GALT1 signal peptide), aa	MRLREPLLSGSAAMP GASLQACRLLVAVCALHLGVTLVYYLAGRDL S RLPQLVGVS TPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGGGGGSGGGGSGGGGSN VVAPSLEVYVDHASLPTLQQLMDIIKSEENPTAQRYIAWGRIVPTDEQMKELNITSFA LINNHTPADLVQEIVKQAQTKHRLNVKLSSNTAHSFDNLVPILKELNSFNNTVTNID LYDDGSMEYVNLYNWRDTLNKTDNLKIGKDYLEDVINGINEDTSNTGTSSVYNWQK LYPANYHFLRKDYLTLEPSLHEL RDYIGDSLKQMQWDGFKKFNSKQQLFLSIVNFD KQKLQNEYNSSNLPNFVFTGTTVWGGNHEREYYAKQQINVINNAINESSPHYLGNSY DLFFKGHPGGGIINTLIMQNYPSMVDIPSKISFEVLMMTDMLPDAVAGIASSLYFTIPAE KIKFIVFTSTETITDRETALRSPLVQVMIKLGIVKEENVLFWADLPNCETGVC GSGATN FSLKQAGDVEENPGPMRLREPLLSGSAAMP GASLQACRLLVAVCALHLGVTLVYY LAGRDL S RLPQLVG VSTPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGGD SSPVVDSGPGPASNLTSVPVPHTTALSLPACPEESPLLVGPMLEFNMPVDLELVAKQN PNVKMGGRYAPRDCVSPHKVAIIPFRNRQEHLKYWLYYLHPVLQRQQLDYGIYVIN

	QAGDTIFNRAKLLNVGFQEALKDYDYTCFVFSVDLIPMNDHNAYRCFSQPRHISVAMDKFGFSLPYVQYFGGVSALSKQQFLTINGFPNNYWGWWGGEDDDIFNRLVFRGMSISRPNVVGRCRMIRHSRDKKNEPNPQRFDRIAHTKETMLSDGLNSLTYQVLDVQRYPLYTQITVDIGTPS
SEQ ID NO: 05 B4GALT1, aa	MRLREPLLSGSAAMPGLQACRLLVAVCALHLGVTLVYYLAGRDL SRLPQLVGVS TPLQGGSNSAAAIGQSSGELRTGGARPPPPLGASSQPRPGDSSPVVDSGPGPASNLTS VPVPHTTALSLPACPEESPLLVGPMLEFNMPVDLELVAKQNPVVKMGGRYAPRDCVS PHKVAHIIPFRNRQEHLKYWLYLHPVLQRQQLDYGIYVINQAGDTIFNRAKLLNVGF QEALKDYDYTCFVFSVDLIPMNDHNAYRCFSQPRHISVAMDKFGFSLPYVQYFGGV SALSKQQFLTINGFPNNYWGWWGGEDDDIFNRLVFRGMSISRPNVVGRCRMIRHSRDKKNEPNPQRFDRIAHTKETMLSDGLNSLTYQVLDVQRYPLYTQITVDIGTPS
SEQ ID NO: 06 Ribosomal Shifting peptide – P2A, aa	GSGATNFSLLKQAGDVEENPGP
SEQ ID NO: 07 Ribosomal Shifting peptide – T2A, aa	GSGEGRGSLLTCGDVEENPGP
SEQ ID NO: 08 Ribosomal Shifting peptide – E2A, aa	GSGQCTNYALLKLAGDVESNPGP
SEQ ID NO: 09 Ribosomal Shifting peptide – F2A, aa	GSGVKQTLNFDLLKLAGDVESNPGP
SEQ ID NO: 10 ST6GAL1, nt	ATGATCCACACCAATCTGAAGAAGAAGTTTAGCTGTTGCGTGCTGGTGTTCCTGCT GTTTCGCTGTGATCTGCGTGTTGGAAGGAGAAGAAGAAGGGCAGCTATTATGACTCC TTTAAGCTGCAGACCAAGGAGTTCAGGTGCTGAAGTCCCTGGGCAAGCTGGCC ATGGGCAGCGACTCCCAGTCCGTGAGCTCCTCCAGCACCCAGGACCCCCACCGG GGAAGGCAGACCCTGGGATCCCTGAGGGGCCTGGCTAAGGCCAAGCCTGAGGCT TCCTTCCAGGTGTGGAATAAGGATAGCTCCAGCAAGAACCTGATCCCTAGGCTGC AGAAGATCTGGAAGAAGTATCTGAGCATGAACAAGTATAAGGTGAGCTATAAGGG CCCTGGCCCTGGCATCAAGTTCTCCGCCGAGGCCCTGAGGTGCCACCTGAGAGAT CACGTGAATGTGAGCATGGTGGAGGTGACCGACTTCCCTTTTAACACCTCCGAGT GGGAGGGCTACCTGCCCAAGGAGAGCATCCGGACCAAGGCCGGCCCTTGGGGCA GATGTGCCGTGGTGTCCAGCGCCGGCTCCCTGAAGAGCTCCAGCTGGGCCGGG AGATCGATGACCACGATGCTGTGCTGAGGTTCAATGGCGCTCCTACCGCTAACTTT CAGCAGGATGTGGGCACCAAGACCACCATCCGGCTGATGAATAGCCAGCTGGTGA CCACCGAGAAGCGGTTTCTGAAGGACTCCCTGTACAACGAGGGGCATCCTGATCGT GTGGGACCCCTCCGTGTACCACAGCGACATCCCCAAGTGATCAGAACCCCGAC TACAACTTCTTCAATAATTACAAGACCTACCGGAAGCTGCACCCCAACCAGCCCTT TTATATCCTGAAGCCCCAGATGCCTTGGGAGCTGTGGGACATCCTGCAGGAGATCT CCCCGAGGAGATCCAGCCTAACCCCTTAGCAGCGGCATGCTGGGCATCATCATC ATGATGACCTGTGCGATCAGGTGGATATCTACGAGTTCTGCGCCAGCAAGCGGA AGACCGACGTGTGTTATTATTATCAGAAGTTCTTCGATTCCGCCTGCACCATGGGC GCCTATCACCTCTGCTGTACGAGAAGAATCTGGTGAAGCACCTGAACCAGGGCA CCGACGAGGACATCTATCTGCTGGGCAAGGCCACCCTGCCCGGCTTTAGGACCAT CCACTGCTGA
SEQ ID NO: 11 PspST only, nt	ATGAAAACTTTTTATTATTAACCTTAAATATTACTTACTGCTTGTATAATTGAGAAG AAAATACACAATCTATTATTAATAATGATATTAATAAACTATTATTGATGAGGAGTA TGTTAATTTAGAGCCAATTAATCAATCAAACATCTCTTTTACAAAACACTCTTGGGT

	ACAAACTTGTGGTACGCAACAACTATTAACAGAACAAAATAAAGAGTCAATATCA TTATCTGTAGTGGCGCCACGATTAGATGACGATGAAAAGTACTGCTTTGATTTTAAT GGTGTAGTAATAAAGGTGAAAAATATATAACAAAAGTAACATTAACGTAAGTGGC TCCATCTTTAGAGGTTTATGTTGATCATGCATCTCTTCCAACCTTCAGCAGCTAAT GGATATTATTAATCGGAAGAAGAAAATCCTACAGCACAAAGATATATAGCTTGGG GGAGAATAGTTCGACTGATGAGCAAATGAAAGAGTTAAATATTACATCGTTTGCA TTGATAAATAACCATAACACCAGCTGACTTAGTACAAGAAATTGTTAAGCAAGCACA AACAAAGCATAGATTGAATGTTAACTTAGCTCTAACACTGCTCATTCAATTTGATAA TTTAGTGCCAATACTAAAAGAATTAAATTTCGTTTAATAACGTTACGGTAACAAATAT AGATTTATATGATGATGGTTCAGCAGAATATGTAAATTTATATAACTGGAGAGATAC ATTAAATAAAACAGATAATTTAAAAATTGGTAAAGATTATCTTGAGGATGTCATTAA TGGTATCAATGAAGACACTTCAAATACAGGAACATCATCTGTTTATAACTGGCAAA AACTATATCCAGCTAACTACCATTTTTTAAGAAAAGATTATTTAACTTTAGAACCAT CATTACATGAGTTACGAGACTATATTGGTGATAGTTTAAAGCAAATGCAATGGGATG GTTTCAAAAAATTCAATAGCAAACAAGAATTATCTTATCGATTGTTAATTTTG ACAAACAAAAATTACAAAATGAATATAATTCATCTAATTTACCAAACCTTTGTGTTTA CAGGTACGACTGTATGGGCTGGTAACCATGAAAGAGAGTATTATGCGAAACAACA AATTAATGTCATTAATAATGCAATTAATGAATCGAGCCACATTATTTAGGCAATAGT TATGATTTGTTCTTCAAAGGTCACCCTGGTGGCGGTATCATTAATACATTAATAATG CAAACTATCCTTCAATGGTTGATATCCATCAAAAATATCATTGAAGTTTGTATG ATGACAGATATGCTTCTGATGCAGTTGCTGGTATAGCGAGCTCTTATATTTACG ATACCAGCTGAAAAAATTAAATTTATAGTTTTTACATCGACAGAACTATAACTGAT CGTGAAACTGCTTTGAGAAGTCCTTTAGTTCAAGTAATGATAAACTAGGTATTGT AAAAGAAGAGAATGTACTTTTTTGGGCTGATCTGCCAAATTGTGAAACAGGTGTT TGTATTGCAGTCTAG
SEQ ID NO: 12 Modified PspST (ST6GAL1 signal peptide), nt	ATGATCCACACCAACCTGAAGAAGAAGTTCTCCTGCTGTGTGCTGGTGTCTGCT GTTTCGCTGTGATCTGCGTGTGGAAGGAGAAGAAGAAGGGCAGCTATTATGACAGC TTTAAAGCTGCAGACCAAGGAGTTTCAGGTGCTGAAGTCCCTGGGCAAGCTGGCCA TGGGCTCCGACTCCCAGTCCGTGTCCAGCTCCAGCACCCAGGATCCTCACCGGGG CAGGCAGACCCCTGGGCTCTCTGAGAGGCTGGCTAAGGCTAAGCCCGGCGCGG AGGCAGCGGAGGAGGAGGATCTGGCGGCGGAGGATCCAATGTGGTGGCCCTTC CCTGGAGGTGTATGTGGACCACGCCTCCCTGCCACCCTGCAGCAGCTGATGGAT ATCATCAAGAGCGAGGAGGAGAATCCTACCGCCCAGCGGTATATCGCCTGGGGCC GGATCGTGCCTACCGACGAGCAGATGAAGGAGCTGAACATCACCTCCTTCGCTCT GATCAACAATCACACCCCGCCGACCTGGTGCAGGAGATCGTGAAGCAGGCCCA GACCAAGCACCGGCTGAACGTGAAGCTGTCTCCAACACCGCCCACTCCTTTGAC AACCTGGTGCCTATCCTGAAGGAGCTGAATTCTTTAATAACGTGACCGTGACCA CATCGACCTGTACGACGACGGCTCCATGGAGTATGTGAACCTGTATAACTGGAGG GACACCCTGAATAAGACCGACAATCTGAAGATCGGCAAGGACTATCTGGAGGACG TGATCAATGGCATCAATGAGGACACCTCCAACACCGGCACCAGCAGCGTGTATAA CTGGCAGAAGCTGTACCCTGCTAATTACCACTTTCTGAGGAAGGATTATCTGACCC TGGAGCCTTCCCTGCACGAGCTGAGGGACTACATCGGCGATAGCCTGAAGCAGAT GCAGTGGGATGGCTTTAAGAAGTTCAACAGCAAGCAGCAGGAGCTGTTTCTGAG CATCGTGAATTTGACAAGCAGAAGCTGCAGAACGAGTATAACTCCTCCAATCTG CCTAACTTTGTGTTACCGGCACACCGTGTGGGGCGGCAATCACGAGCGGGAGT ACTATGCCAAGCAGCAGATCAACGTGATCAATAATGCCATCAATGAGAGCAGCCC CCACTACCTGGGCAATAGCTACGATCTGTTCTTCAAGGGCCACCCTGGCGGCGGC ATCATCAACACCCTGATCATGCAGAACTATCCTTCCATGGTGGATATCCCTAGCAAG ATCTCCTTCGAGGTGCTGATGATGACCGACATGCTGCCCCAGCGCGTGGCTGGCAT CGCCTTAGCCTGTATTTTACCATCCCCGCTGAGAAGATCAAGTTCATCGTGTTTAC CAGCACCGAGACCATACCGACCGGGAGACCGCCCTGAGGTCCCCACTGGTGCA GGTGATGATCAAGCTGGGCATCGTGAAGGAGGAGAACGTGCTGTTTTGGGCCGAC CTGCCAACTGTGAGACCGGCGTGTGTGGCTCCGCGCTACCAACTTCTCCCTGC TGAAGCAGGCTGGCGATGTGGAGGAGAATCCCGGCCCTATGCGGCTGCGGGAGC CCCTGTTGTCCGGCTCTGCCGCTATGCCCGCGCTTCCCTGCAGAGAGCCTGTGCG

	CTGCTGGTGGCTGTGTGCGCTCTGCACCTGGGCGTGACCCTGGTGTACTATCTGGC TGGCCGGGATCTGAGCCGGCTGCCTCAGCTGGTGGGCGTGAGCACCCCTGCAG GGAGGATCCAACCTCCGCCGTGCCATCGGCCAGTCCTCCGGAGAGCTGCGGACCG GAGGCGCTAGGCCTCCACCACCACTGGGCGCTTCTTCCAGCCTCGGCCCGGAGG AGATAGCAGCCCCGTGGTGGACTCCGGCCCTGGACCTGCTTCCAACCTGACCAGC GTGCCCGTGCCTCACACCACCGCTCTGTCCCTGCCCGCCTGTCCCGAGGAGTCCC CTCTGCTGGTGGGCCCTATGCTGATCGAGTTTAATATGCCTGTGGACCTGGAGCTG GTGGCTAAGCAGAATCCTAACGTGAAGATGGGCGGCAGGTACGCTCCAGGGACT GCGTGAGCCCTACAAGGTGGCCATCATCATCCCTTTCCGGAACCGGCAGGAGCA CCTGAAGTACTGGCTGTACTACCTGCACCCTGTGCTGCAGAGGCAGCAGCTGGAC TATGGCATCTACGTGATCAACCAGGCCGGCGACACCATCTTTAACGGGCCAAGCT GCTGAATGTGGGCTTTCAGGAGGCCCTGAAGGATTATGACTACACCTGCTTTGTGT TTAGCGATGTGGATCTGATCCCTATGAACGATCACAACGCCTACCGGTGCTTCAGC CAGCCTCGGCACATCAGCGTGGCCATGGACAAGTTCGGCTTCTCCCTGCCTTACGT GCAGTACTTCGGCGGCGTGTCCGCCCTGTCCAAGCAGCAGTTCCTGACCATCAAC GGCTTTCTTAATAACTACTGGGGCTGGGCGGCGAGGATGATGACATCTTTAATCG GCTGGTGTTTAGGGGCATGAGCATCAGCCGGCCTAACGCCGTGGTGGGCAGGTGC AGGATGATCAGGCACTCCCGGGACAAGAAGAACGAGCCTAATCCTCAGAGTTTG ACAGGATCGCTCACACCAAGGAGACCATGCTGAGCGACGGCCTGAATAGCCTGAC CTACCAGGTGCTGGACGTGCAGAGGTACCCTCTGTATACCCAGATCACCGTGGATA TCGGCACCCCTAGCTGA
SEQ ID NO: 13 Modified PspST (B4GALT1 signal peptide), nt	ATGAGGCTGCGGGAGCCATTGCTGAGCGGCTCCGCTGCCATGCCTGGCGCTTCTC TGCAGAGGGCTTGTAGGCTGCTGGTGGCCGTGTGCGCCCTGCACCTGGGAGTGAC CCTGGTGTACTACCTGGCTGGCCGGGACCTGTCCCGGCTGCCTCAGTTGGTGGGC GTGTCCACCCCTCTGCAGGGCGGCGGAGGCAGCGGAGGAGGAGGATCTGGCGGC GGAGGATCCAATGTGGTGGCCCCCTTCCCTGGAGGTGTATGTGGACCACGCCTCCC TGCCCAACCCTGCAGCAGCTGATGGATATCATCAAGAGCGAGGAGGAGAATCCTAC CGCCAGCGGTATATCGCTGGGGCCGATCGTGCCTACCGACGAGCAGATGAAG GAGCTGAACATCACCTCCTTCGCTCTGATCAACAATCACACCCCGCCGACCTGG TGAGGAGATCGTGAAGCAGGCCAGACCAAGCACCGGCTGAACGTGAAGCTGT CCTCCAACACCGCCCACTCCTTTGACAACCTGGTGCCTATCCTGAAGGAGCTGAA TTCCTTTAATAACGTGACCGTGACCAACATCGACCTGTACGACGACGGCTCCATGG AGTATGTGAACCTGTATAACTGGAGGGACACCCTGAATAAGACCGACAATCTGAA GATCGGCAAGGACTATCTGGAGGACGTGATCAATGGCATCAATGAGGACACCTCC AACACCGGCACCAGCAGCGTGTATAACTGGCAGAAGCTGTACCCTGCTAATTACC ACTTTCTGAGGAAGGATTATCTGACCCTGGAGCCTTCCCTGCACGAGCTGAGGGA CTACATCGGCGATAGCCTGAAGCAGATGCAGTGGGATGGCTTTAAGAAGTTCAAC AGCAAGCAGCAGGAGCTGTTTCTGAGCATCGTGAATTTTGACAAGCAGAAGCTGC AGAACGAGTATAACTCCTCCAATCTGCCTAACTTTGTGTTACCGGCACCACCGTG TGGGGCGGCAATCACGAGCGGGAGTACTATGCCAAGCAGCAGATCAACGTGATCA ATAATGCCATCAATGAGAGCAGCCCCACTACCTGGGCAATAGCTACGATCTGTTT TTCAAGGGCCACCCTGGCGGCGGCATCATCAACACCCTGATCATGCAGAACTATCC TTCCATGGTGGATATCCCTAGCAAGATCTCCTTCGAGGTGCTGATGATGACCGACA TGCTGCCCCGACGCCGTGGCTGGCATCGCCTCTAGCCTGTATTTTACCATCCCCGCT GAGAAGATCAAGTTCATCGTGTTTACCAGCACCGAGACCATCACCGACCGGGAGA CCGCCCTGAGGTCCCCACTGGTGCAGGTGATGATCAAGCTGGGCATCGTGAAGGA GGAGAACGTGCTGTTTTGGGCCGACCTGCCCCAACTGTGAGACCGGCGTGTGT
SEQ ID NO: 14 B4GALT1, nt	ATGAGGCTGCGGGAGCCATTGCTGAGCGGCTCCGCTGCCATGCCTGGCGCTTCTC TGCAGAGGGCTTGTAGGCTGCTGGTGGCCGTGTGCGCCCTGCACCTGGGAGTGAC CCTGGTGTACTACCTGGCTGGCCGGGACCTGTCCCGGCTGCCTCAGTTGGTGGGC GTGTCCACCCCTCTGCAGGGCGGAAGCAATAGCGCCGCTGCCATCGGCCAGTCCT CCGGAGAGCTGCGGACCGGAGGCGCTAGGCCACCTCCACCTCTGGGCGCTTCCCTC CCAGCCTCGGCCTGGAGGAGATAGCAGCCCCGTGGTGGATAGCGGCCCTGGCCCA GCTTCTAACCTGACCAGCGTGCCTGTGCCCCACACCACCGCCCTGAGCCTGCCTG CTTGCCCCGAGGAGTCCCCCTGCTGGTGGGACCAATGCTGATCGAGTTCAATATG

	CCTGTGGATCTGGAGCTGGTGGCCAAGCAGAATCCCAATGTGAAGATGGGCGGCC GGTACGCTCCCCGGGATTGTGTGTCCCCCACAAGGTGGCTATCATCATCCCTTTC CGGAACCGGCAGGAGCACCTGAAGTACTGGCTGTATTACCTGCACCCCGTGCTGC AGAGGCAGCAGCTGGACTATGGCATCTACGTGATCAATCAGGCTGGCGACACCAT CTTCAATCGGGCTAAGCTGCTGAACGTGGGCTTTTCAGGAGGCTCTGAAGGACTAC GACTACACCTGCTTTGTGTTCAGCGATGTGGACCTGATCCCCATGAACGACCACA ACGCTTATAGGTGCTTCTCCCAGCCTAGGCACATCTCCGTGGCTATGGACAAGTTT GGCTTCTCCCTGCCCTACGTGCAGTATTTTGGCGGCGTGTCCGCCCTGAGCAAGCA GCAGTTTCTGACCATCAATGGCTTTCCTAACAATTACTGGGGCTGGGGCGGCGAG GATGATGACATCTTCAACCGGCTGGTGTTCAGGGGCATGTCCATCAGCAGGCCTAA CGCTGTGGTGGGCCGGTGTAGGATGATCAGGCACTCCCGGGACAAGAAGAATGA GCCCAATCCTCAGCGTTTGACCGGATCGCCACACCAAGGAGACCATGCTGTCC GACGGCCTGAATTCCTGACCTACCAGGTGCTGGACGTGCAGAGGTATCCTCTGT ACACCCAGATCACCGTGGATATCGGCACCCCTTCTCTGA
SEQ ID NO: 15 Endo H, aa	MFTPVRRRVRTAALALSAALVLGSTAASGASATPSPAPAPAPVKQGPTSVAYVE VNNNSMLNVGKYTLADGGGNFADVAVIFAANINYDTGKTKAYLHFNENVQRLDNA VTQIRPLQQQGIKVLISVLGNHQGAGFANFPSQQAASAFKQLSDAVAKYGLDGVD DDEYAEYGNNGTAQPNDSFVHLVTALRANMPDKIISLYNIGPAASRLSYGGVDVSD KFDYAWNPPYYGTWQVPGIALPKAQLSPAIVEIGRTSRSTVADLARRTVDEGYGVYLT YNLDGGDRADVSAFTRELYGSEAVRTP
SEQ ID NO: 16 Endo S2, aa	MDKHLVLRKTLGCVCAATLMGAALATHHDSLNTVKAEEKTVQTKTDQQVGAKLV QEIREGKRGPYAGYFRTWHDRASTGIDGKQHPENTMAEVPKEVDILFVFDHTAS DSPFWSELKDSYVHKLHQQTALVQTIGVNELNGRTGLSKDYPDTPEGNKALAAIIV KAFVTDRGVDGLDIDIEHEFTNKRTPEEDARALNVFKEIAQLIGKNGSDKSKLLIMDT TLSVENNPFIKGAEDLDYLLRQYYGSQGGAEVDTINSDWNQYQNYIDASQFMIGF SFEEESASKGNLWFDVNEYDPNNPEKGKDIEGTRAKKYAEWQSTGGLKAGIFSAYI DRDGVAVHPSTYKNRTSTNLQRHEVDNISHTDYTVSRKLKTLMTEDKRYDVIDQKDI PDPALREQIIQQVGQYKGDRLERYNKTIVLTGDKIQNLKGLEKLSKLQKLELRQLSNV KEITPELLPESMKKDAELVMVGMTGLEKLNLSGLNRQTLGDIDVNSITHLTSFDISHN SLDLSEKSEDRKLLMTLMEQVSNHQKITVKNTAFENQPKGYYPQTYDTKEGHYDV DNAEHDILTDFVFGTVTKRNTFIGDEEAFIYKEGAVDGRQYVSKDITYEAFRKDYK GYKVHLTASNLGETVTSKVTATTDETYLVLDVSDGEKVVHMKLNIGSGAIMMENLA KGAKVIGTSGDFEQAKKIFDGEKSDRFFTWGQTNWIAFDLGEINLAKEWRLFNAETN TEIKTDSLNVAKGRLQILKDDTTIDLEKMDIKNRKEYLSNDENWTDVAQMDDAKAIF NSKLSNVLSRYWRFCDVGGSYYYPQYTELQILGQRLSNDVANTLKD
SEQ ID NO: 17 Endo H, nt	GACTCGAAGCACTCGCTCGGCGCTTCCGACTGGCGGGGCGCCGACCATCCCGTTG GGAGACCATCAGCCATGTTCACTCCGGTTCGCAGAAGGGTGCAGGACGGCTGCGCT CGCGCTCTCGGCCGCCGCGGCCCTCGTCCTCGGTTCCACCGCCGCGAGCGGCGCG TCAGCGACCCCTCACCCGCTCCGGCCCCGGCCCCGGCCCCGGTGAAGCAGGGG CCGACCTCGGTGGCCTACGTCGAGGTGAACAACAACAGCATGCTCAACGTCGGC AAGTACACCCTGGCGGACGGAGGCGGCAACGCCTTCGACGTAGCCGTGATCTTCG CGGCGAACATCAACTACGACACCGGCACGAAGACGGCCTACCTGCACTTCAACG AGAACGTGCAGCGCGTCCTTGACAACGCTGTCACGCAGATACGGCCGTTGCAGCA ACAGGGCATCAAGGTCCTCTCTCGGTGCTCGGCAACCACAGGGCGCCGGGTTG GCGAACTTCCCCTCACAGCAGGCGGCTTCGGCGTTTCGGAAGCAGCTCTCGGAC GCCGTGGCGAAGTACGGCCTCGACGGCGTCGACTTCGACGACGAATACGCCGAG TACGGCAACAACGGCACCGCGCAGCCCAACGACAGTTCGTTCTGTGCACCTGGTG ACGGCACTGCGCGCGAACATGCCCCGACAAGATCATCAGCCTCTACAACATCGGCC CGGCCGCGTCCCGCCTGTCTGACGGCGGTGTCGACGTCTCCGACAAGTTCGACTA CGCCTGGAATCCCTACTACGGCACCTGGCAGGTCCCCGGCATCGCACTGCCAAG GCGCAGCTGTGCGCGGCGGCCGTCGAGATCGGCCGGACCTCACGGAGCACCGTC GCCGACCTCGCCCGTCGCACCGTCGACGAGGGGTACGGCGTCTATCTGACGTACA ACCTCGACGGCGCGGATCGCACCGCCGACGTCTCCGCGTTACACAGGGAGCTGTA CGGCAGCGAGGCGGTCCGGACGCCGTAGGGGCGTCGGGGCCTGCCGTACGTCCA

	GTACGAAGGTGCCGCCGGCGGTGGTCGCCTGGCCGTGCCCGAAAGCGGCCGCCG GCGTCCAGGATCC
SEQ ID NO: 18 Endo S2, nt	GGCGAACTATAGGAATGCGCTTACATAGATGGTATATCAGATGGGAAGCCATGACT TAGTACCAAAAATAAGGAGTGTCCAAATGGATAAACATTTGTTGGTAAAAAGAAC ACTAGGGTGTGTTTGTGCTGCAACGTTGATGGGAGCTGCCTTAGCGACCCACCAT GATTCACTCAATACTGTAAAGCGGAGGAGAAGACTGTTCAAACAGGAAAGACA GATCAGCAGGTTGGTGCTAAATTGGTACAGGAAATCCGTGAAGGAAAACGCGGA CCACTATATGCTGGTTATTTTAGGACATGGCATGATCGTGCTTCAACAGGAATAGAT GGTAAACAGCAACATCCAGAAAATACTATGGCTGAGGTCCCAAAGAAGTTGATA TCTTATTTGTTTTTCATGACCATACAGCTTCAGATAGTCCATTTTGGTCTGAATTA GGACAGTTATGTCCATAAATTACATCAACAGGGAACGGCACTTGTTCAGACAATTG GTGTTAACGAATTAATGGACGTACAGGTTTATCTAAAGATTATCCTGATACTCCTG AGGGGAACAAAGCTTTAGCAGCAGCCATTGTCAAGGCATTTGTAAGTATGATCGTGG TGTCGATGGACTAGATATTGATATTGAGCACGAATTTACGAACAAAAGAACACCTG AAGAAGATGCTCGTGCTCTAAATGTTTTAAAGAGATTGCGCAGTTAATAGGTAAA AATGGTAGTGATAAATCTAAATGCTCATCATGGACACTACCCTAAGTGTGAAAA TAATCCAATATTTAAAGGGATAGCGGAAGATCTTGATTATCTTCTTAGACAATATTAT GGTTCACAAGGTGGAGAAGCTGAAGTGGATACTATAAACTCTGATTGGAACCAAT ATCAGAATTATATTGATGCTAGCCAGTTCATGATTGGATTCTCCTTTTTTGAAGAATC TGCGTCCAAAGGGAATTTATGGTTTGATGTTAACGAATACGACCCTAACAACTCCTG AAAAAGGGAAAGATATTGAAGGAACACGTGCTAAAAAATATGCAGAGTGGCAAC CTAGTACAGGTGGTTTAAAAGCAGGTATATTCTCTTATGCTATTGATCGTGATGGAG TGGCTCATGTTCCCTTCAACATATAAAAATAGGACTAGTACAAATTTACAACGGCAT GAAGTCGATAATATCTCACATACTGACTACACCGTATCTCGAAAATTA AAAACATTG ATGACCGAAGACAAACGCTATGATGTCATTGATCAAAAAGACATTCCTGACCCAG CATTAAAGAGAACAAATCATTCAACAAGTTGGACAGTATAAAGGCGATTTGGAACG TTATAACAAGACATTGGTGCTTACAGGAGATAAGATTCAAAATCTTAAAGGACTAG AAAAATTAAGCAAGTTACAAAAATTAGAGTTGCGCCAGCTATCTAACGTTAAAGA AATTACTCCAGAACTTTTGCCGGAAGCATGAAAAAAGATGCTGAGCTTGTTATG GTAGGCTGACTGGTTTAGAAAACTAAACCTTAGTGGTCTAAATCGTCAAACTTT AGACGGTATAGACGTGAATAGTATTACGCATTTGACATCATTGATATTTACATAAT AGTTTGGACTTGTCGGAAGAGAGTGAAGACCGTAAACTATTAATGACTTTGATGG AGCAGGTTTCAAATCATCAAAAAATAACGGTGAAAAATACGGCTTTTGAAAAATCA AAAACCGAAAGGTTATTATCCTCAGACGTATGATACCAAAGAAGGTCATTATGATG TTGATAATGCAGAACATGATATTTAACTGATTTTGTGTTTTGGAACGTGTTACTAAAC GTAATACCTTTATTGGAGACGAAGAAGCATTTGCTATCTATAAAGAAGGAGCTGTC GATGGTCGACAATATGTGTCTAAAGACTATACTTATGAAGCTTTTCGTAAAGACTAT AAAGGTTACAAGGTTCAATTAAGTCTTCTAACCTAGGAGAAACAGTTACTTCTAA GGTAACTGCTACTACTGATGAACTTACTTAGTAGATGTTTCTGATGGGGA AAAAG TTGTTACACCATGAAACTCAATATAGGATCTGGTGCCATCATGATGGAAAATCTG GCAAAAGGGGCTAAAGTGATTGGTACATCTGGGGACTTTGAGCAAGCAAAGAAG ATTTTCGATGGTGAAAAGTCAGATAGATTCTTCACTTGGGGACAACTAACTGGAT AGCTTTTGATCTAGGAGAAATTAATCTTGCGAAGGAATGGCGTTTATTTAATGCAG AGACAAATACTGAAATAAAGACAGATAGTAAACGTGGCTAAAGGACGTCT TCAGATTTTAAAGATACAACCTATTGATTTAGAAAAAATGGACATAAAAAATCGTA AAGAGTATCTGTGAATGATGAAAATTGGACTGATGTTGCTCAGATGGATGATGCA AAAGCGATATTTAATAGTAAATTATCCAATGTTTTATCTCGGTATTGGCGGTTTTGTG TAGATGGTGGAGCTAGCTCTTATTACCCTCAATATACCGAACTTCAAATCCTCGGAC AACGTTTATCAAATGATGTCGCTAATACGCTGAAGGATTGATATTAAGTCCTACGA TTACAAAGATAAACAGTTTCTCAAAGTTGATTTAAGCACTTTAAACCTAACTAAA AATCTGAGATGAATAGTCCCAGATTTTATAGTCTTTTATAGGTTTTGATGACATAAAG CTAAATAATCGTTAGACTACCAGAAAGTGCGCG
SEQ ID NO: 19	GGCTCCGGCGCTACCAACTTCTCCCTGCTGAAGCAGGCTGGCGATGTGGAGGAGA ATCCCGGCCCT

Ribosomal Shifting peptide – P2A, nt	
SEQ ID NO: 20 Ribosomal Shifting peptide – T2A, nt	GGTTCAGGTGAGGGCCGCGGCAGTCTGCTGACTTGCGGCGACGTCGAGGAGAAC CCCCGGTCCT
SEQ ID NO: 21 Ribosomal Shifting peptide – E2A, nt	GGGAGTGGGCAATGCACTAACTATGCATTGCTTAAGTTGGCTGGTGATGTAGAATC TAATCCTGGGCCC
SEQ ID NO: 22 Ribosomal Shifting peptide – F2A, nt	GGCAGTGGGGTGAAGCAGACCCTCAATTTTGACTTGTTGAAACTCGCAGGCGAC GTAGAATCTAACCCCGGACCT
SEQ ID NO: 23 SWG-006, nt	GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACCTCAGTACAATCTGCTCT GATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTGCTGA GTAGTGC GCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGC ATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAG ATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA TTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCC CCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATG GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAATTACCATGGTGAT GCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGACTCACGGGGATTTC CAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACG GGACTTTCCAAAATGTCGTAACAACCTCCGCCCATGACGCAATGGGCGGTAGG CGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCA CTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAAGTGGC TAGCGCCGCCACCATGATCCACACCAATCTGAAGAAGAAGTTTAGCTGTTGCGTG CTGGTGTTCCTGCTGTTTCGCTGTGATCTGCGTGTGGAAGGAGAAGAAGAAGGGCA GCTATTATGACTCCTTTAAGCTGCAGACCAAGGAGTTCCAGGTGCTGAAGTCCCTG GGCAAGCTGGCCATGGGCAGCGACTCCAGTCCGTGAGCTCCTCCAGCACCCAG GACCCCCACCGGGAAGGCAGACCCTGGGATCCCTGAGGGGCTGGCTAAGGCC AAGCCTGAGGCTTCCITCCAGGTGTGGAATAAGGATAGCTCCAGCAAGAACCTGA TCCCTAGGCTGCAGAAGATCTGGAAGAACTATCTGAGCATGAACAAGTATAAGGT GAGCTATAAGGGCCCTGGCCCTGGCATCAAGTTCTCCGCCGAGGCCCTGAGGTGC CACCTGAGAGATCACGTGAATGTGAGCATGGTGGAGGTGACCGACTTCCCTTTTA ACACCTCCGAGTGGGAGGGCTACCTGCCCAAGGAGAGCATCCGGACCAAGGCCG GCCCTTGGGGCAGATGTGCCGTGGTGTCCAGCGCCGGCTCCCTGAAGAGCTCCCA GCTGGGCGGGAGATCGATGACCACGATGCTGTGCTGAGGTTCAATGGCGCTCCT ACCGCTAACTTTACGACAGGATGTGGGCACCAAGACCACCATCCGGCTGATGAATA GCCAGCTGGTGACCACCGAGAAGCGGTTTCTGAAGGACTCCCTGTACAACGAGG GCATCCTGATCGTGTGGGACCCCTCCGTGTACACAGCGACATCCCCAAGTGGTAT CAGAACCCCGACTACAACCTTCTTCAATAATTACAAGACCTACCGGAAGCTGCACC CCAACCAGCCCTTTTATATCCTGAAGCCCCAGATGCCTTGGGAGCTGTGGGACATC CTGCAGGAGATCTCCCCCGAGGAGATCCAGCCTAACCCCCCTAGCAGCGGCATGC TGGGCATCATCATCATGATGACCCTGTGCGATCAGGTGGATATCTACGAGTTCCTGC CCAGCAAGCGGAAGACCGACGTGTGTTATTATTATCAGAAGTTCTTCGATTCCGCC TGCACCATGGGCGCCTATCACCTCTGCTGTACGAGAAGAATCTGGTGAAGCACC TGAACCAGGGCACCGACGAGGACATCTATCTGCTGGGCAAGGCCACCCTGCCCGG CTTTAGGACCATCCACTGCGGCAGCGGCGCTACCAATTTACGCTGCTGAAGCAG GCTGGCGATGTGGAGGAGAACCCCGGCCCATGAGGCTGCGGGAGCCATTGCTG

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SEQ ID NO: 24 SWG-015, nt	GACGGATCGGGAGATCTCCCGATCCCTATGGTGCACTCTCAGTACAATCTGCTCT GATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTGCTGA GTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGC ATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAG ATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA TTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCG CCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT

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SEQ ID NO: 27 pcDNA2 TADA (Adalimumab), nt	GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCATCTCAGTACAATCTGCTCT GATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGA GTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGC ATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAG ATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCA TTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCG CCTGGCTGACCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT AAACTGCCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT

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What is claimed is:

1. A cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity.
2. The cell of claim 1, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are expressed in a single transcript.
3. The cell of claim 1, comprising a first nucleic acid configured to express the exogenous sialyltransferase catalytic peptide and a second nucleic acid configured to express the exogenous galactosyltransferase catalytic peptide, wherein the first nucleic acid and the second nucleic acid are transcriptionally controlled by the same promoter.
4. The cell of claim 3, wherein the first nucleic acid and the second nucleic acid are connected to each other via a connecting nucleic acid, which is configured to encode a ribosomal shifting peptide.
5. The cell of claim 3, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are co-configured to be expressed as a fusion protein.
6. The cell of claim 5, wherein the fusion protein comprises a first portion having the sialyltransferase catalytic peptide domain and a second portion having the galactosyltransferase catalytic peptide domain, wherein the first portion and the second portion are connected to each other via a cleavable linker, further wherein the cleavable linker is configured to be cleavable post-translation of the fusion protein, thereby upon cleavage releasing the sialyltransferase catalytic peptide and the galactosyltransferase catalytic peptide as separate proteins.
7. The cell of claim 3, wherein the first nucleic acid is derived from an ST6Gal1 gene or a PspST gene.
8. The cell of claim 3, wherein the second nucleic acid is derived from an B4GALT1 gene.
9. The cell of claim 3, wherein the promoter is a constitutive promoter or an activable promoter.

10. The cell of claim 1, wherein the sialyltransferase is a beta-galactoside alpha-2,6-sialyltransferase 1.
11. The cell of claim 1, wherein the galactosyltransferase catalytic peptide is a beta-1,4-galactosyltransferase 1.
12. The cell of claim 1, wherein the cell is deficient in fucosyltransferase activity.
13. A method for glycoengineering a recombinant glycoprotein comprising:

delivering an expression vector into a cell according to any one of claims 1 to 12, wherein the expression vector comprises a payload nucleic acid configured to encode the recombinant glycoprotein; and

expressing the payload nucleic acid in the cell, thereby obtaining a plurality of recombinant glycoproteins, where at least one recombinant glycoprotein of the plurality is conjugated with a sialylated glycan.
14. The method of claim 13, wherein the sialylated glycan is an α 2-6 sialyl complex type (SCT) glycan.
15. The method of claim 13, wherein the sialylated glycan lacks a core-fucose.
16. The method of claim 13, wherein at least 50% of the plurality of the recombinant glycoproteins is conjugated with the sialylated glycan.
17. The method of claim 13, further comprising harvesting the plurality of recombinant glycoproteins within 200 hours from the expression of the payload nucleic acid in the cell.
18. The method of claim 13, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.
19. The method of claim 18, wherein the glycan is on an N46, N402, and/or N297 site of the antibody.
20. The method of claim 18, wherein the antibody is Adalimumab (Humira[®]), Adalimumab-atto (Amjevita[®]), Rituximab (Rituxan[®]), Rituximab -atto (Truxima[®]), Cetuximab (Erbix[®]), Bevacizumab (Avastin[®]), Infliximab (Remicade[®]), Trastuzumab (Herceptin[®]), Pembrolizumab

(Keytruda[®]), Etanercept (Enbrel[®]), Ipilimumab (Yervoy[®]), Ofatumumab (Arzerra), Golimumab (Simponi[®]), Atezolizumab (Tecentriq[®]), Ocrelizumab (OCREVUS[®]), Durvalumab (Durvalumab[®]), Avelumab (Bavencio[®]), Nivolumab (Opdivo[®]), Pertuzumab (Perjeta[®]), Obinutuzumab (Gazyva[®]), Gazyvaro Infliximab (Remicade[®]), or Trastuzumab emtansine (Kadcyla[®]).

21. A plurality of enriched recombinant glycoproteins, wherein at least 50% of the plurality of recombinant glycoproteins is configured with a sialylated glycan.
22. The plurality of enriched recombinant glycoproteins of claim 21, wherein at least 70% of the plurality of enriched recombinant glycoproteins is configured with the sialylated glycan.
23. The plurality of enriched recombinant glycoproteins of claim 21, wherein the plurality of the enriched recombinant glycoproteins is conjugated with the sialylated glycan at homogeneity of 40% to 99%
24. The plurality of enriched recombinant glycoproteins of claim 21, wherein the sialylated glycan is an α 2-6 sialyl complex type (SCT) glycan.
25. The plurality of enriched recombinant glycoproteins of claim 21, wherein the recombinant glycoprotein is an antibody or an antigen-binding fragment thereof.
26. The plurality of enriched recombinant glycoproteins of claim 25, wherein the glycan is on an N46, N402, and/or N297 site of the antibody.
27. The plurality of enriched recombinant glycoproteins of claim 21, being obtained by using the method for glycoengineering a recombinant glycoprotein of claim 13.

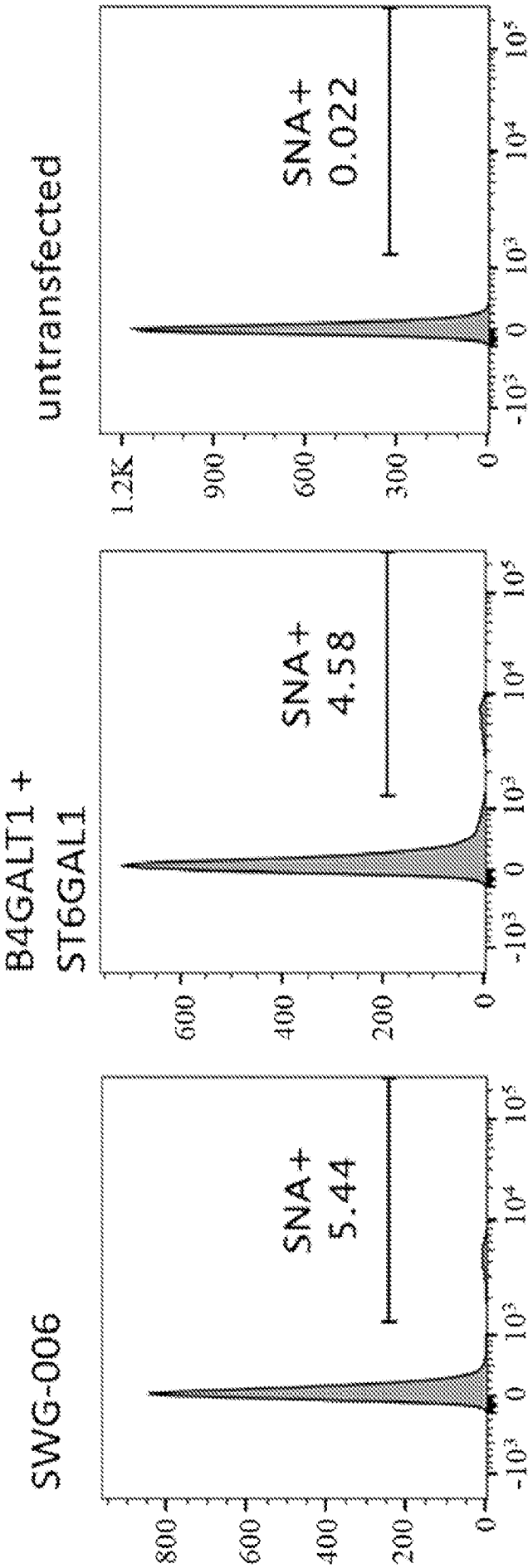


FIG. 1

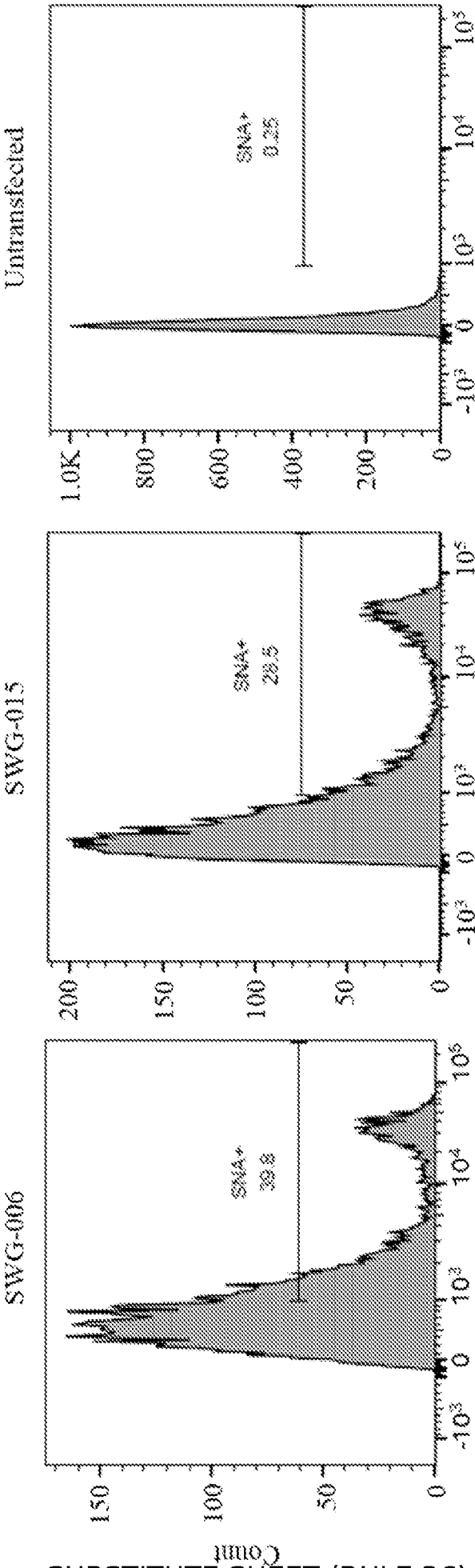


FIG. 2

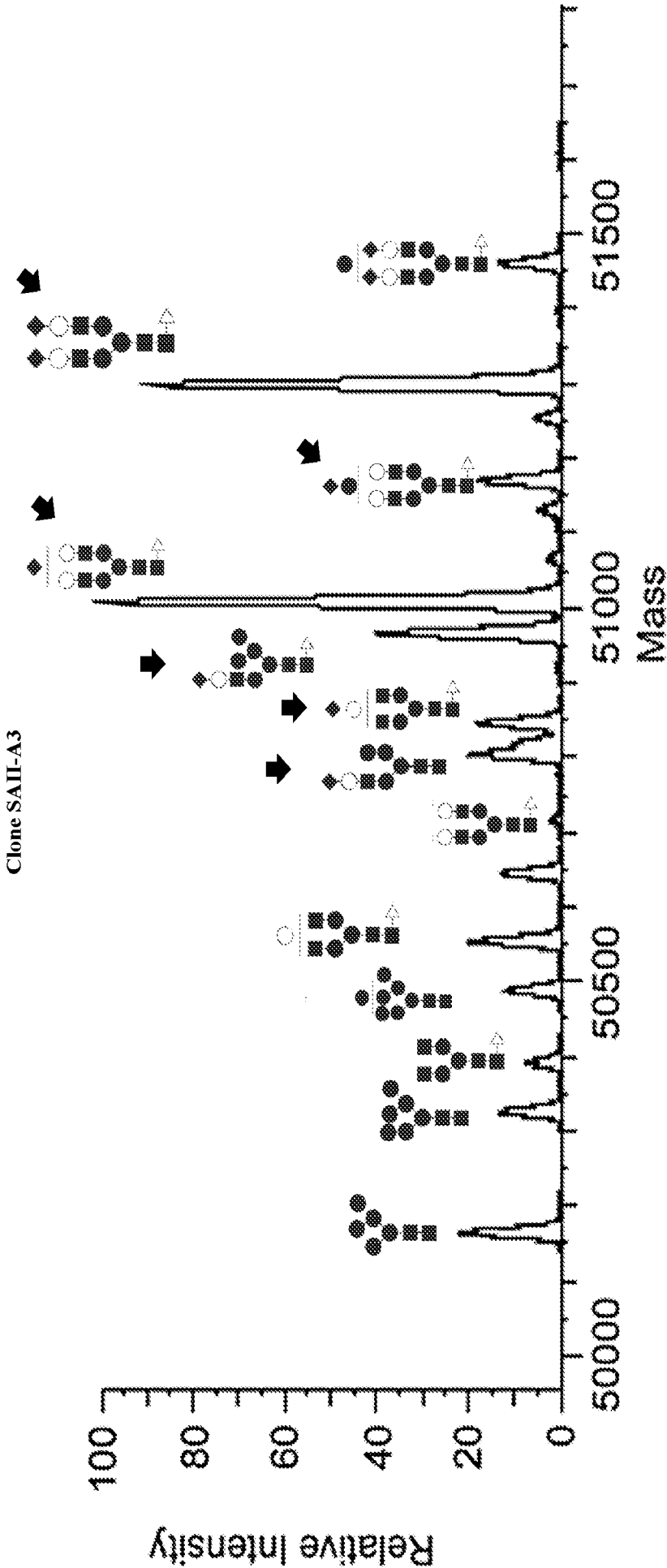


FIG. 3A

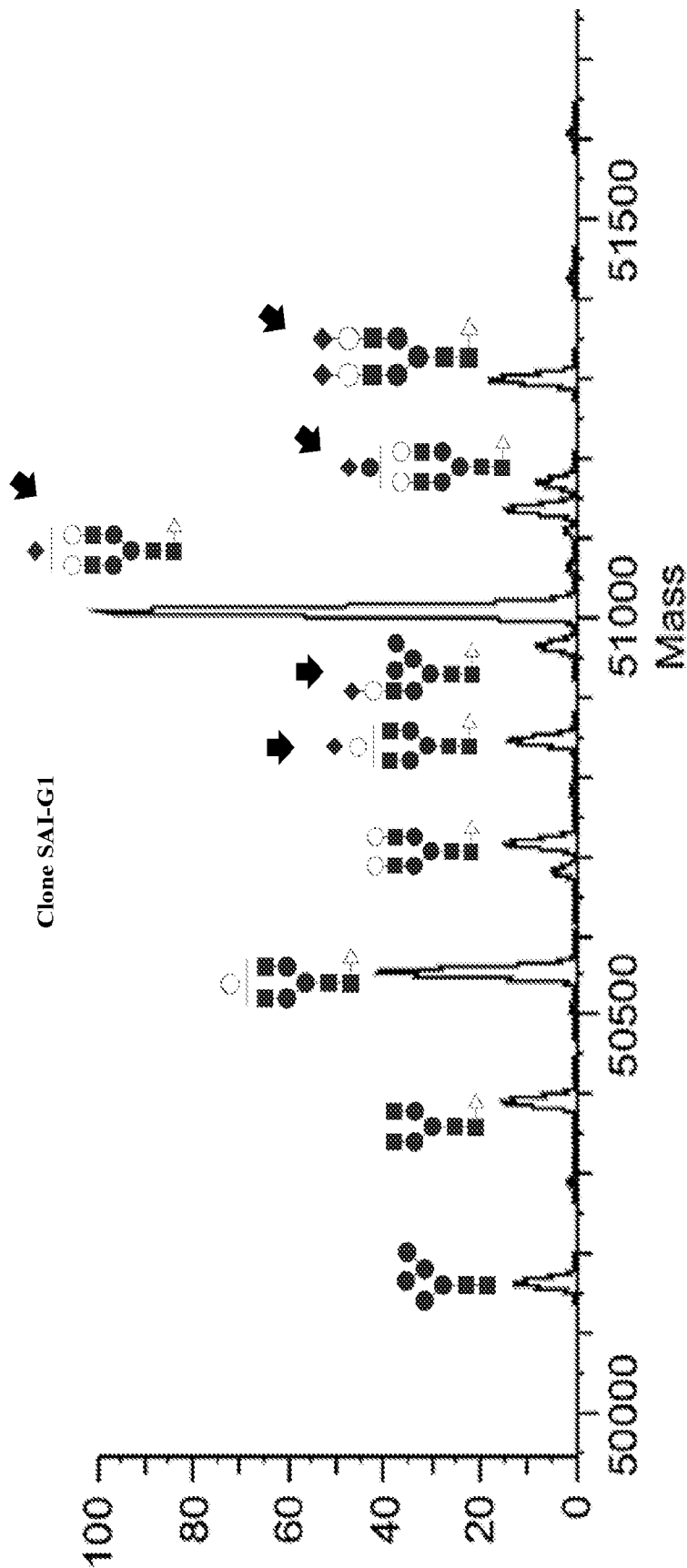


FIG. 3B

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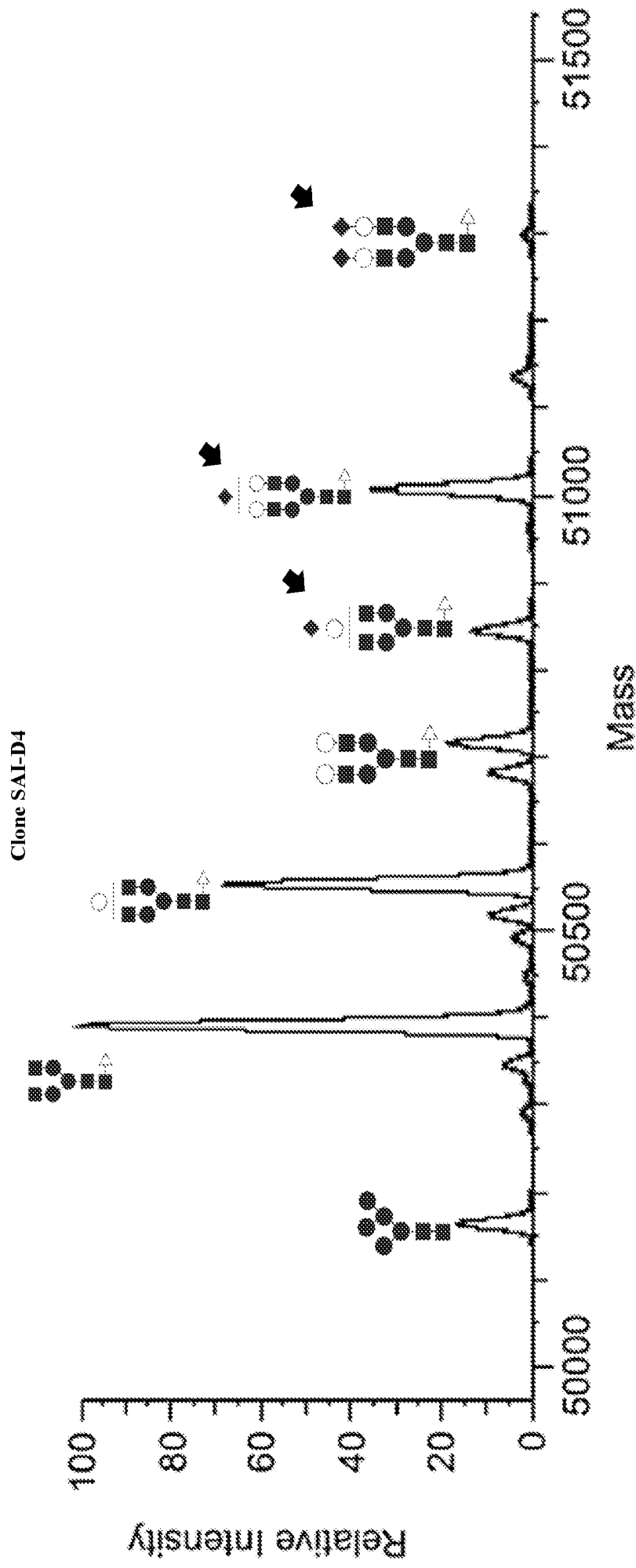


FIG. 3C

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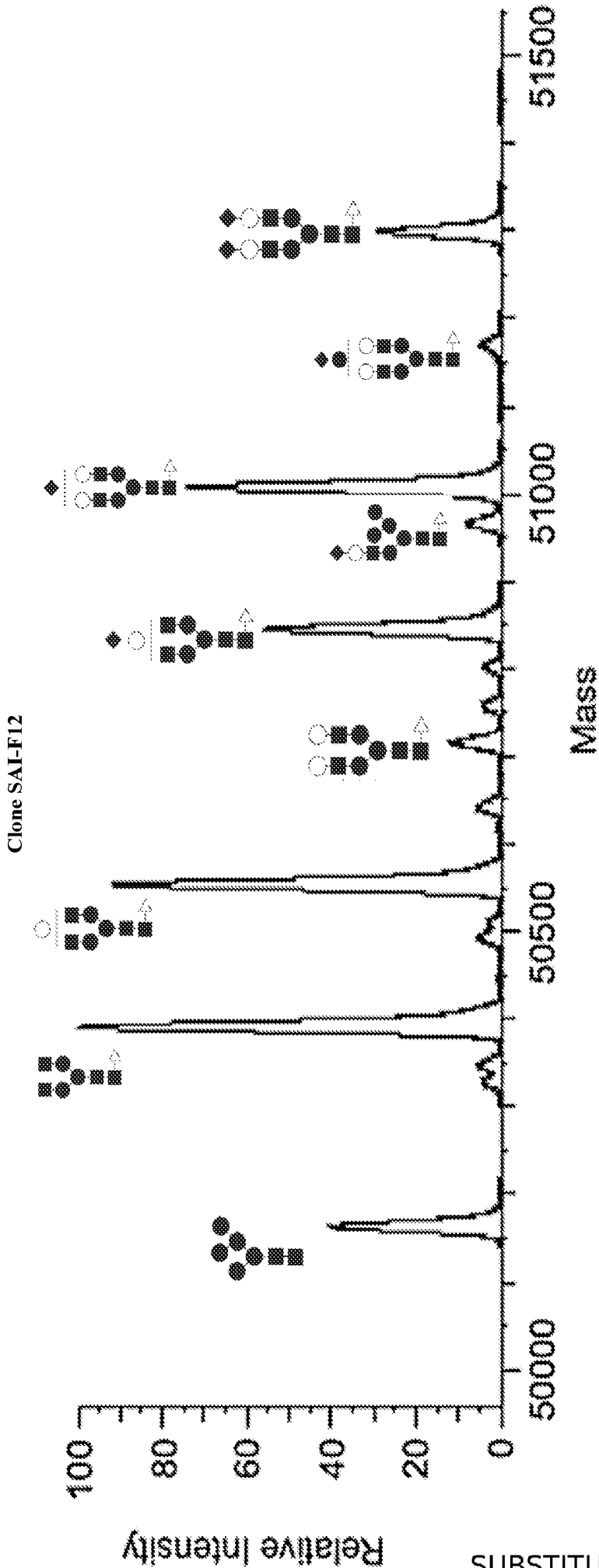


FIG. 3D

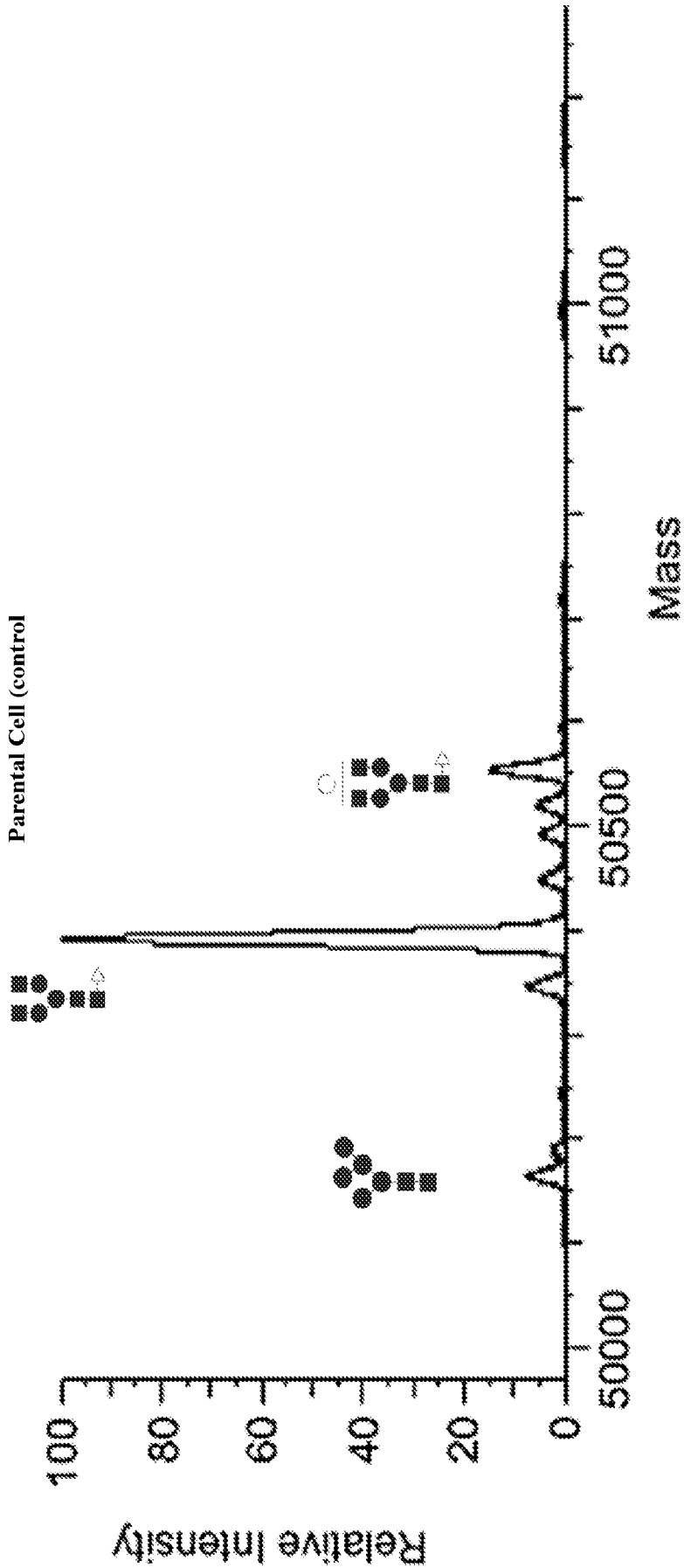


FIG. 3E

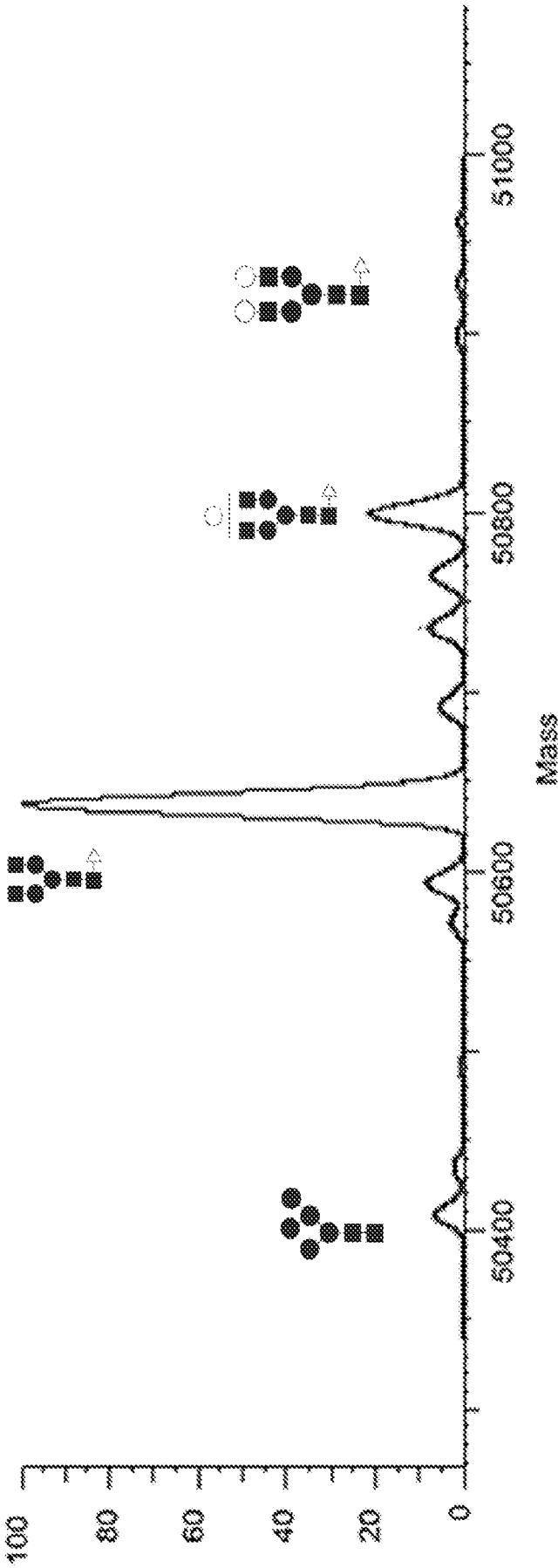


FIG. 4A

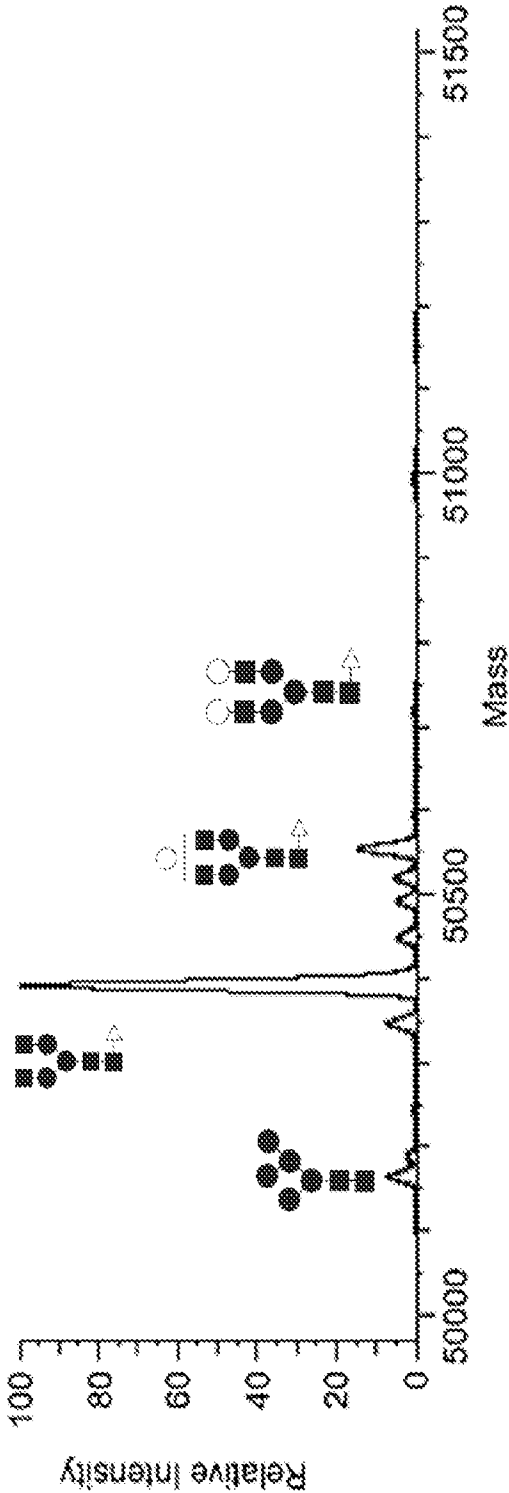


FIG. 4B

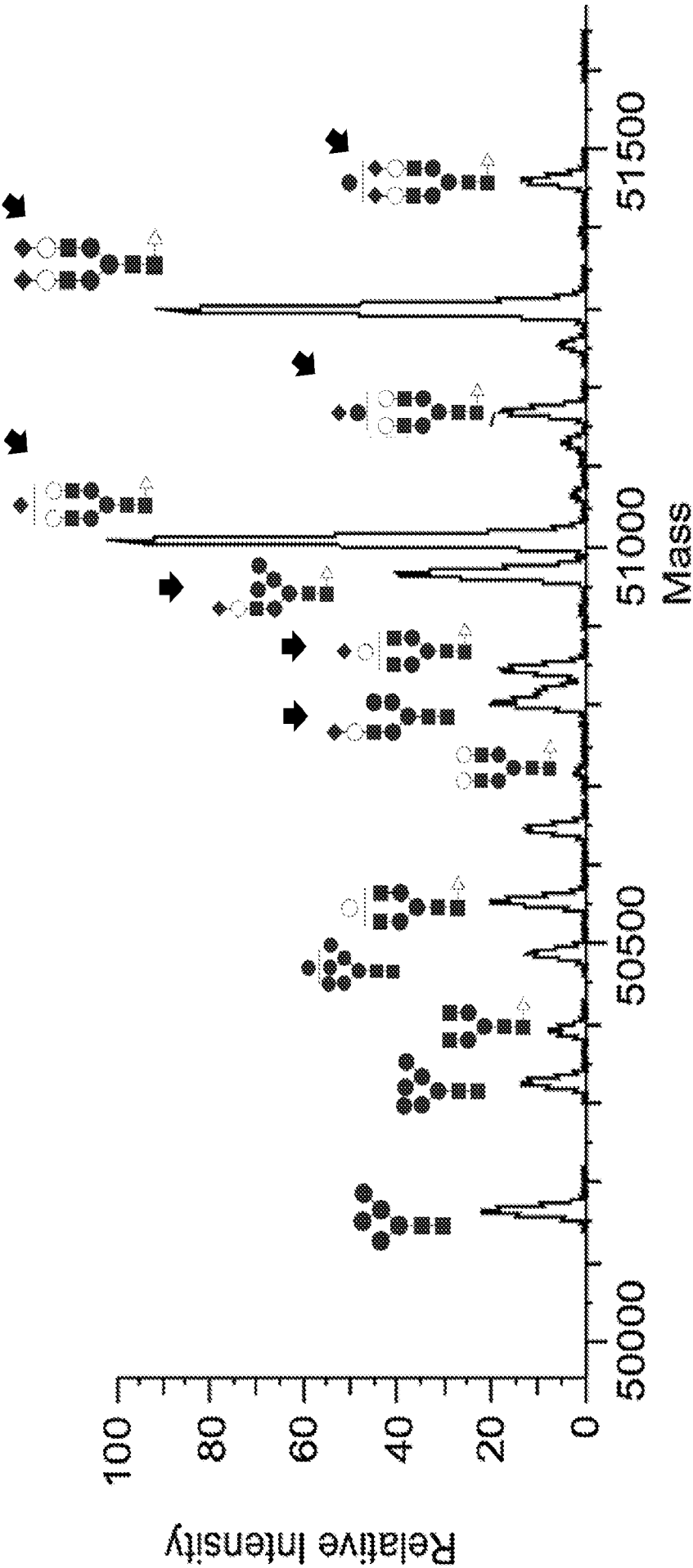
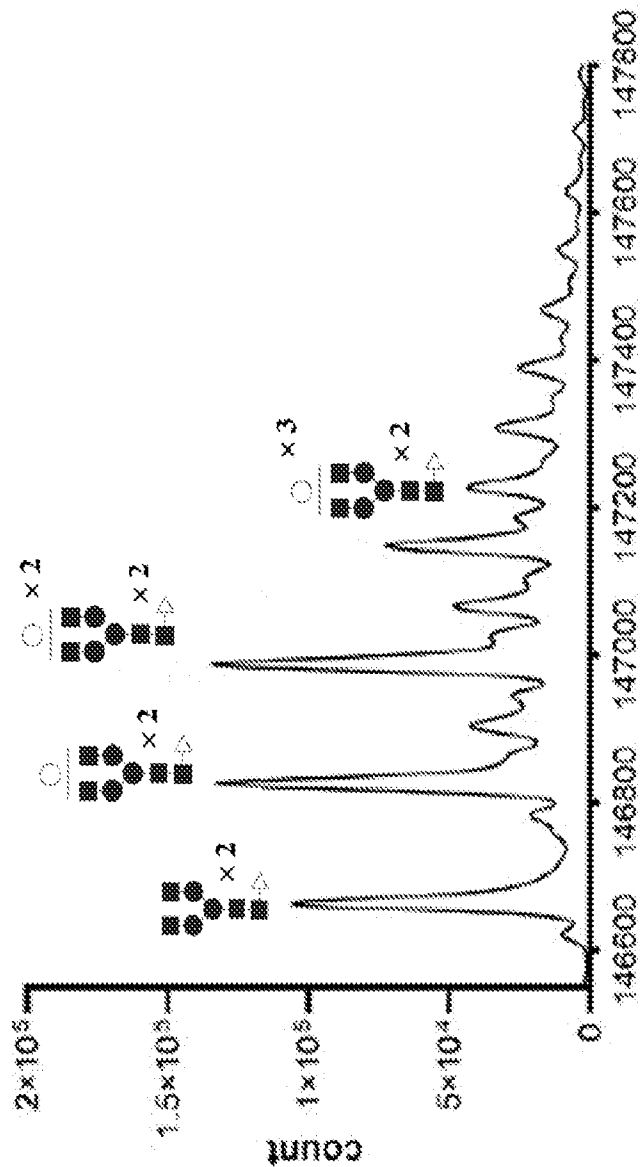


FIG. 4C



Deconvoluted Mass (amu)

FIG. 5A

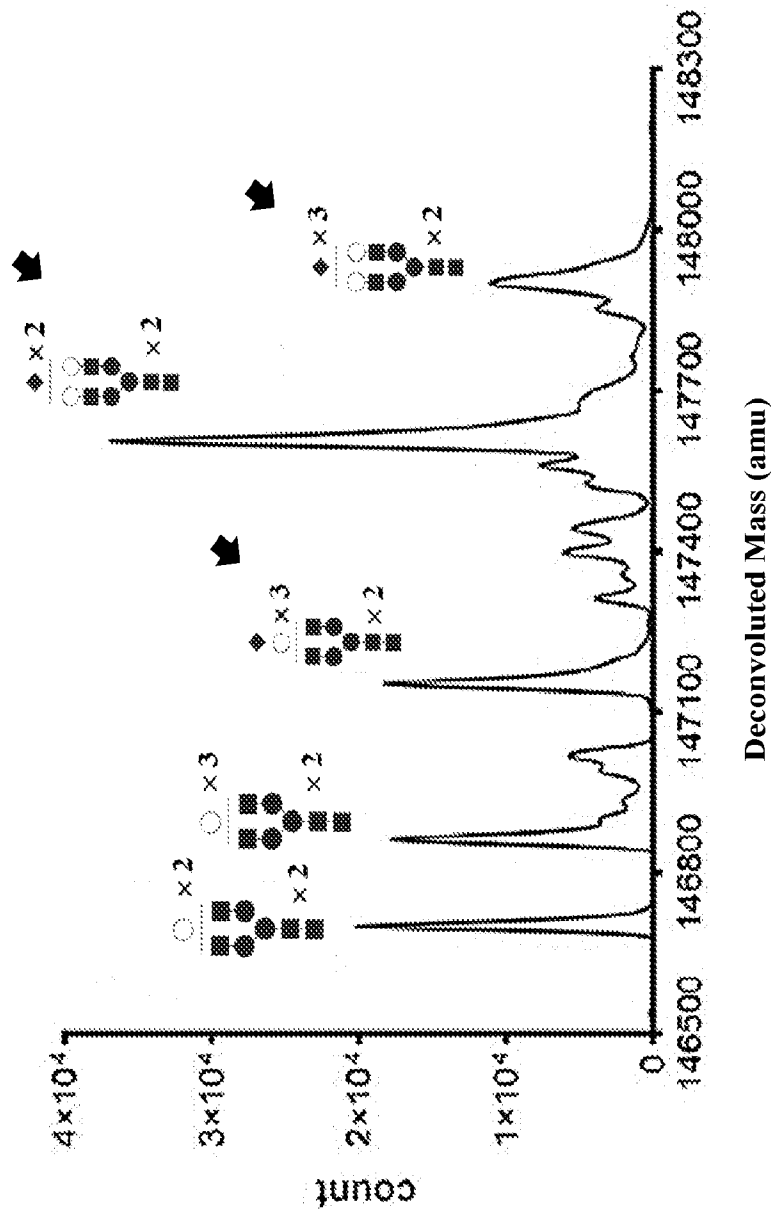


FIG. 5B

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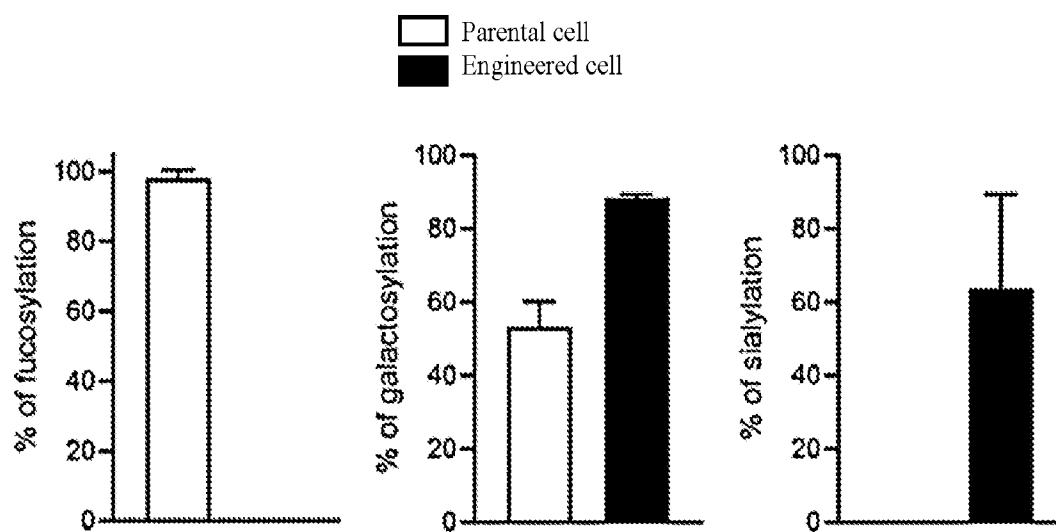


FIG. 6

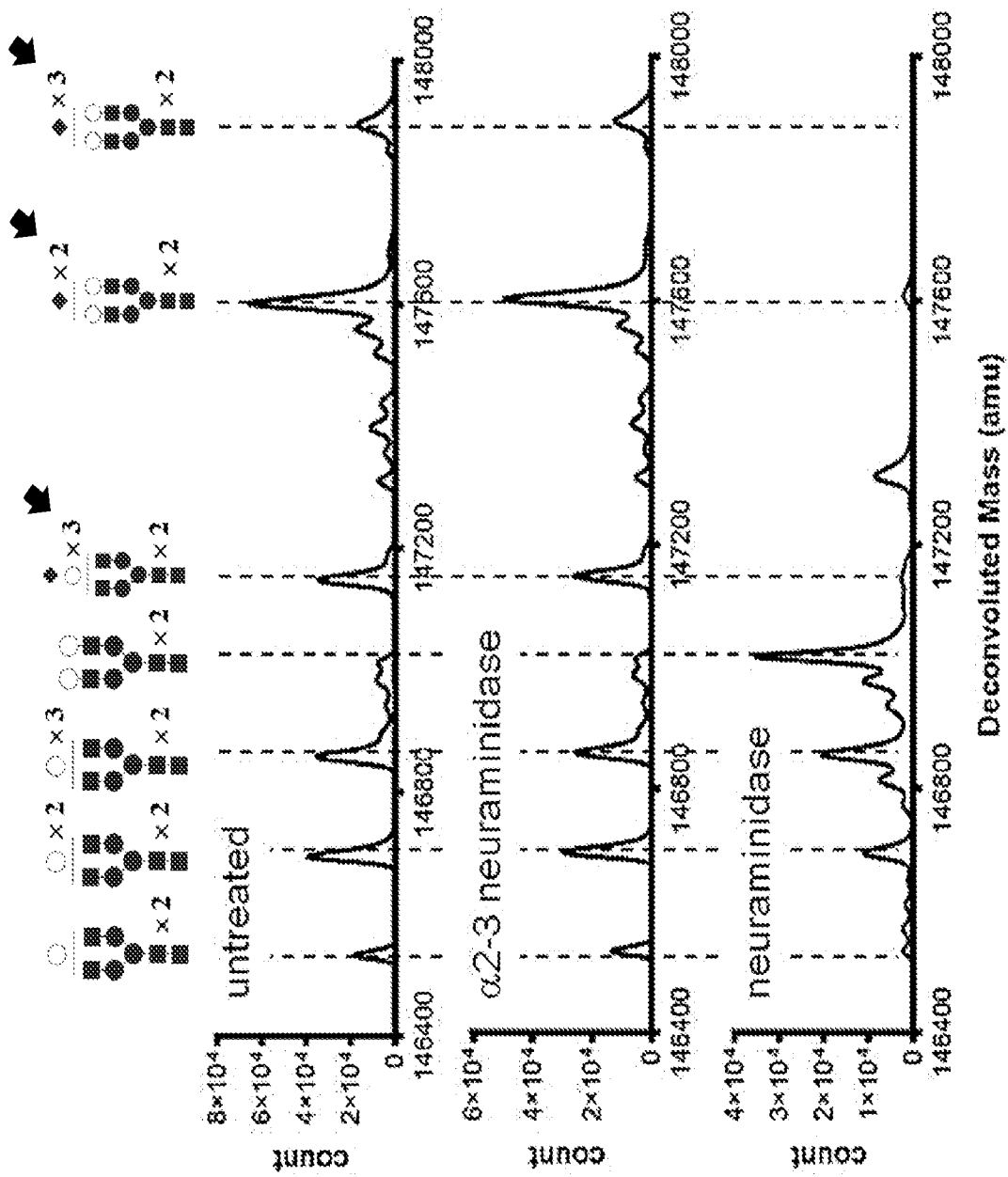


FIG. 7

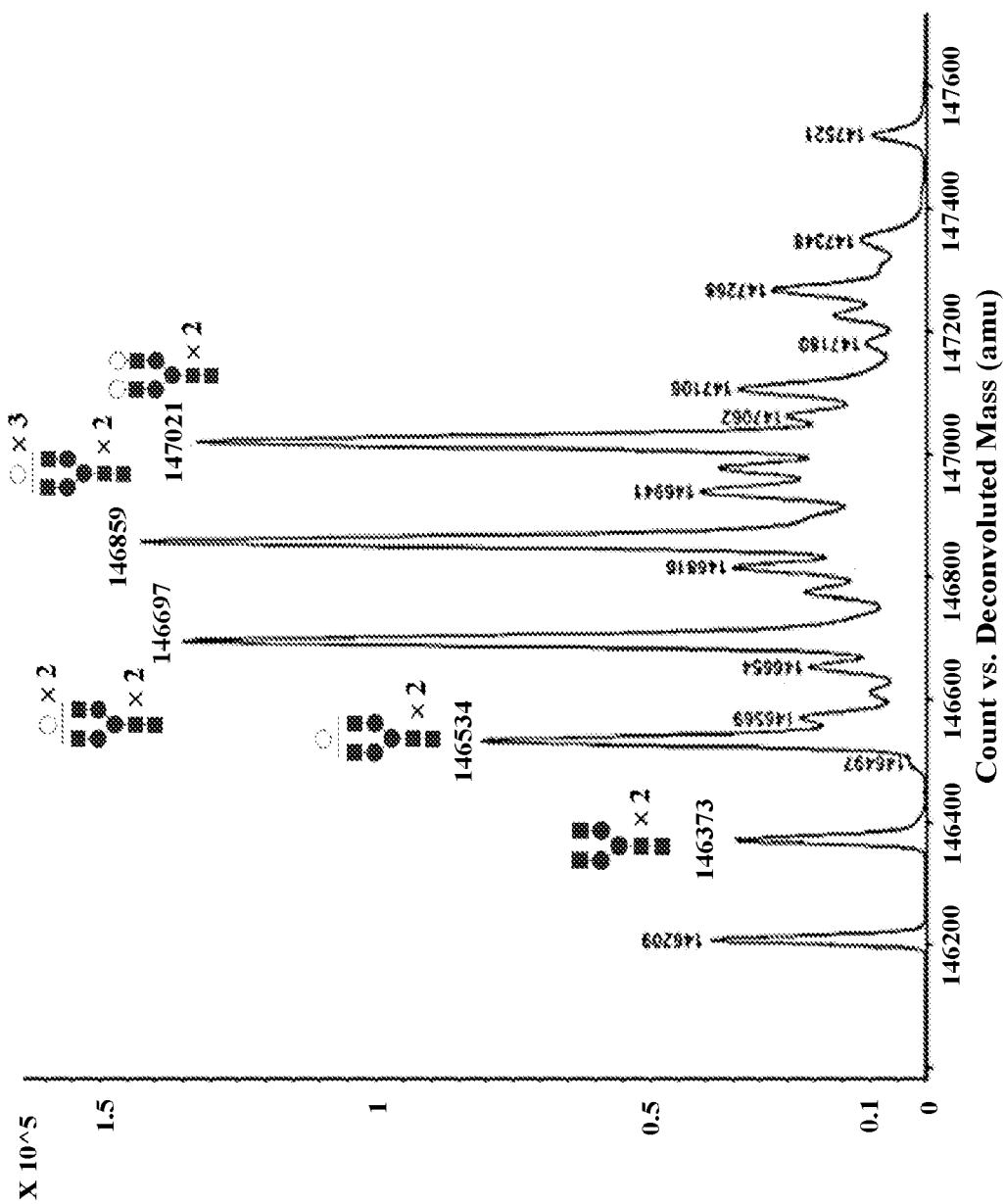


FIG. 8

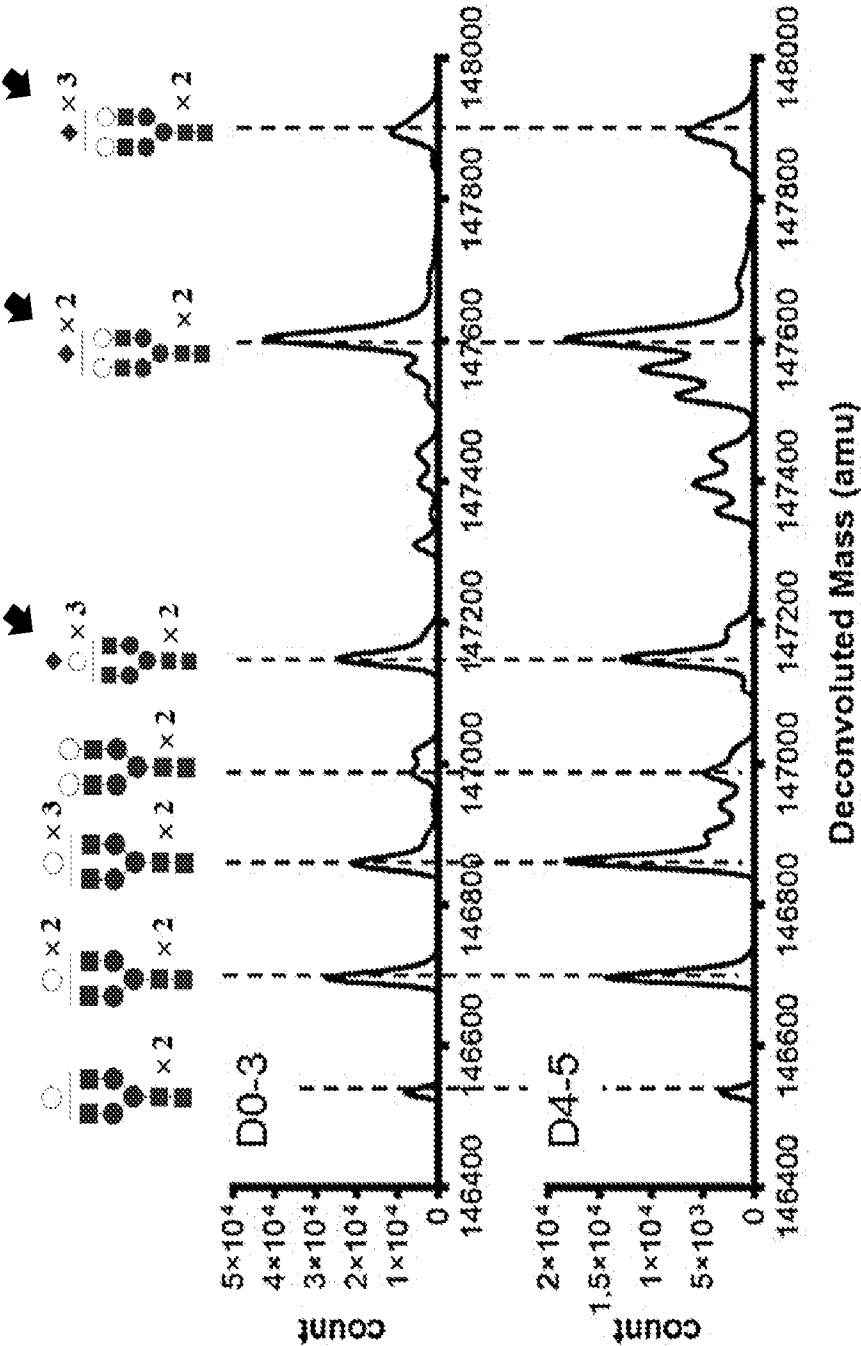
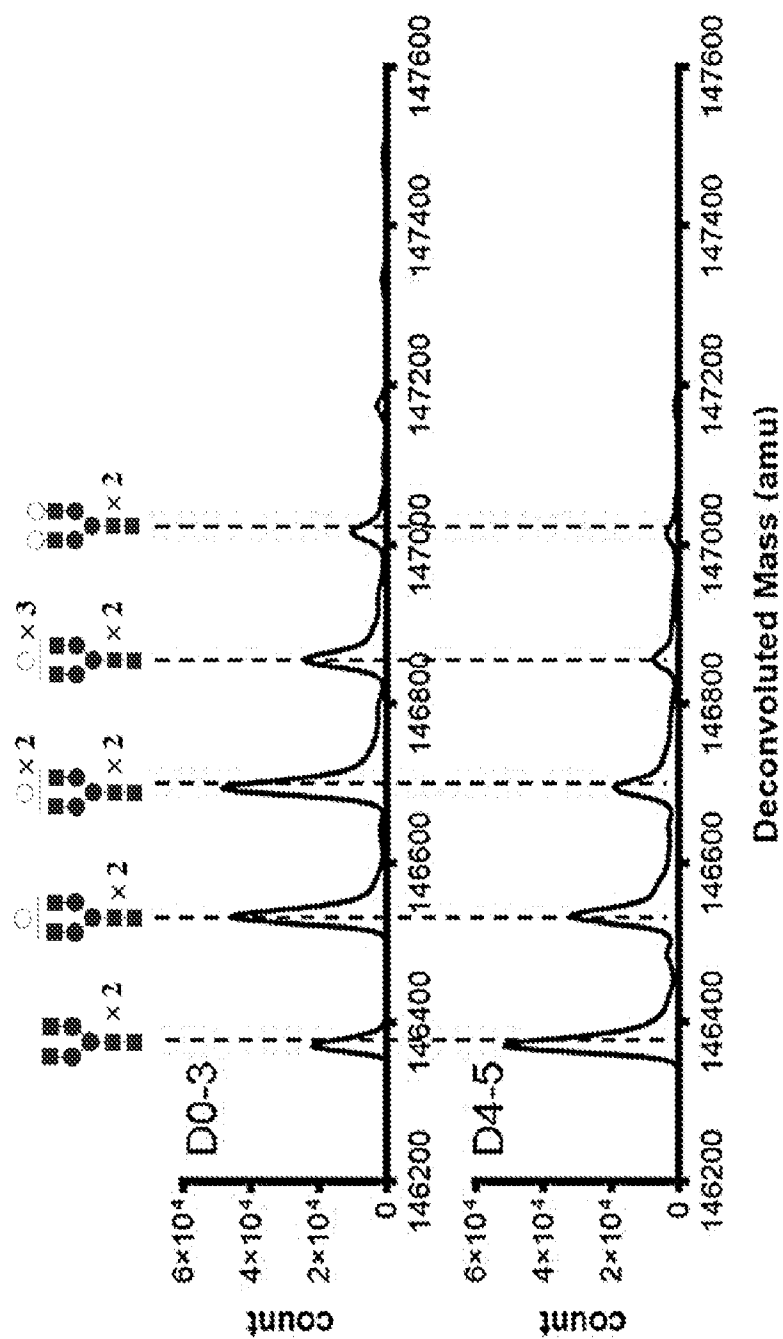


FIG. 9A



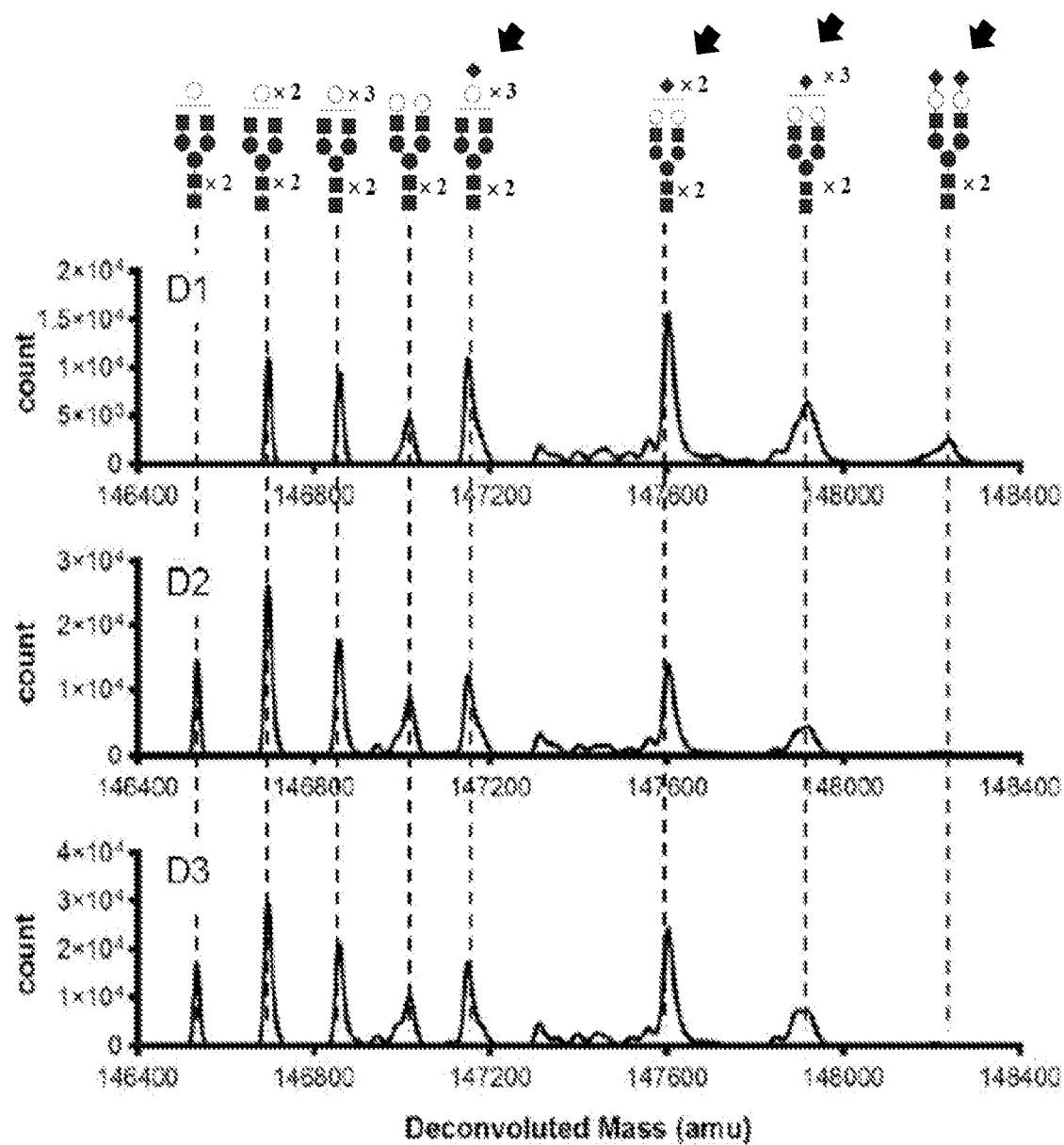


FIG. 10

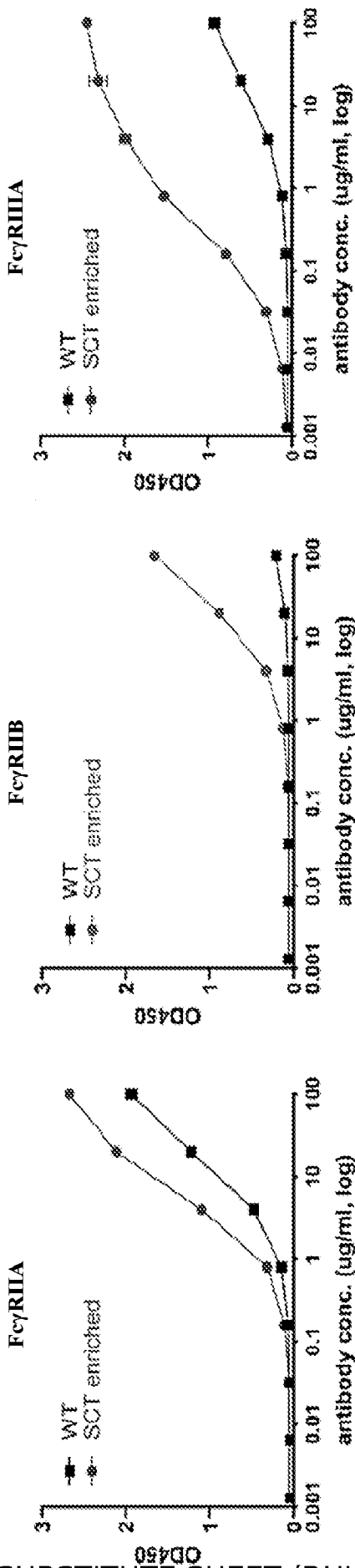


FIG. 11

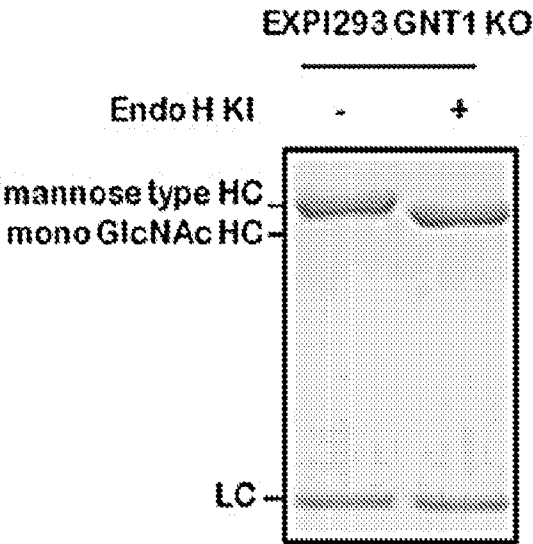


FIG. 12A

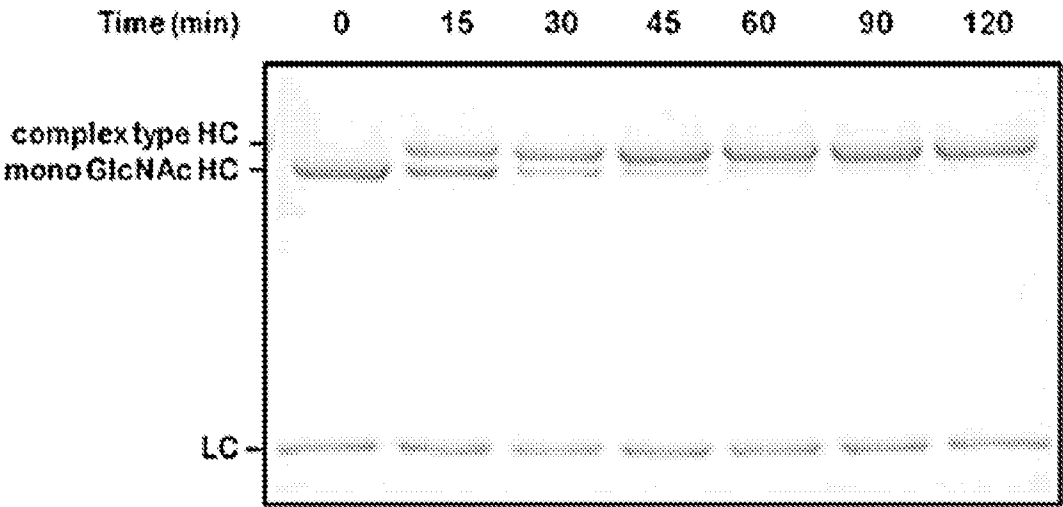


FIG. 12B

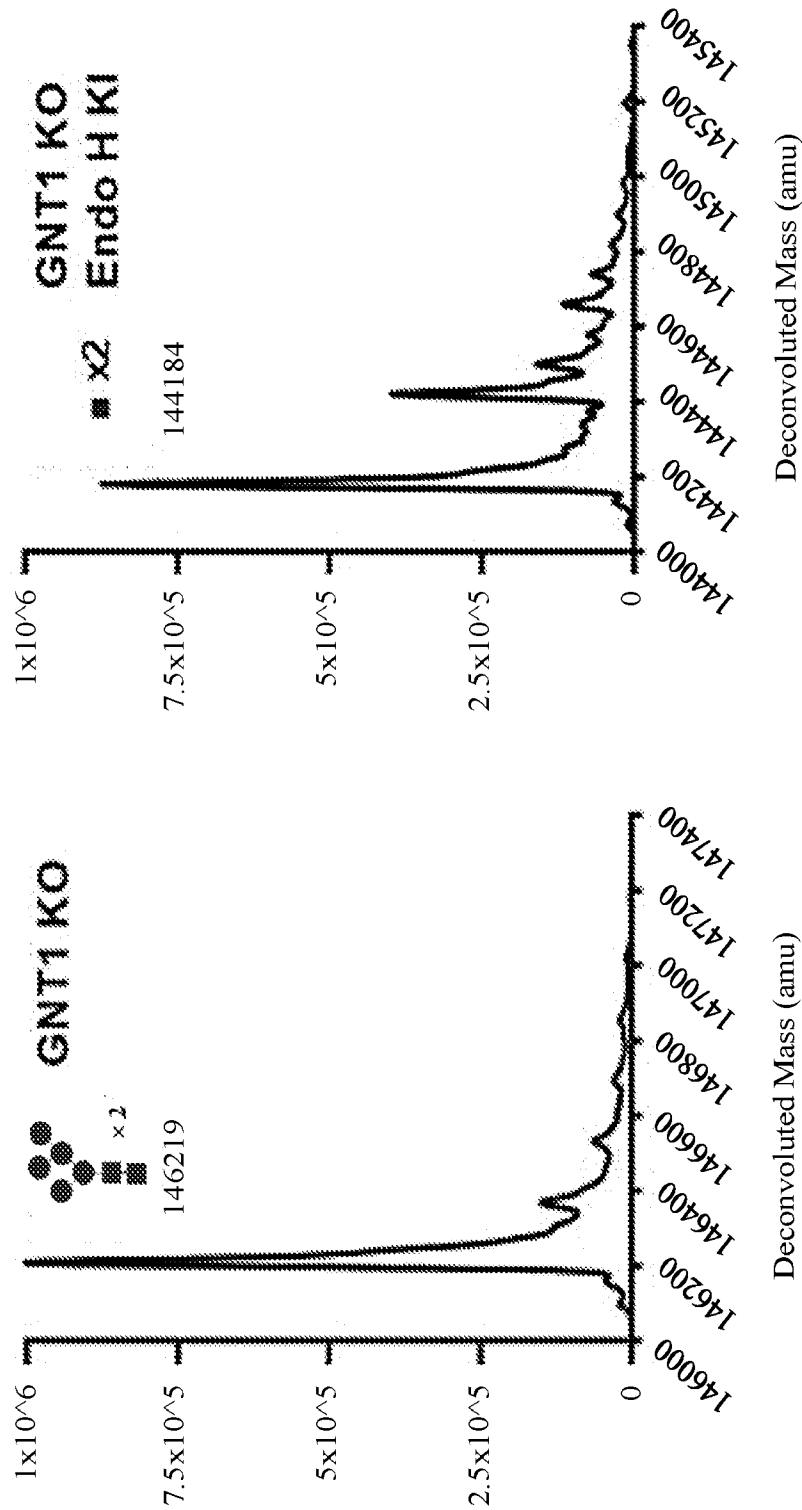


FIG. 12D

FIG. 12C

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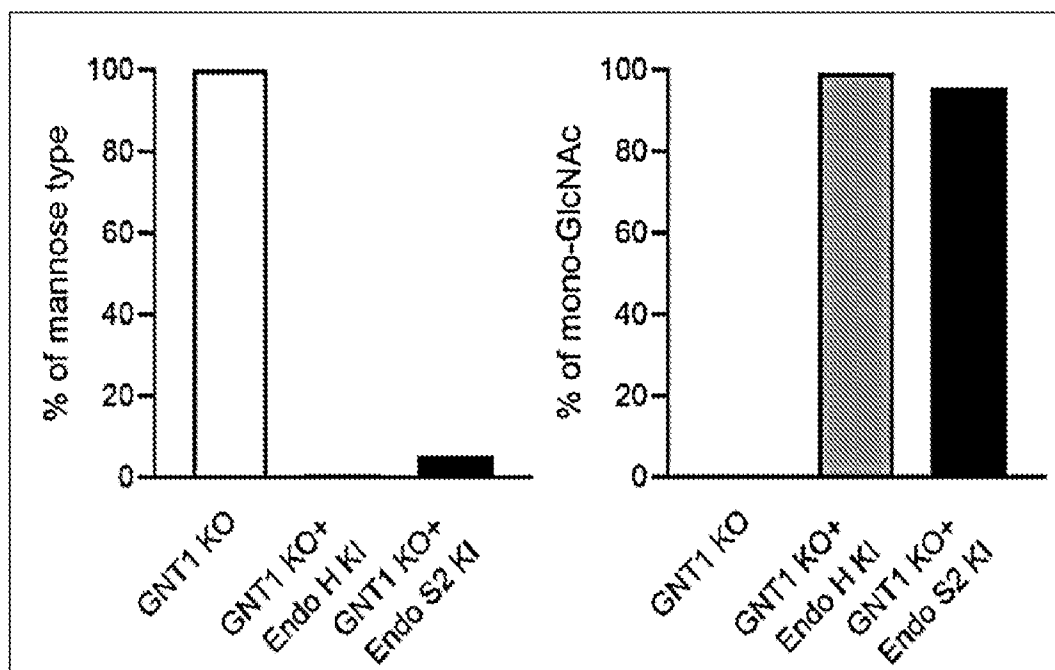


FIG. 12E

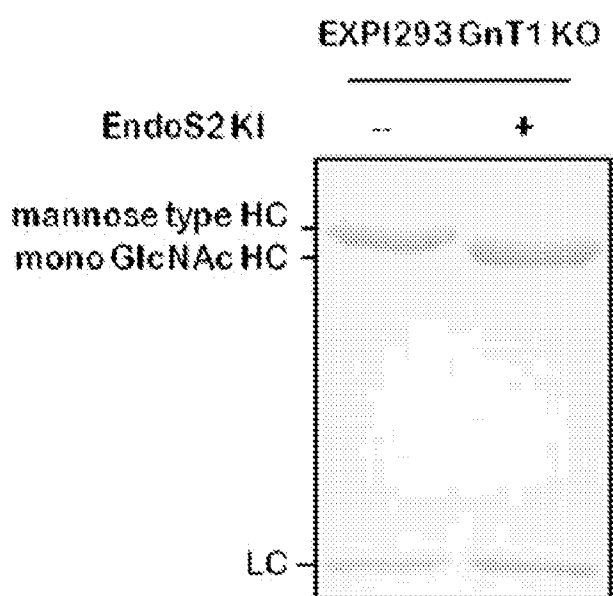


FIG. 13A

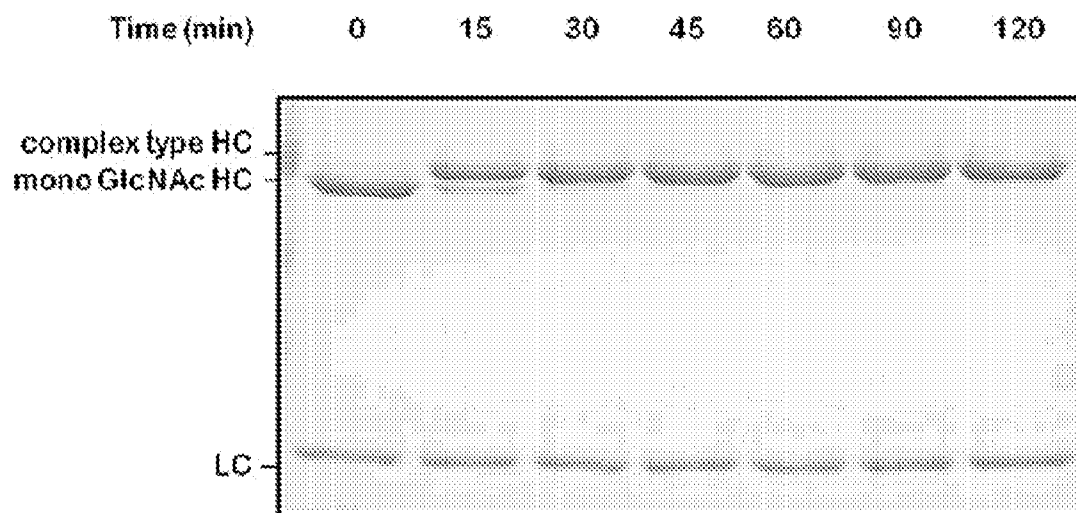


FIG. 13B

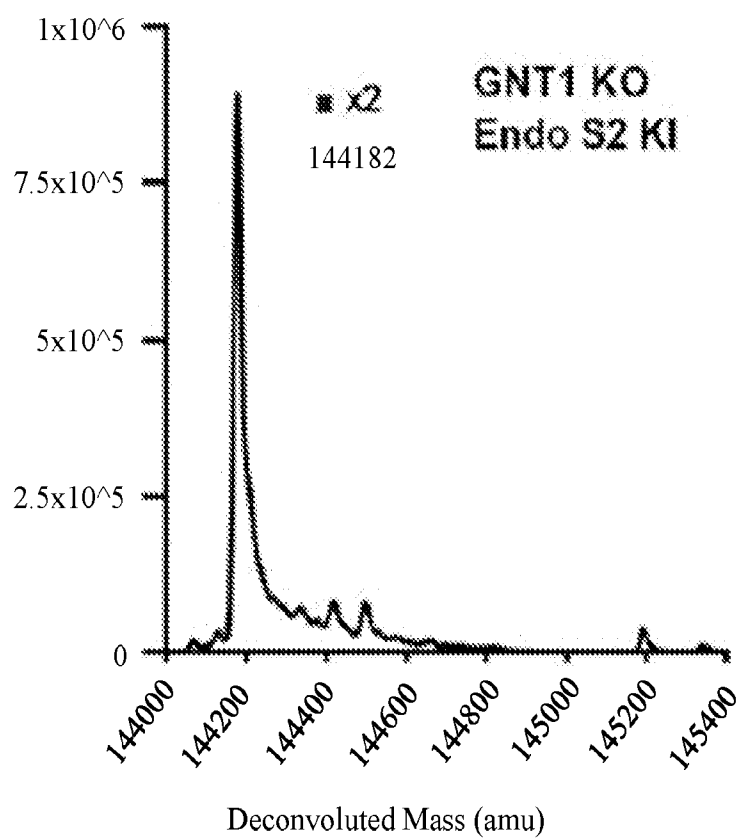


FIG. 13C

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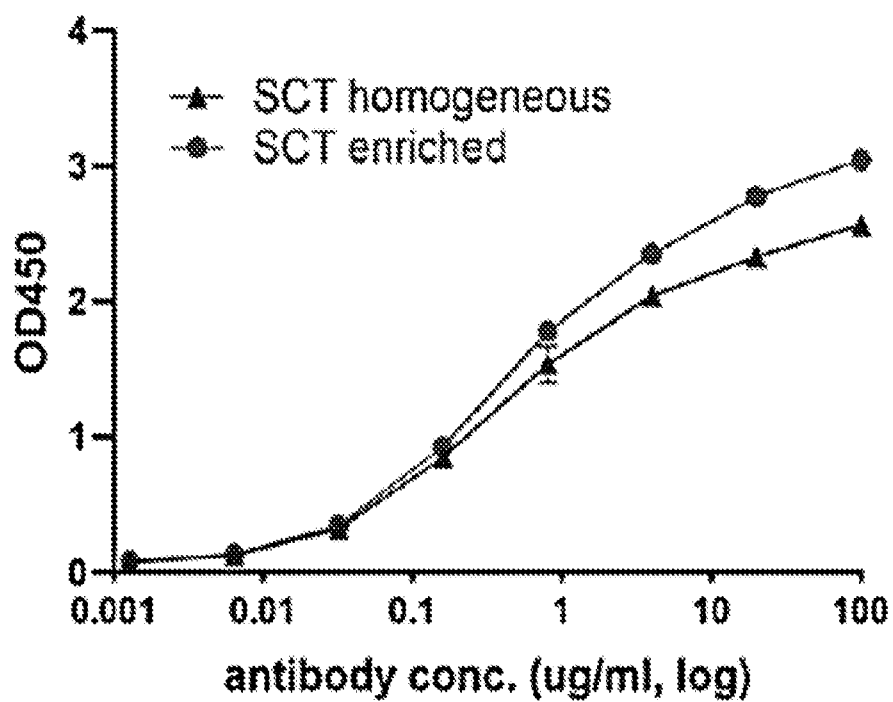


FIG. 14A

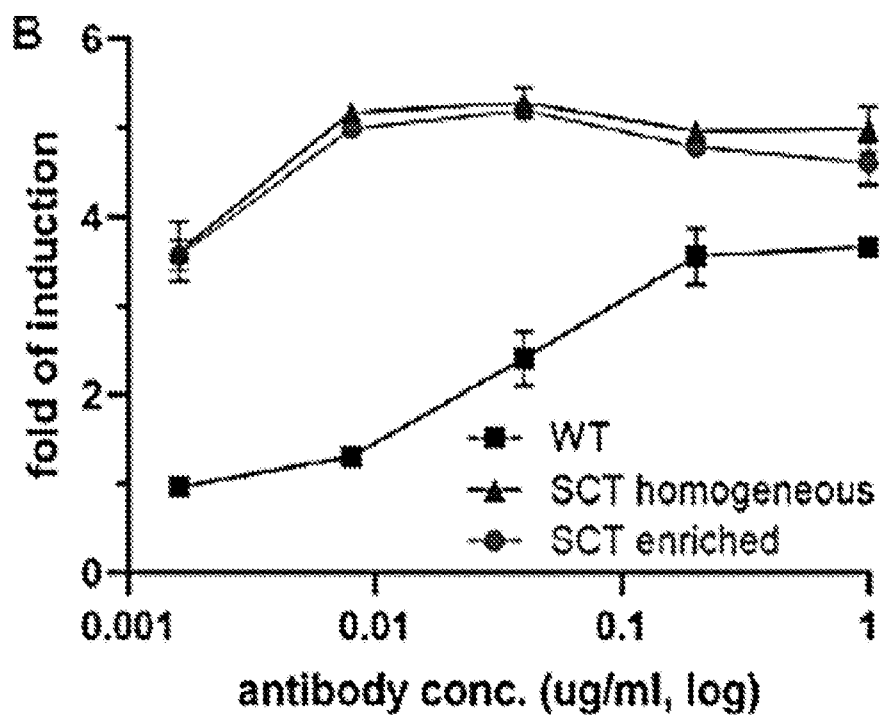


FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034588

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C12N 9/10** (2024.01); **C12P 21/00** (2024.01); **C07K 19/00** (2024.01); **C12N 15/52** (2024.01); **C12N 5/10** (2024.01); **C07K 16/24** (2024.01); **C12N 15/62** (2024.01); **C12N 15/63** (2024.01); **C12N 1/15** (2024.01); **C12N 1/19** (2024.01); **C12N 1/21** (2024.01)

CPC: **C12N 9/1051**; **C12N 15/52**; **C12N 5/10**; **C12P 21/005**; **C07K 2319/00**; **C07K 2319/03**; **C07K 2319/30**; **C12N 9/1081**; **C12Y 204/01038**; **C12Y 204/99001**; **C07K 16/241**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/229854 A1 (NOVARTIS AG) 03 November 2022 (03.11.2022) entire document	1-4, 7-11
Y	entire document	5, 6, 12
Y	US 2006/0073542 A1 (BAYER et al.) 06 April 2006 (06.04.2006) entire document	5, 6
A	US 2021/0207106 A1 (THE GENERAL HOSPITAL CORPORATION) 08 July 2021 (08.07.2021) entire document	1-12
Y	YANG et al. "Glycoproteomic Characterization of FUT8 Knock-Out CHO Cells Reveals Roles of FUT8 in the Glycosylation," Frontiers in Chemistry, 28 October 2021 (28.10.2021), Vol. 9, No. 755238, Pgs. 1-9. entire document	12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance
“D” document cited by the applicant in the international application
“E” earlier application or patent but published on or after the international filing date
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
“O” document referring to an oral disclosure, use, exhibition or other means
“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

29 August 2024 (29.08.2024)

Date of mailing of the international search report

24 October 2024 (24.10.2024)

Name and mailing address of the ISA/US

**COMMISSIONER FOR PATENTS
MAIL STOP PCT, ATTN: ISA/US
P.O. Box 1450
Alexandria, VA 22313-1450 UNITED STATES OF AMERICA**

Authorized officer

TAINA MATOS

Facsimile No. **571-273-8300**

Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034588

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034588

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-12 are drawn to cells expressing sialylated glycoproteins.

Group II: claims 13-20 are drawn to methods for glycoengineering recombinant glycoproteins.

Group III: claims 21-26 are drawn to recombinant glycoproteins.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase, are not present in Groups II or III; the special technical features of Group II, method for glycoengineering a recombinant glycoprotein, are not present in Groups I and III; and the special technical features of Group III, enriched recombinant glycoproteins, are not present in Groups I or II.

Additionally, even if Groups I-III were considered to share the technical features of a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2021/0207106 A1 to The General Hospital Corporation discloses a cell for expressing a sialylated glycoprotein (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; wherein the catalytic domain of sialyltransferase catalyzes sialylation of a glycoprotein; and a second fusion polypeptide having an antibody heavy chain CH2 region, an antibody heavy chain CH3 region, and a catalytic domain of galactosyltransferase, wherein the catalytic domain of galactosyltransferase catalyzes galactosylation of a glycoprotein, Para. [0016]) wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

galactosyltransferase catalytic peptide (this disclosure provides a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of sialyltransferase or the catalytic domain of sialyltransferase, Para. [0103]; a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of galactosyltransferase or the catalytic domain of galactosyltransferase, Para. [0104]), wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; a first fusion polypeptide having ...a catalytic domain of sialyltransferase ...a second fusion polypeptide having ...a catalytic domain of galactosyltransferase, Para. [0016]).

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-12**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and
Administrative Instructions, Sections 402(c) and 409)

To:

WADE, Lin
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 06 September 2024 (06.09.2024)	
Applicant's or agent's file reference A1000-01700PCT	IMPORTANT NOTIFICATION
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)
Applicant ROCK BIOMEDICAL INC. et al	

The applicant is hereby **notified** of the following in respect of the priority claim identified as item () in Box No. VI of the Request Form (PCT/RO/101).

1. ☐ **Correction of priority claim.** In accordance with the applicant's notice received on the following priority claim has been corrected to read as follows:

☐ even though the indication of the number of the earlier application is missing.

☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

☐ even though the international application has an international filing date which is later than the date on which the priority period expired, but is within two months from that date.

2. ☒ **Addition of priority claim.** In accordance with the applicant's notice received on 30 August 2024 (30.08.2024) the following priority claim has been added:

US 08 September 2023 (08.09.2023) 63/581,389

☐ even though the indication of the number of the earlier application is missing.

☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

☐ even though the international application has an international filing date which is later than the date on which the priority period expired, but is within two months from that date.

3. ☐ The priority claim is **considered void** (Rule 26bis.2(b)) because:

☐ **at the expiration of the prescribed time limit under Rule 26bis.1(a)**, the International Bureau had **not received a notice** from the applicant correcting the priority claim so as to comply with the requirements of Rule 4.10, as notified in the invitation (Form PCT/RO/110 or PCT/IB/316).

☐ the applicant's **notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a)**; and consequently, the priority claim could not be corrected so as to comply with the requirements of Rule 4.10.

☐ the applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Abdulaziz Ismail e-mail pct.team4@wipo.int Telephone No. +41 22 338 74 04
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The applicant may, before the technical preparations for international publication have been completed, submit information concerning the priority claim, which the International Bureau will publish together with the international application (Rule 26bis.2(d)).

4. ☐ As a **result of the correction and/or addition** of priority claim(s) under items 1 and/or 2, or of the priority claim(s) under item 3 **being considered void**, the (earliest) priority date is:
5. ☐ The priority claim cannot be corrected/added since the applicant's notice was received on that is, after the expiration of the prescribed time limit under Rule 26bis.1(a). However, where the applicant's notice concerns a correction, in accordance with Rule 26bis.2(c)(i) to (iii), the priority claim(s) indicated in Box VI of the request **will not be considered void**.
- The applicant may, prior to the expiration of 30 months from the priority date and subject to the payment of a fee, request the International Bureau to publish information concerning the matter. See Rule 26bis.2(e) and the *PCT Applicant's Guide*, Annex B2(IB).
6. ☐ In the case where **multiple priorities** have been claimed, the above item(s) relate(s) to the following priority claim(s):

A copy of this Notification has been sent to the receiving Office and

- ☒ to the International Searching Authority (*where the international search report and the written opinion of the International Searching Authority have not yet been issued*).
- ☐ to the Authority(ies) specified for supplementary search (*where a request for supplementary search has been submitted and the supplementary international search report has not yet been issued*).
- ☐ to the International Preliminary Examining Authority (*where a demand for international preliminary examination has been made and the IPRP (Chapter II) has not yet been issued*).
- ☐ to the designated Offices (*in accordance with Rule 93bis*).

From the INTERNATIONAL BUREAU

PCT

SECOND AND SUPPLEMENTARY NOTICE
INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES
WHICH APPLY THE 30 MONTH TIME
LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

WADE, Lin
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 08 January 2026 (08.01.2026)		
Applicant's or agent's file reference A1000-01700PCT		IMPORTANT NOTICE
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)	Priority date (day/month/year) 08 September 2023 (08.09.2023)
Applicant ROCK BIOMEDICAL INC. et al		

- ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does not apply**, please see Form PCT/IB/308(First Notice) issued previously.
- Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
13 March 2025 (13.03.2025)

EP

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

- The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1 :

AE, AG, AL, AM, AO, AP, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OA, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. TIME LIMITS for entry into the national phase

For the designated or elected Office(s) listed above, the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **30 MONTHS** from the priority date.

In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For **regular updates on the applicable time limits** (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/guide/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Fiona Doherty e-mail pct.team4@wipo.int Telephone No. +41 22 338 74 04
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From the INTERNATIONAL BUREAU

PCT

FIRST NOTICE INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES WHICH
DO NOT APPLY THE 30 MONTH TIME LIMIT
UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

WADE, Lin
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 10 April 2025 (10.04.2025)		
Applicant's or agent's file reference A1000-01700PCT		IMPORTANT NOTICE
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)	Priority date (day/month/year) 08 September 2023 (08.09.2023)
Applicant ROCK BIOMEDICAL INC. et al		

- ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), **does apply**, please see Form PCT/IB/308(Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).
- Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
13 March 2025 (13.03.2025)

None

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).
- The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, **does not apply**, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1 :

LU, TZ

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.
- TIME LIMITS for entry into the national phase**

For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of **19 months** from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, **subject to what is said in the following paragraph**, be **20 MONTHS** from the priority date.

In practice, **time limits other than the 20-month time limit** will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For **regular updates on the applicable time limits** (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/guide/index.html>.

It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Fiona Doherty e-mail pct.team4@wipo.int Telephone No. +41 22 338 74 04
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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

WADE, Lin
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 12 February 2026 (12.02.2026)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference A1000-01700PCT	
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)

1. The following indications appeared on record concerning:

☒ the applicant

☐ the inventor

☐ the agent

☐ the common representative

Name and Address ROCK BIOMEDICAL INC. Rm. 301, Bldg. A No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City, 115202 TAIWAN PROVINCE OF CHINA	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	E-mail address	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person

☒ the name

☐ the address

☐ the nationality

☐ the residence

Name and Address ROCK BIOMEDICAL, INC. Rm. 301, Bldg. A No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City, 115202 TAIWAN PROVINCE OF CHINA	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	E-mail address ☐ Notifications by e-mail authorized	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office

☐ the International Searching Authority

☐ the Authority(ies) specified for supplementary search

☐ the International Preliminary Examining Authority

☒ the designated Offices concerned

☐ the elected Offices concerned

☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Deblue Natalia e-mail pct.team4@wipo.int Telephone No. +41 22 338 74 04
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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION,
OBTENTION OR TRANSMITTAL
OF PRIORITY DOCUMENT
(PCT Administrative Instructions, Section 411)

To:

WADE, Lin
Prosyla Group PC
530 Technology Dr., Suite 100
Irvine, CA 92618
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 09 September 2024 (09.09.2024)	
Applicant's or agent's file reference A1000-01700PCT	IMPORTANT NOTIFICATION
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 08 September 2023 (08.09.2023)
Applicant ROCK BIOMEDICAL INC. et al	

The applicant is hereby notified of the date of receipt (or of obtaining by the International Bureau) of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to the date of receipt, **the priority document concerned was submitted or transmitted to or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis)**. This Form replaces any previously issued notification concerning submission, transmittal or obtaining of priority documents.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
26 April 2024 (26.04.2024)	63/639,286	US	13 July 2024 (13.07.2024)
08 September 2023 (08.09.2023)	63/581,389	US	30 August 2024 (30.08.2024)

The letters "NR" denote a priority document which, on the date of mailing of this Form, had not yet been received or obtained by the International Bureau in compliance with Rule 17.1(a), (b) or (b-bis). Where the applicant has failed to either submit, request to prepare and transmit, or to request the International Bureau to obtain the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

An asterisk "*" next to a date of receipt, denotes a priority document submitted or transmitted to or obtained by the International Bureau but not in compliance with Rule 17.1(a), (b) or (b-bis) (the priority document was received after the time limit prescribed in Rule 17.1(a); the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b) or the request to the International Bureau to obtain the priority document was made after the applicable time limit under Rule 17.1(b-bis)). Even though the priority document was not furnished in compliance with Rule 17.1(a), (b) or (b-bis), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Fiona Doherty e-mail pct.team4@wipo.int Telephone No. +41 22 338 74 04
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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference (if desired) (25 characters maximum)

A1000-01700PCT

Box No. I TITLE OF INVENTION	
CELLS FOR GLYCOENGINEERING AND METHODS OF USE	
Box No. II APPLICANT	
☐ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
ROCK BIOMEDICAL INC. Rm. 301, Bldg. A No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei City 115202 TAIWAN (R.O.C.)	
E-mail address*	
Telephone No.	
Facsimile No.	
Applicant's registration No. with the Office	
* E-mail authorization: Indicating an e-mail address above authorizes the receiving Office, the International Searching Authority and the International Bureau, if they provide such a service, to send notifications exclusively by e-mail to that address, unless the following box is marked: ☐ notifications are requested to be sent exclusively by postal mail.	
State (that is, country) of nationality: TW	State (that is, country) of residence: TW
This person is applicant for the purposes of: ☒ all designated States ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
Wade Lin PROSYLA GROUP PC 530 Technology Dr., Suite 100 Irvine, California 92618 UNITED STATES	
E-mail address* ipdocketing@prosylogroup.com	
Telephone No. 949-352-4590	
Facsimile No. 949-506-3690	
Agent's registration No. with the Office L1383	
* E-mail authorization: Indicating an e-mail address above authorizes the receiving Office, the International Searching Authority and the International Bureau, if they provide such a service, to send notifications exclusively by e-mail to that address, unless the following box is marked: ☐ notifications are requested to be sent exclusively by postal mail.	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WONG, Chi-Huey
P.O. Box 8154
Rancho Santa Fe, California 92067
UNITED STATES

This person is:

- ☐ applicant only
☐ applicant and inventor
☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:
US

State *(that is, country)* of residence:
US

This person is applicant
for the purposes of:

- ☐ all designated States ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

WANG, Szu-Wen
Rm. 301, Bldg. A, No. 99, Ln. 130, Sec. 1, Academia Rd.,
Nangang Dist., Taipei City 115202
TAIWAN (R.O.C.)

This person is:

- ☐ applicant only
☐ applicant and inventor
☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:
TW

State *(that is, country)* of residence:
TW

This person is applicant
for the purposes of:

- ☐ all designated States ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LEE, Jeng Shin
9 Bedford Lane
Lincoln, Massachusetts 01773
UNITED STATES

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:
US

State *(that is, country)* of residence:
US

This person is applicant
for the purposes of:

- ☐ all designated States ☒ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant
for the purposes of:

- ☐ all designated States ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Supplemental Box*If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No...." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) **if more than one person is to be indicated as applicant and/or inventor** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant intends to make an indication of the wish that the international application be treated, in certain designated States, as an application for a patent of addition, certificate of addition, inventor's certificate of addition or utility certificate of addition: in such case, write the name or two-letter code of each designated State concerned and the indication **"patent of addition," "certificate of addition," "inventor's certificate of addition" or "utility certificate of addition,"** the number of the parent application or parent patent or other parent grant and the date of grant of the parent patent or other parent grant or the date of filing of the parent application (Rules 4.11(a) (i) and 49bis.1(a) or (b)).

3. If the applicant intends to make an indication of the wish that the international application be treated, in the United States of America, as a continuation or continuation-in-part of an earlier application: in such case, write "United States of America" or "US" and the indication **"continuation" or "continuation-in-part"** and the number and the filing date of the parent application (Rules 4.11(a)(ii) and 49bis.1(d)).

Continuation of Box III:

Applicant LEE, Jeng Shin is applicant for purposes of filing in the Republic of Malawi (MW) only.

Box No. V DESIGNATIONS

The filing of this request **constitutes under Rule 4.9(a) the designation** of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.

However,

- ☐ DE Germany **is not designated** for any kind of national protection
- ☐ JP Japan **is not designated** for any kind of national protection
- ☐ KR Republic of Korea **is not designated** for any kind of national protection

(The check-boxes above may only be used to exclude (irrevocably) the designations concerned if, at the time of filing or subsequently under Rule 26bis.1, the international application contains in Box No. VI a priority claim to an earlier national application filed in the particular State concerned, in order to avoid the ceasing of the effect, under the national law, of this earlier national application.)

Box No. VI PRIORITY CLAIM AND DOCUMENT

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application: regional Office	international application: receiving Office
item (1) 26 April 2024 26.04.2024	63/639,286	US		
item (2)				
item (3)				

☐ Further priority claims are indicated in the Supplemental Box.

Furnishing the priority document(s):

☒ The **receiving Office** is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application(s) was filed with the receiving Office which, for the purposes of this international application, is the receiving Office)* identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box

☐ The **International Bureau** is requested to obtain from a digital library a certified copy of the earlier application(s) identified above, using, where applicable, the access code(s) indicated below *(if the earlier application(s) is available to it from a digital library)*:

☐ item (1) ☐ item (2) ☐ item (3) ☐ other, see Supplemental Box
access code _____ access code _____ access code _____

Restore the right of priority: the receiving Office is requested to restore the right of priority for the earlier application(s) identified above or in the Supplemental Box as item(s) (_____). *(See also the Notes to Box No. VI; further information must be provided to support a request to restore the right of priority.)*

Incorporation by reference: where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a), or an element or part of the description, claims or drawings referred to in Rule 20.5bis(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if more than one International Searching Authority is competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/ US

Box No. IX CHECK LIST for electronic filing with RO/US – only to be used when filing via the USPTO electronic filing system			
This international application contains the following:	Number of sheets	This international application is accompanied by the following item(s) (<i>mark the applicable check-boxes below and indicate in right column the number of each item</i>):	Number of items
(a) request form PCT/RO/101 (including any declarations and supplemental sheets)	5	1. ☒ fee calculation sheet.	1
(b) description	71	2. ☐ original separate power of attorney	
(c) claims	3	3. ☐ original general power of attorney.	
(d) abstract	1	4. ☐ copy of general power of attorney; reference number:	
(e) drawings (if any)	18	5. ☐ priority document(s) identified in Box No. VI as item(s)	
Total number of sheets	98	6. ☐ Translation of international application into (language):	
(f) sequence listing part of the description ☒ filed as a WIPO Standard ST.26 XML file ☐ WILL BE filed separately on physical data carrier(s) as a WIPO Standard ST.26 XML file , on the same day Indicate type and number of physical data carrier(s)		7. ☐ separate indications concerning deposited microorganism or other biological material	
		8. ☐ copy of results of earlier search(es) (Rule 12bis.1(a)) ...	
		9. ☐ other (<i>specify</i>):	
Figure of the drawings which should accompany the abstract:	1	Language of filing of the international application:	English
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>			
/Wade Lin/ Wade Lin Reg. No. L1383			

For receiving Office use only			
1. Date of actual receipt of the purported international application:	2. Drawings:		
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	☐ received:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):	☐ not received:		
5. International Searching Authority (if two or more are competent):	6. ☐ Transmittal of search copy delayed until search fee is paid		

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

PCT**FEE CALCULATION SHEET****Annex to the Request**

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's file reference

A1000-01700PCT

Applicant

ROCK BIOMEDICAL INC.**CALCULATION OF PRESCRIBED FEES***(Applicants may be entitled to a reduction of certain fees as indicated in the PCT Fee Tables (www.wipo.int/pct/en/fees.pdf))*1. TRANSMITTAL FEE: 104.00 2. SEARCH FEE: 872.00 International search to be carried out by: US

3. INTERNATIONAL FILING FEE

Enter total number of sheets indicated in Box No IX: 98 Fixed amount for the first 30 sheets 1445.00 68 x 18.00 = 1224.00
number of sheets fee per sheet
in excess of 30Add amounts entered at i1 and i2 and enter total at I: 2669.00 *(Applicants from certain States are entitled to a reduction of 90% of the international filing fee (see www.wipo.int/pct/en/fees/fee_reduction.pdf). Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 10% of the international filing fee.)*4. FEE FOR PRIORITY DOCUMENT (if applicable): 5. FEE FOR RESTORATION OF THE RIGHT OF PRIORITY (if applicable): 6. FEE FOR EARLIER SEARCH DOCUMENTS (if applicable):

7. TOTAL FEES PAYABLE 3645.00

Add amounts entered at T, S, I, P, RP and ES,
and enter total in the TOTAL box**TOTAL****MODE OF PAYMENT** (Not all modes of payment may be available at all receiving Offices)☐ credit card (details should
not be included on this
sheet)☒ authorization to charge deposit
or current account (see below)☐ bank transfer☐ cash☐ postal money order☐ check☐ revenue stamps☐ other (specify):**AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT OR CURRENT ACCOUNT***(This mode of payment may not be available at all receiving Offices)*☒ Authorization to charge the total fees indicated above.☒ (This check-box may be marked only if the conditions for deposit or current
accounts of the receiving Office so permit) Authorization to charge any
deficiency or credit any overpayment in the total fees indicated above.☒ Authorization to charge the fee for priority document.Receiving Office: RO/ USDeposit or Current Account No.: 603395Date: June 19, 2024Name: Wade LinSignature: /Wade Lin/

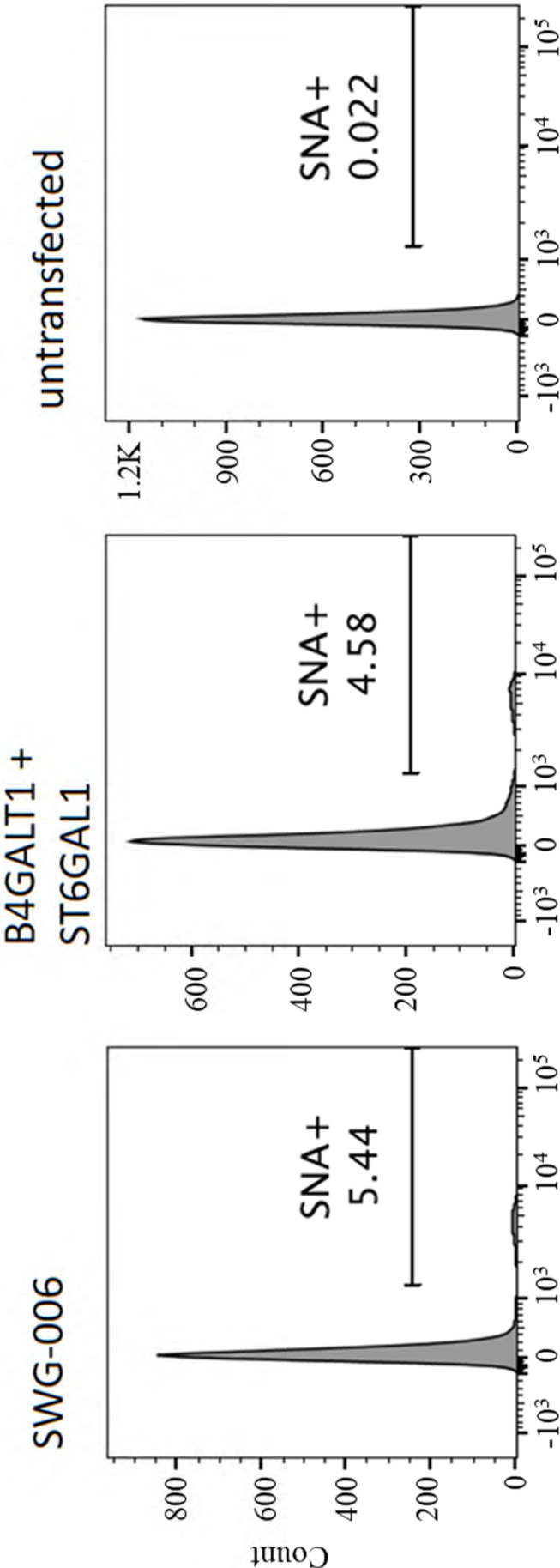


FIG. 1

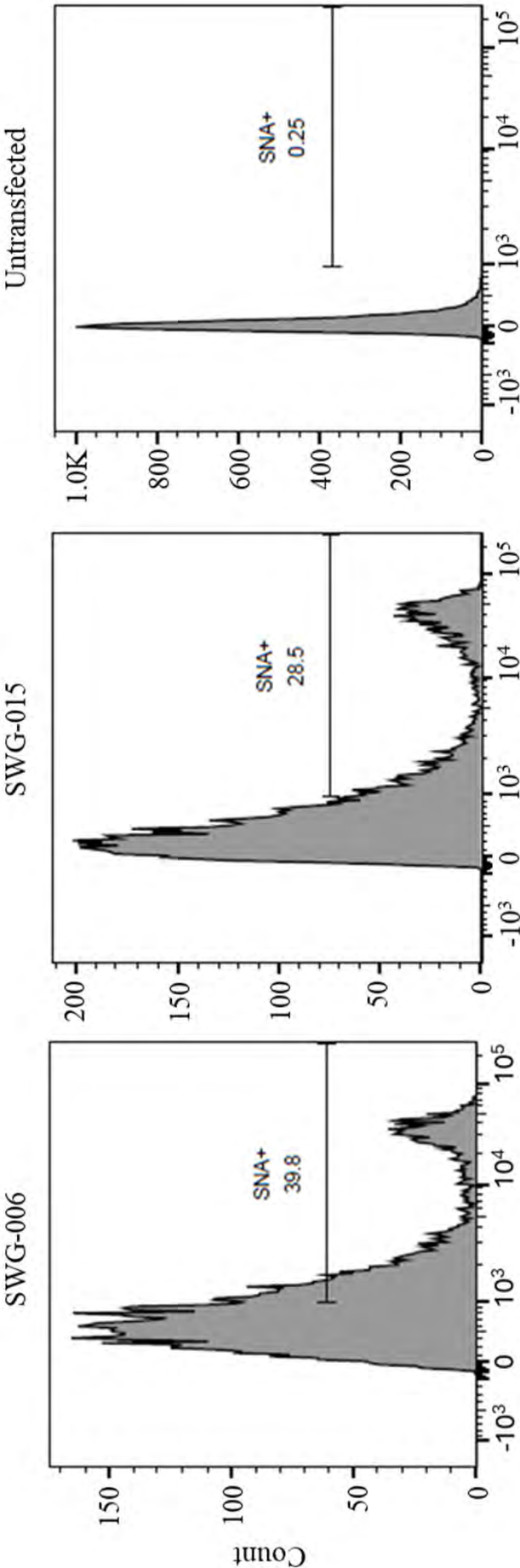


FIG. 2

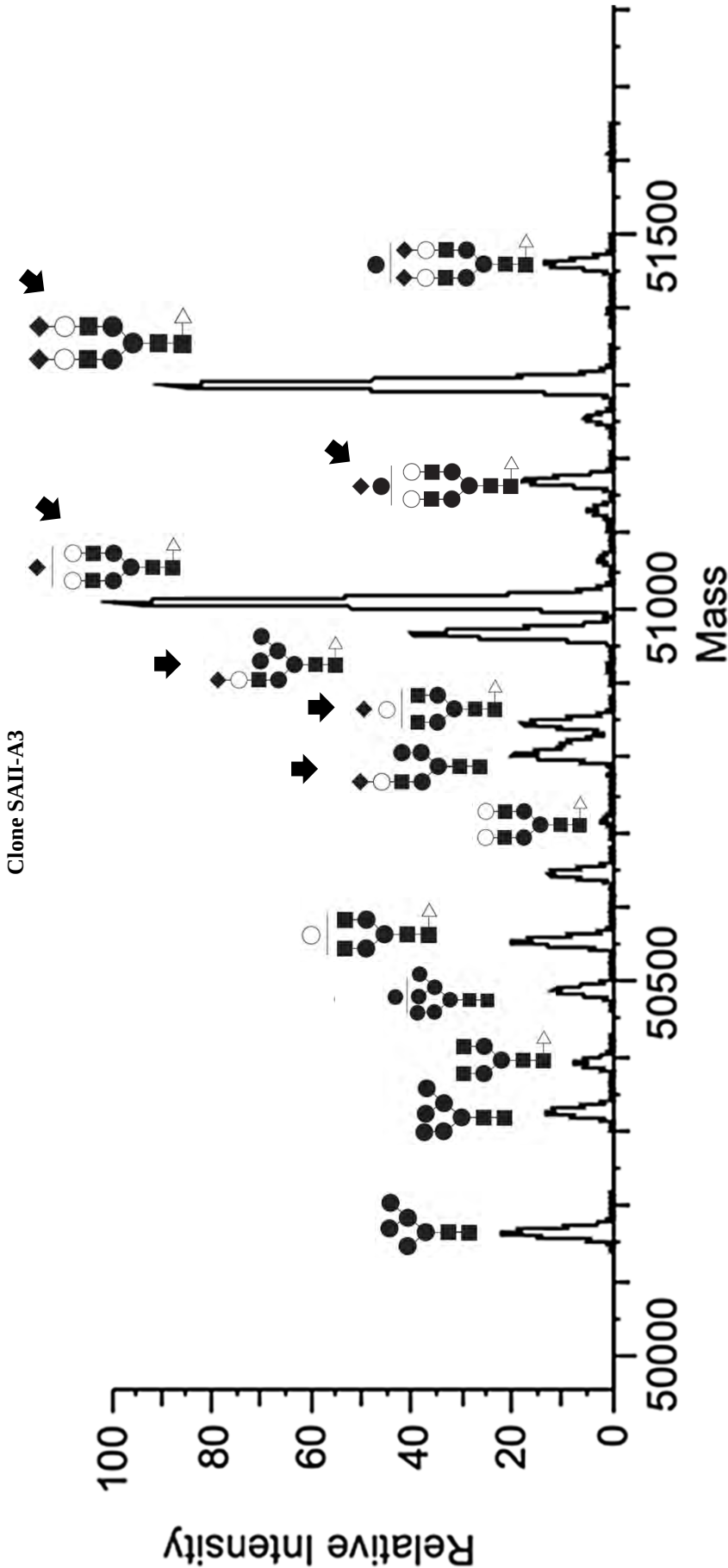


FIG. 3A

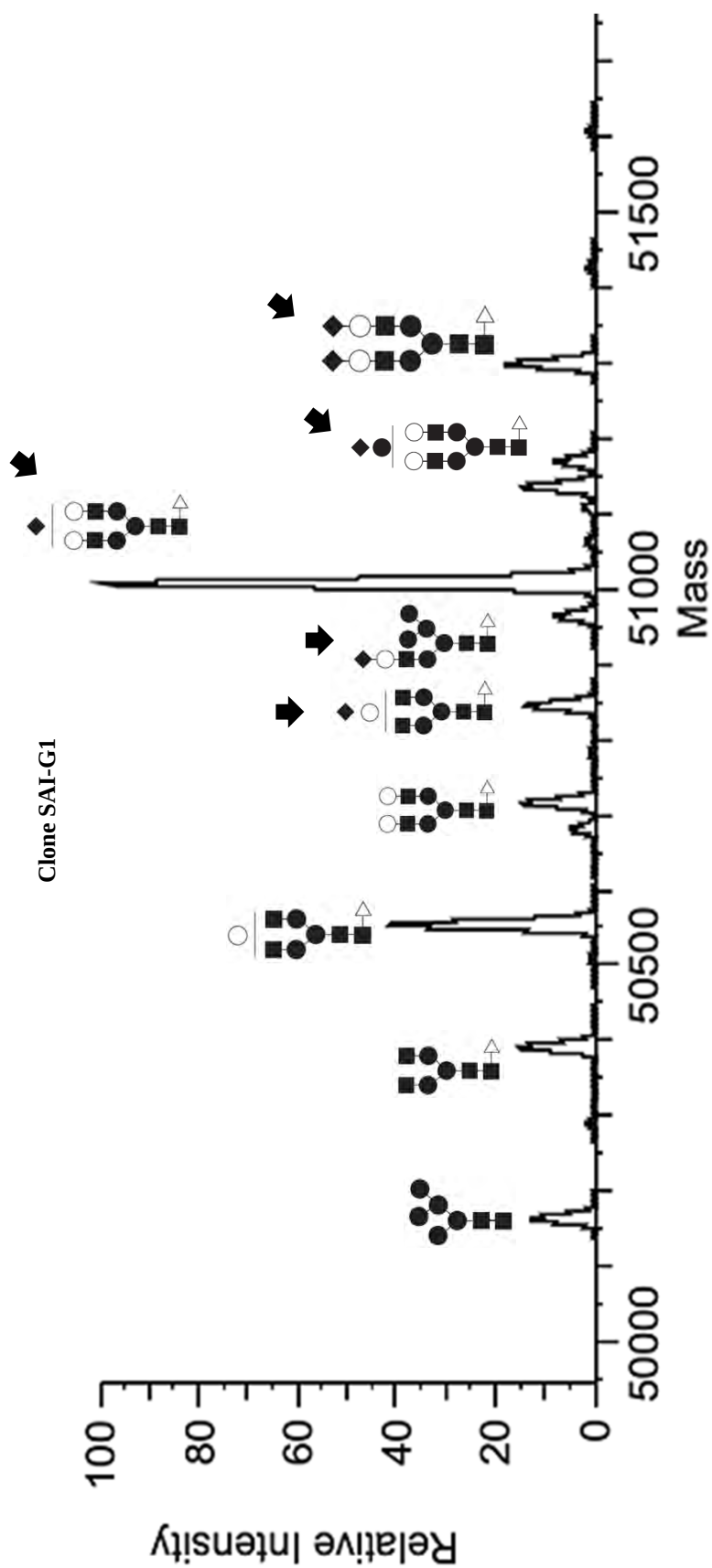


FIG. 3B



6/24

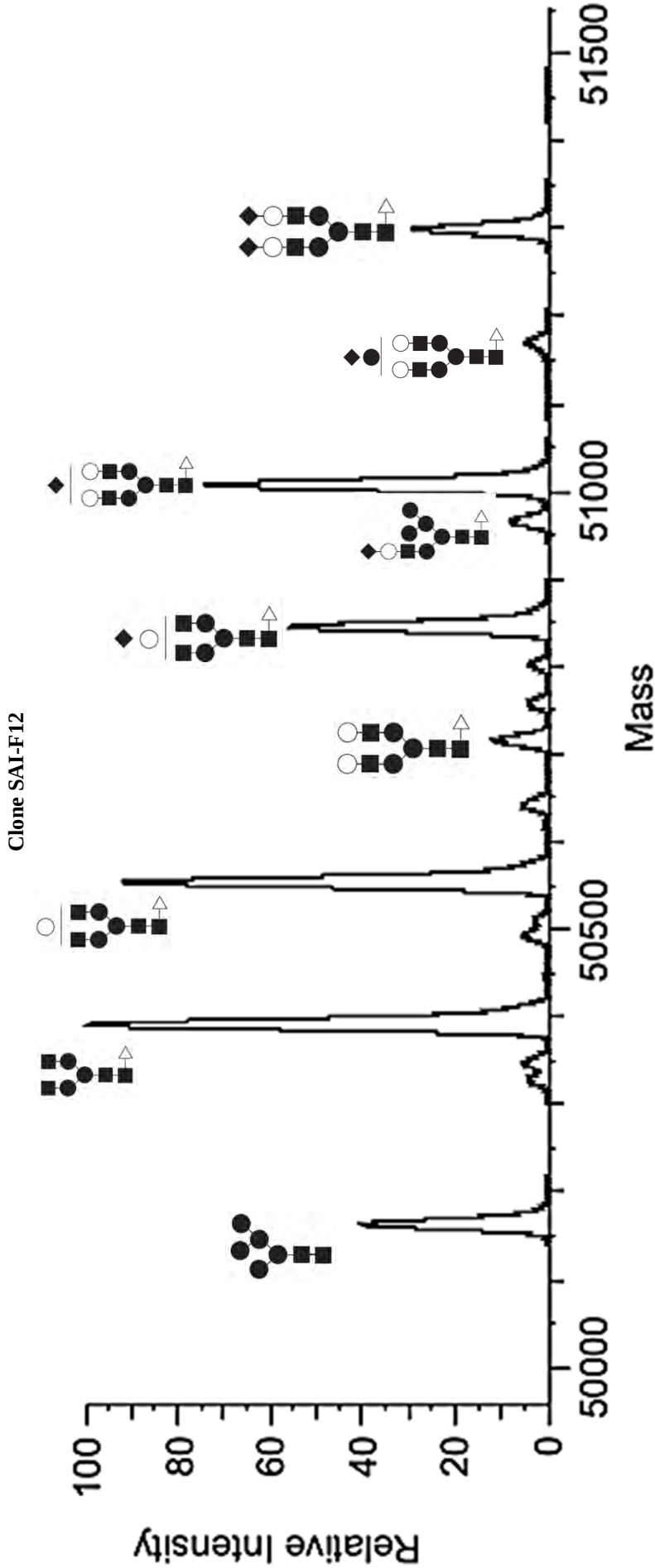


FIG. 3D

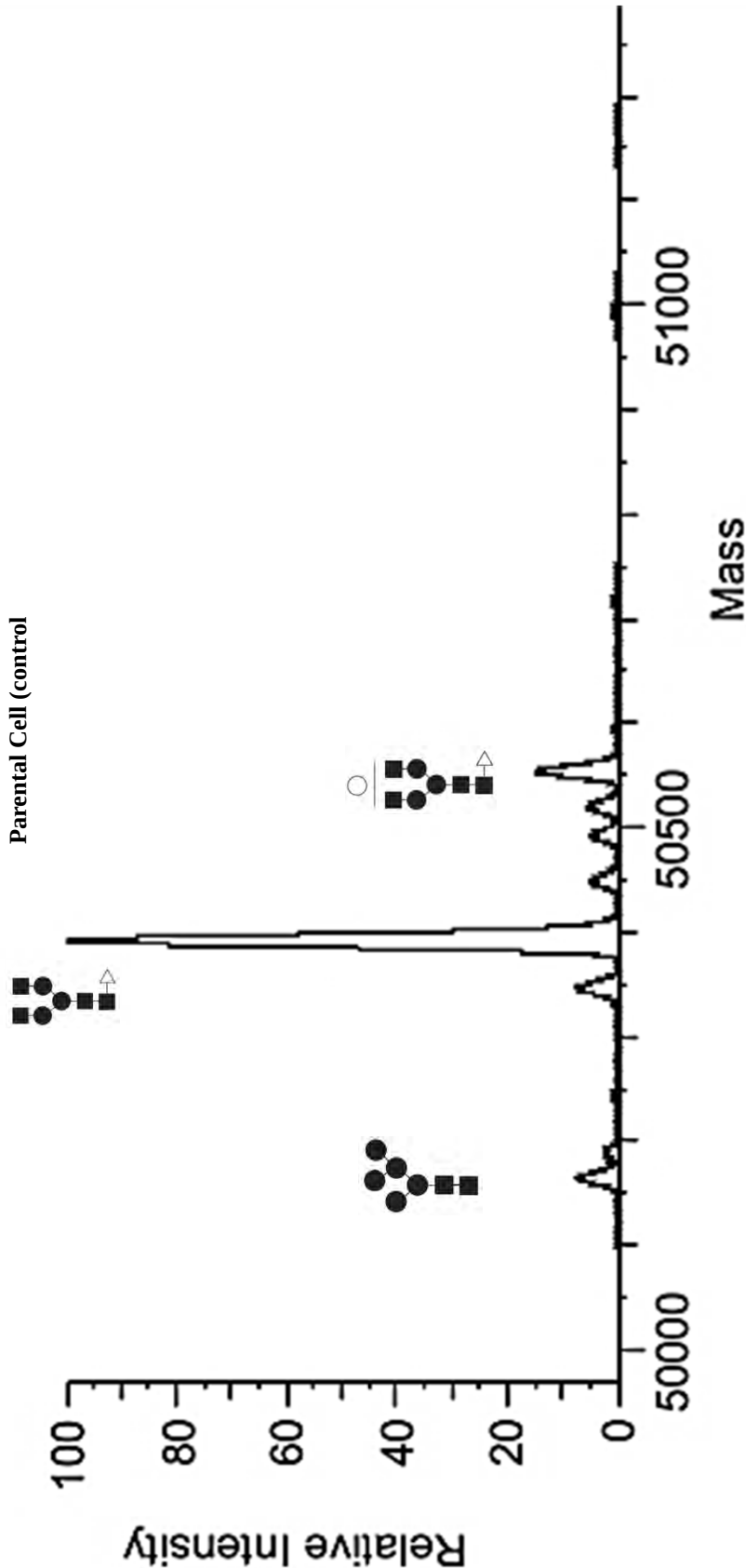


FIG. 3E

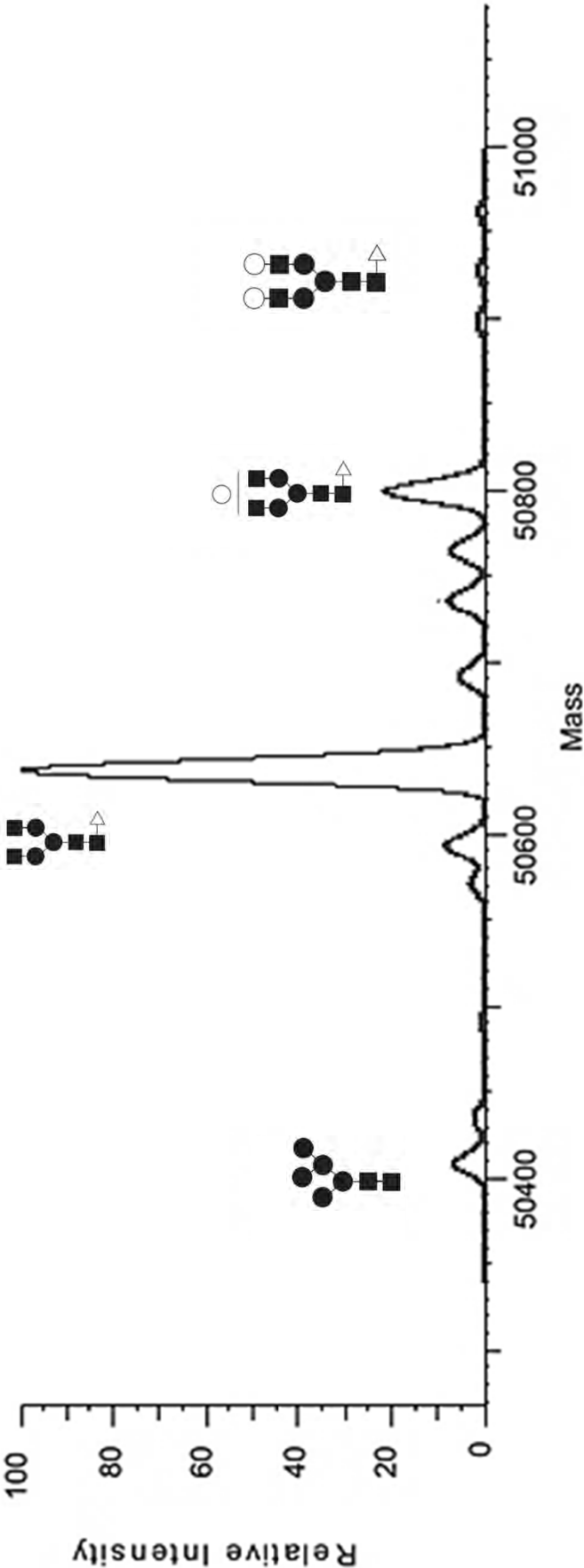


FIG. 4A

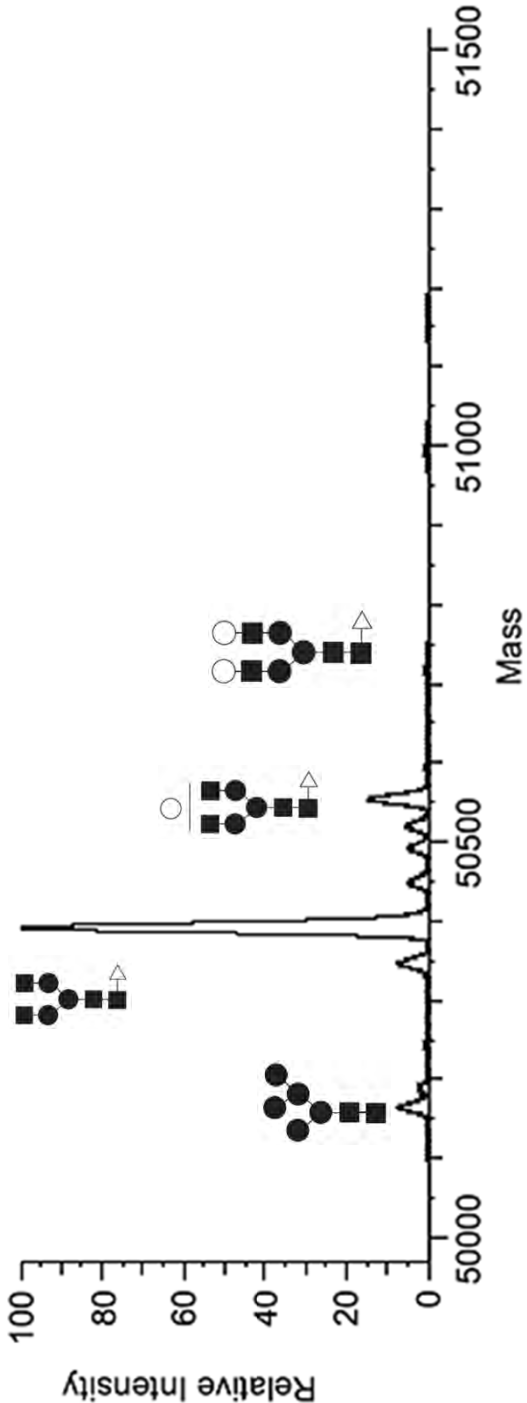


FIG. 4B

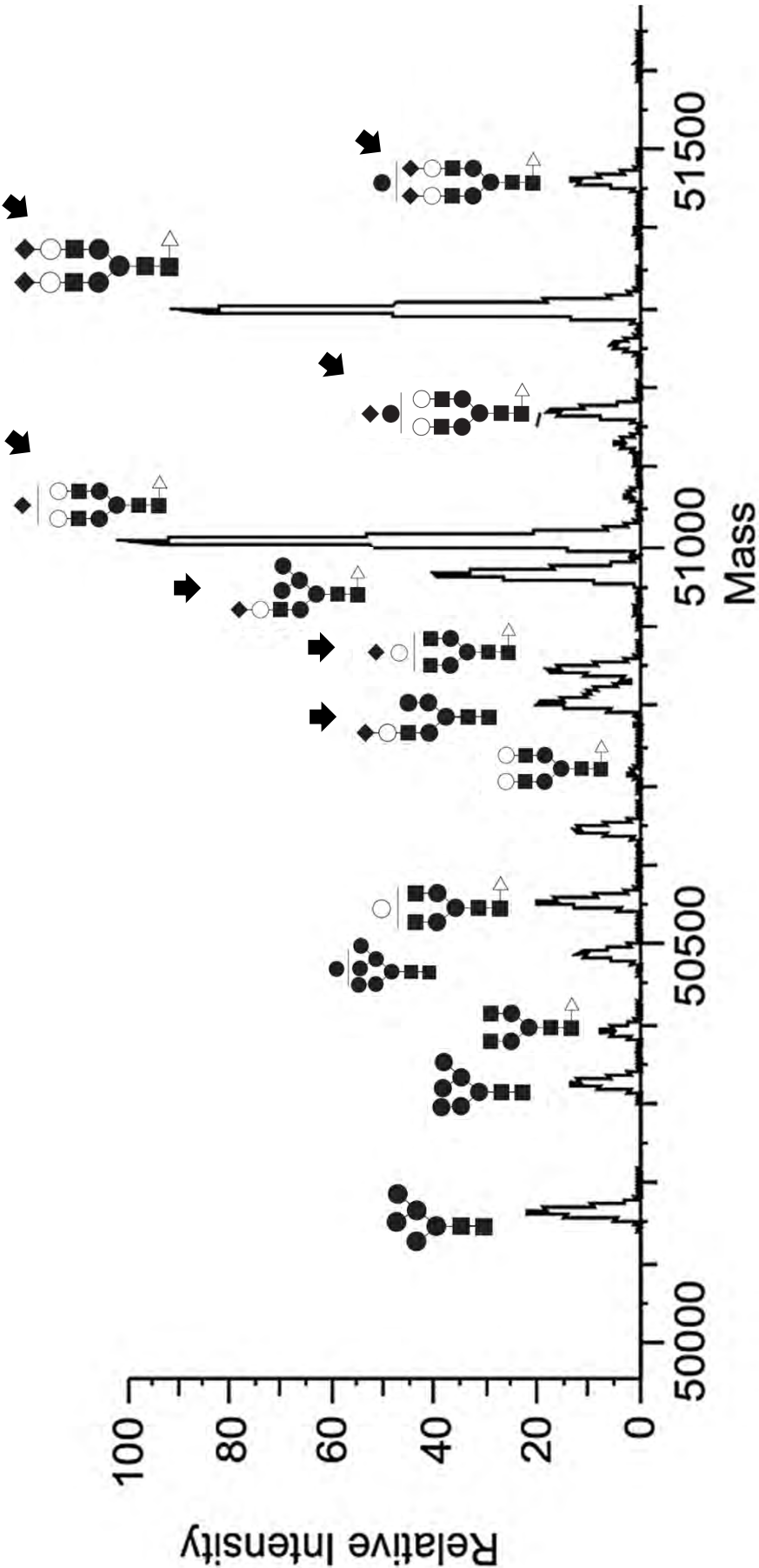
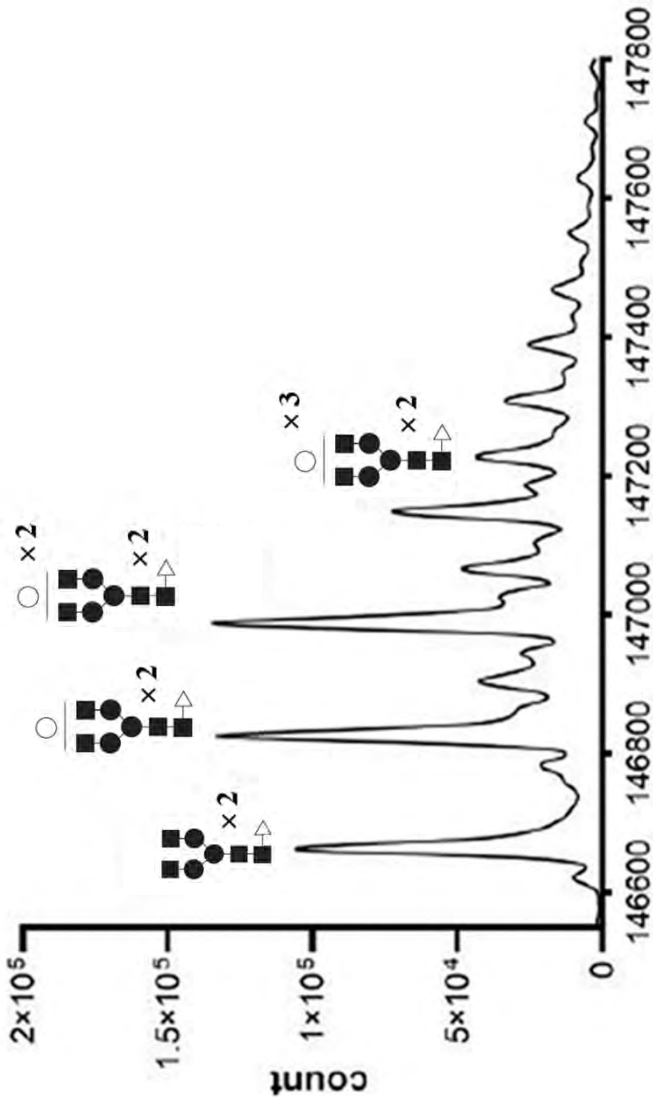


FIG. 4C



Deconvoluted Mass (amu)

FIG. 5A

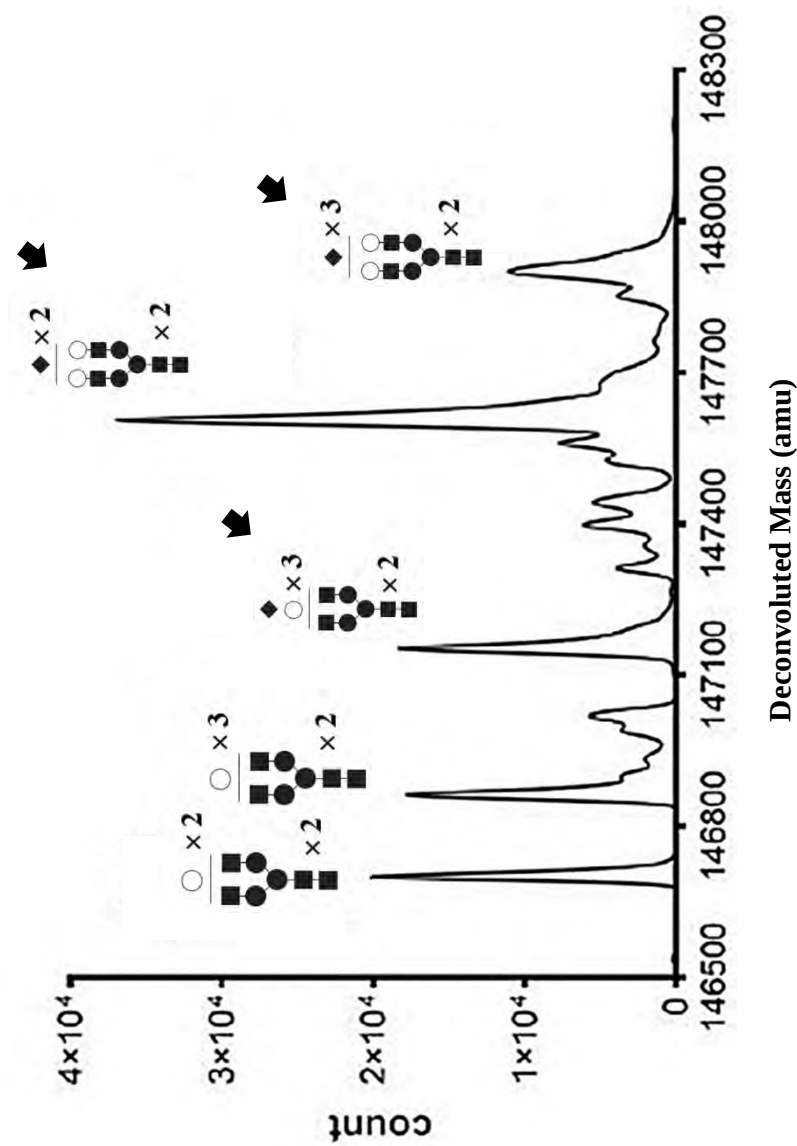


FIG. 5B

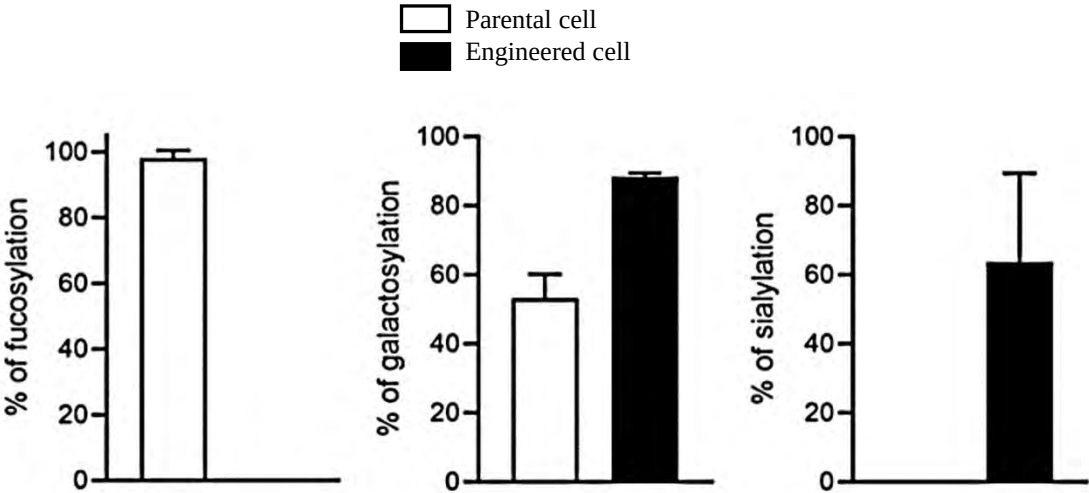


FIG. 6

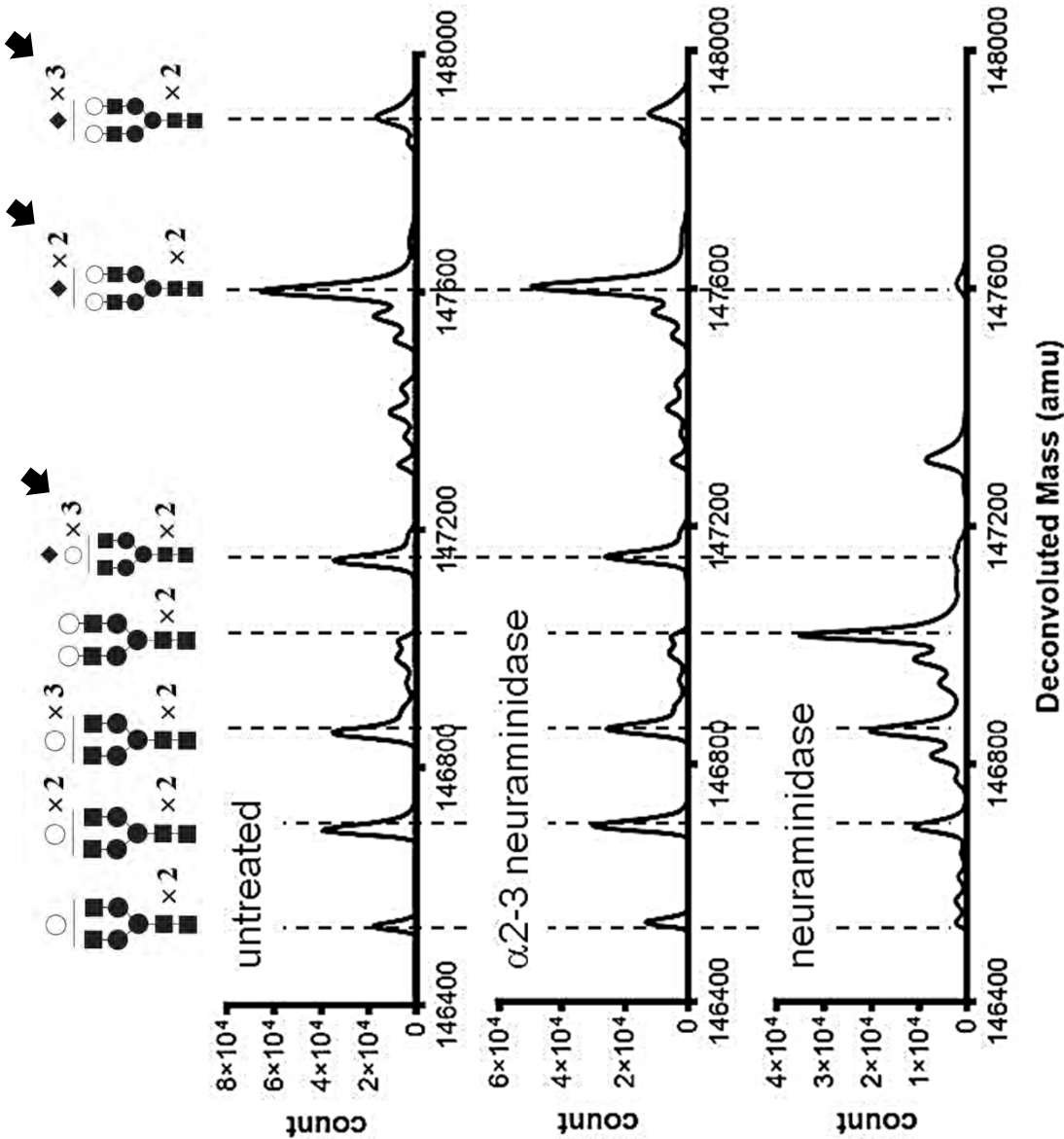


FIG. 7

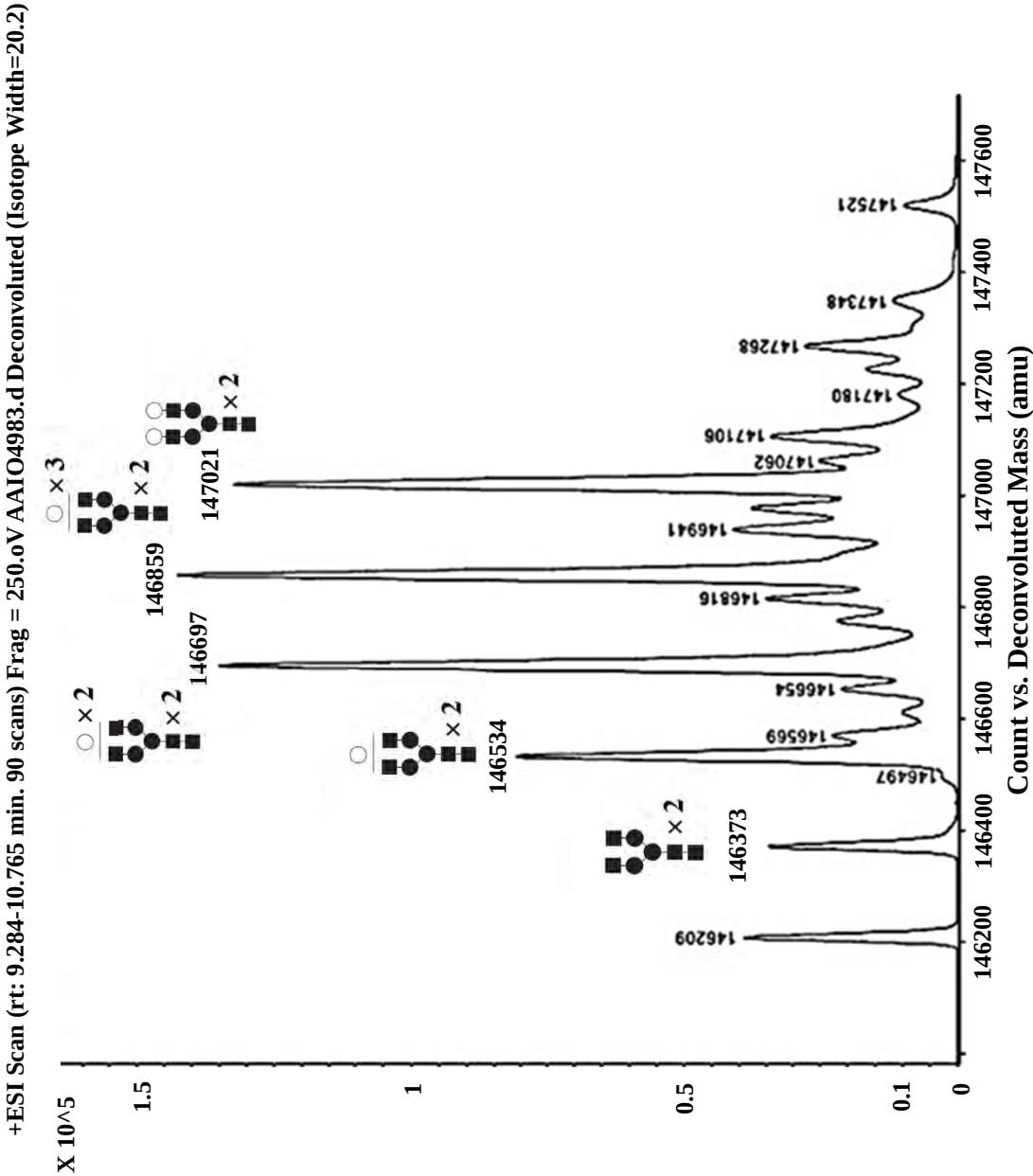


FIG. 8

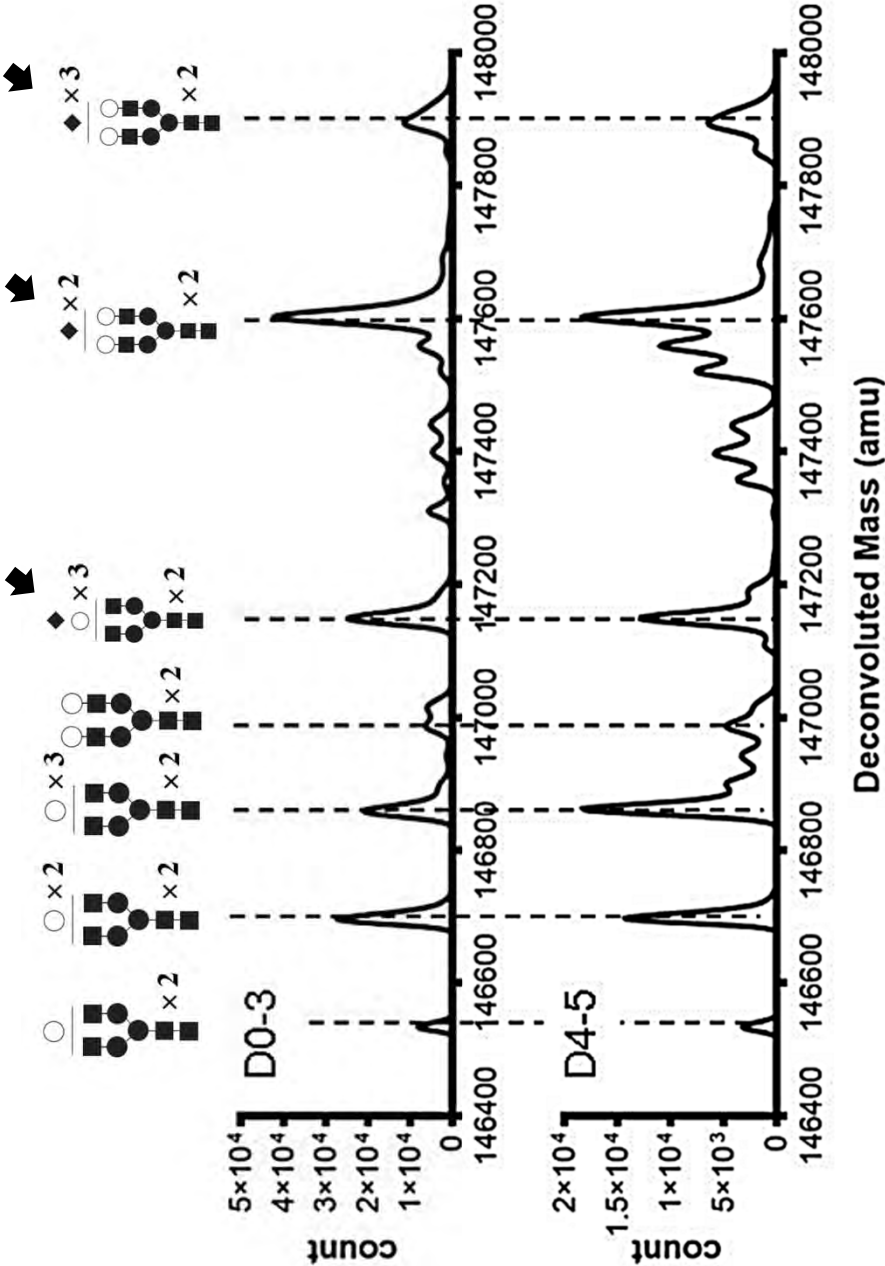


FIG. 9A

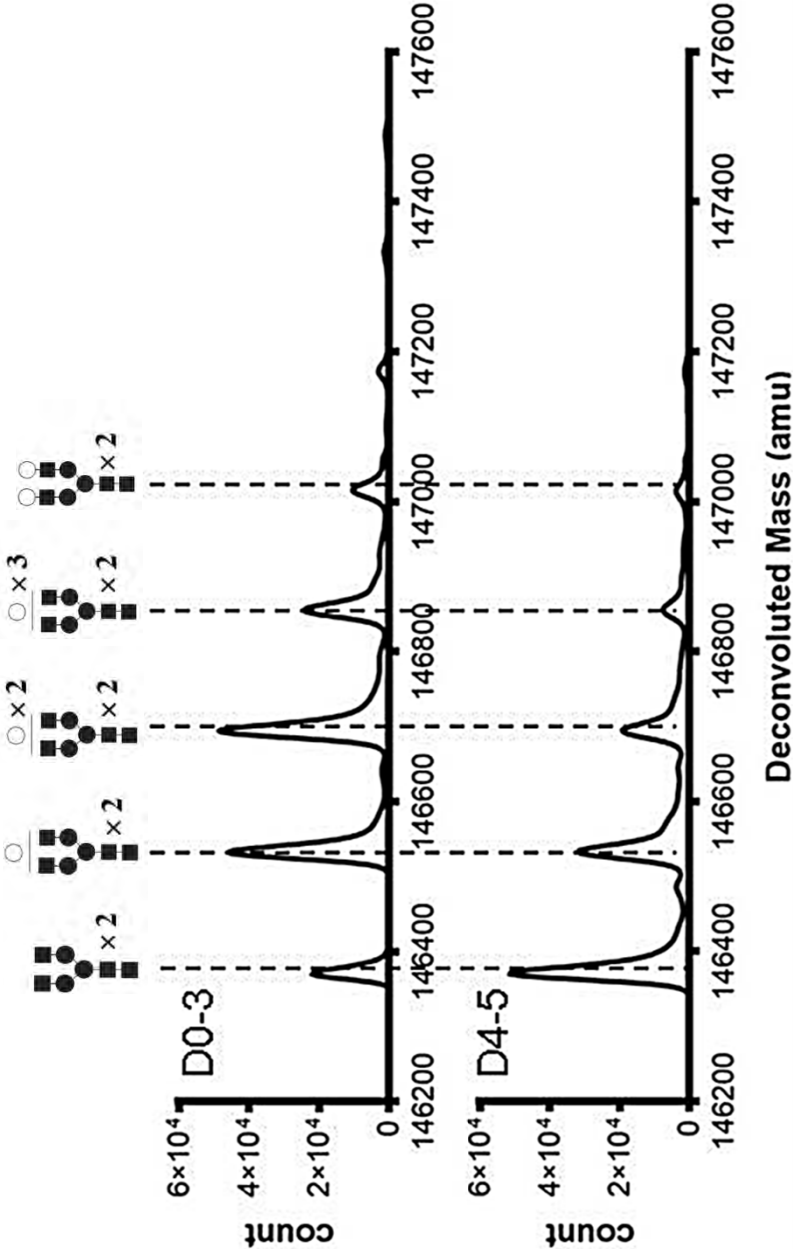


FIG. 9B

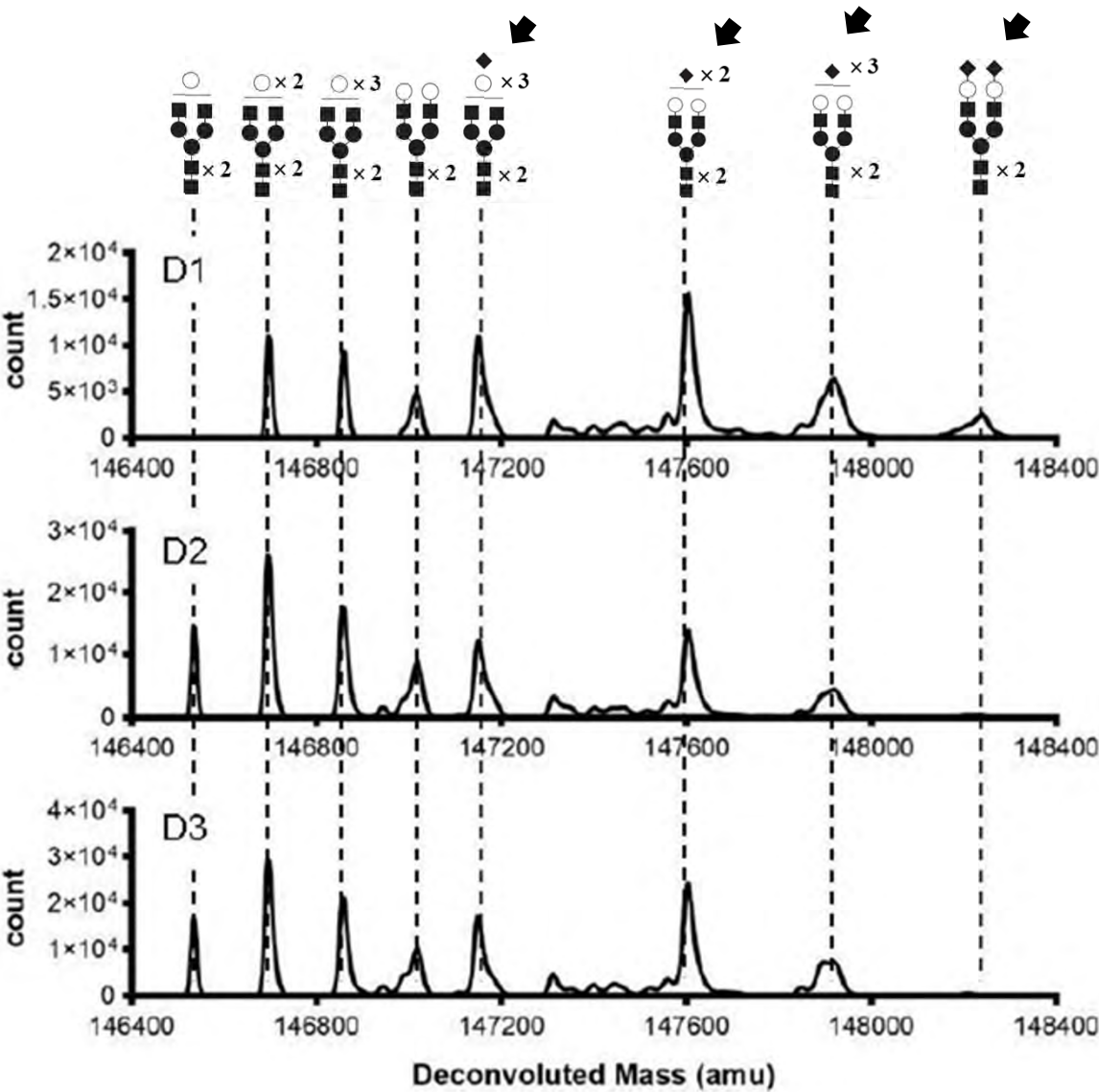


FIG. 10

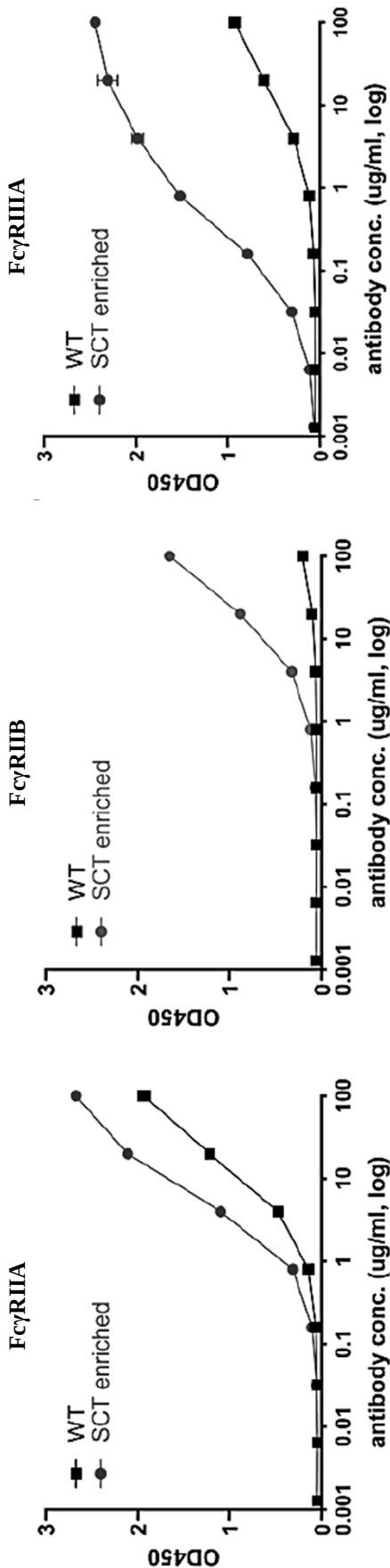


FIG. 11

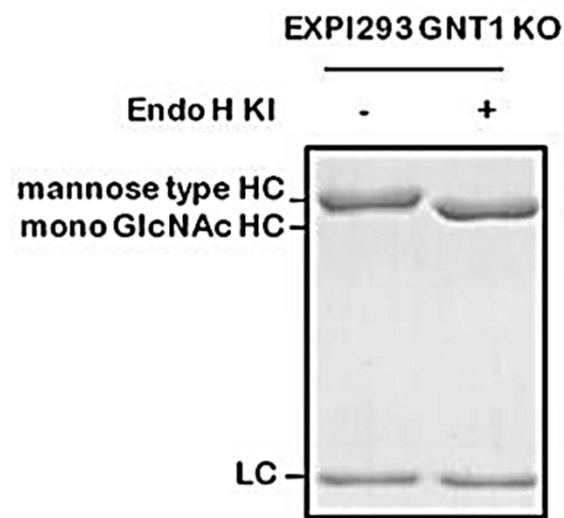


FIG. 12A

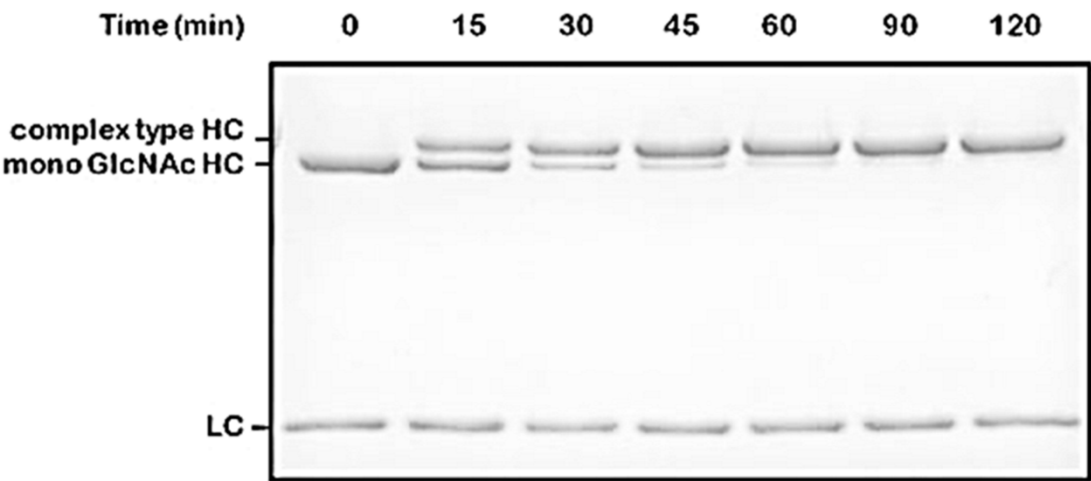


FIG. 12B

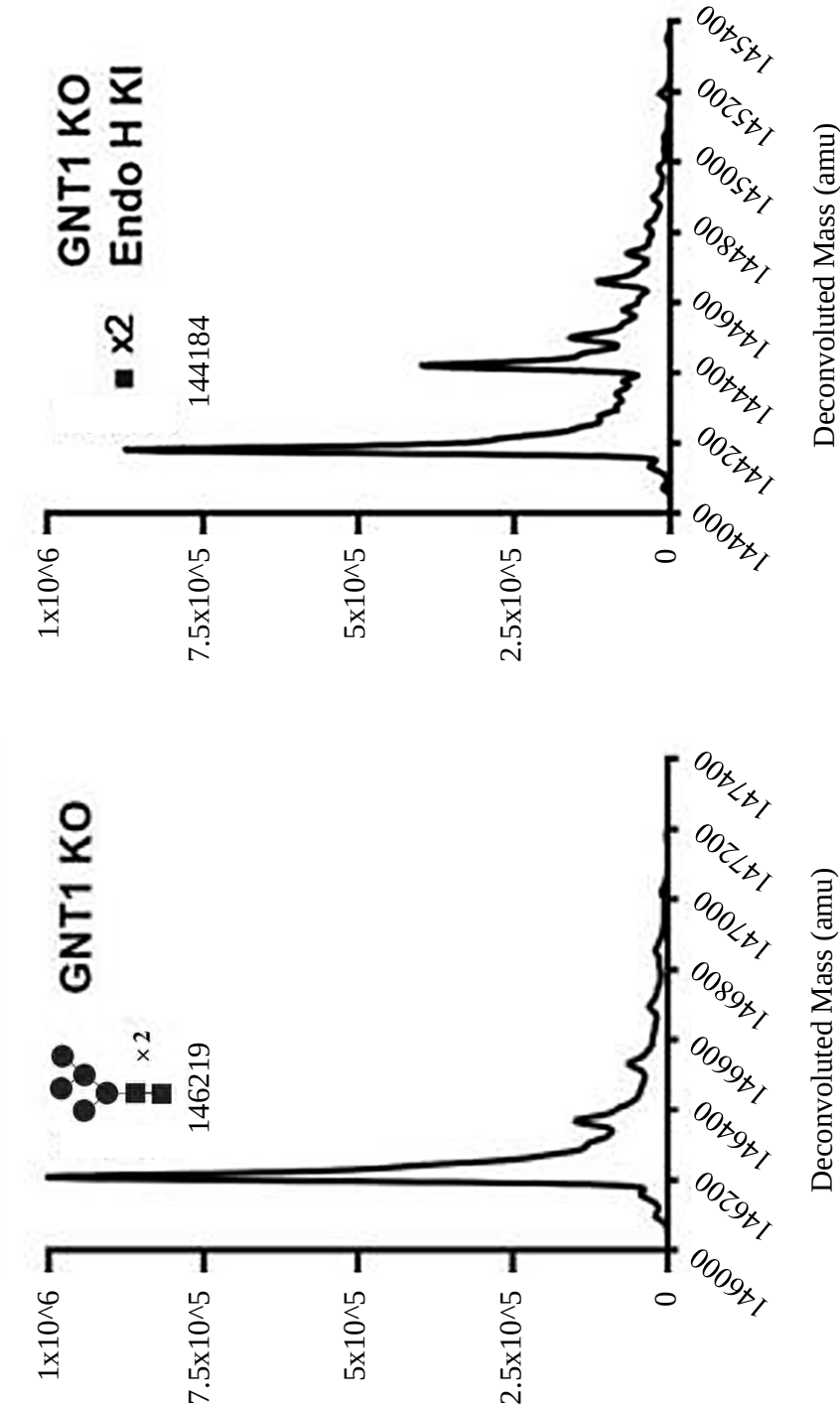


FIG. 12D

FIG. 12C

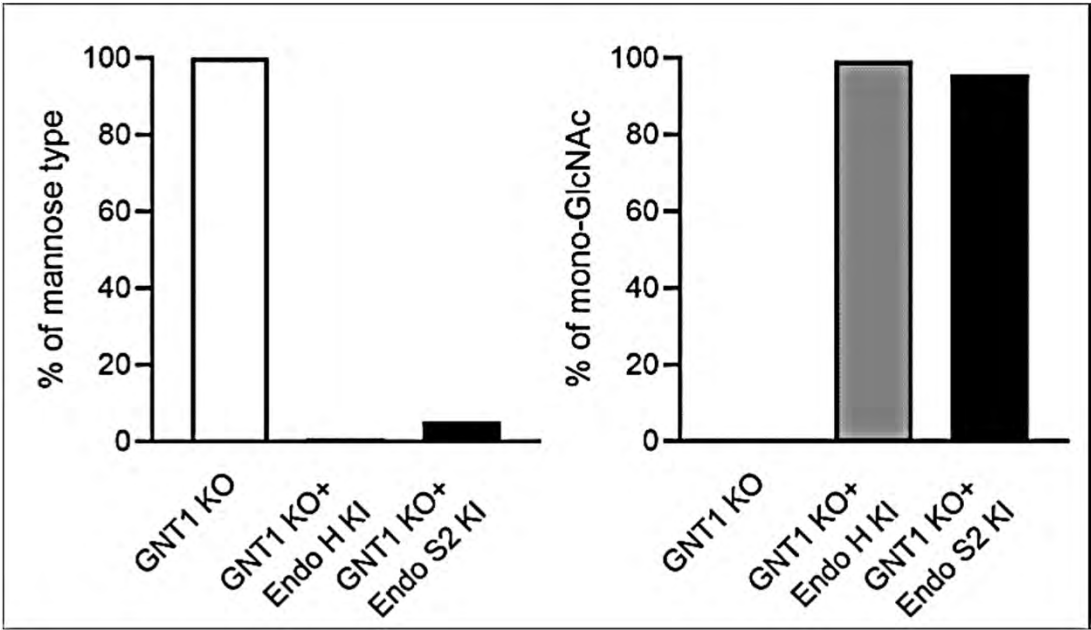


FIG. 12E

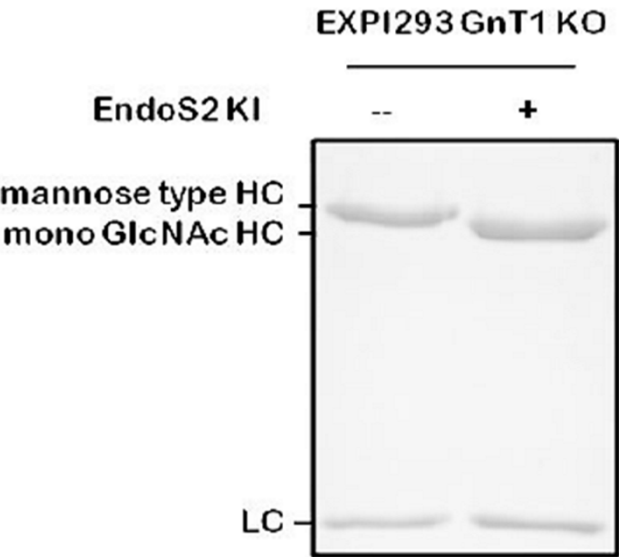


FIG. 13A

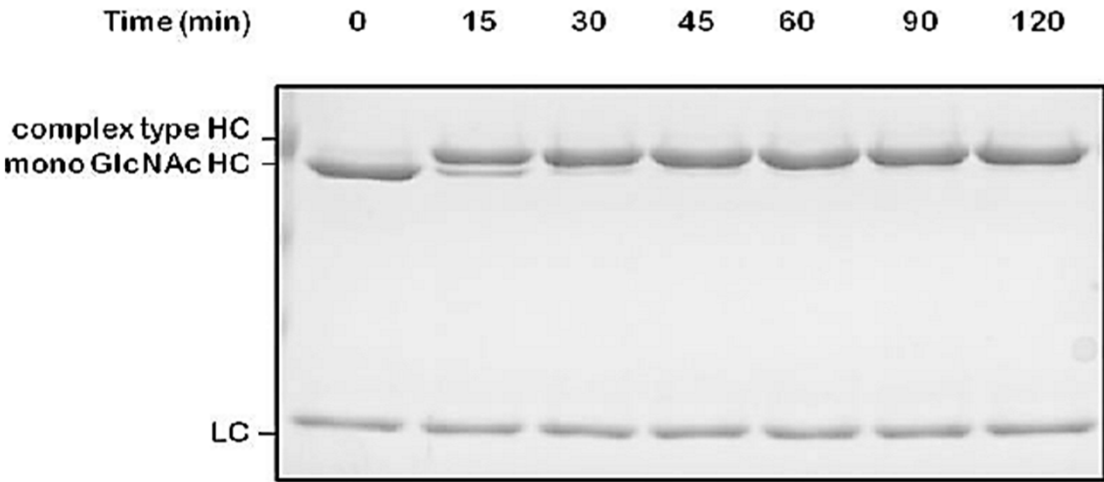


FIG. 13B

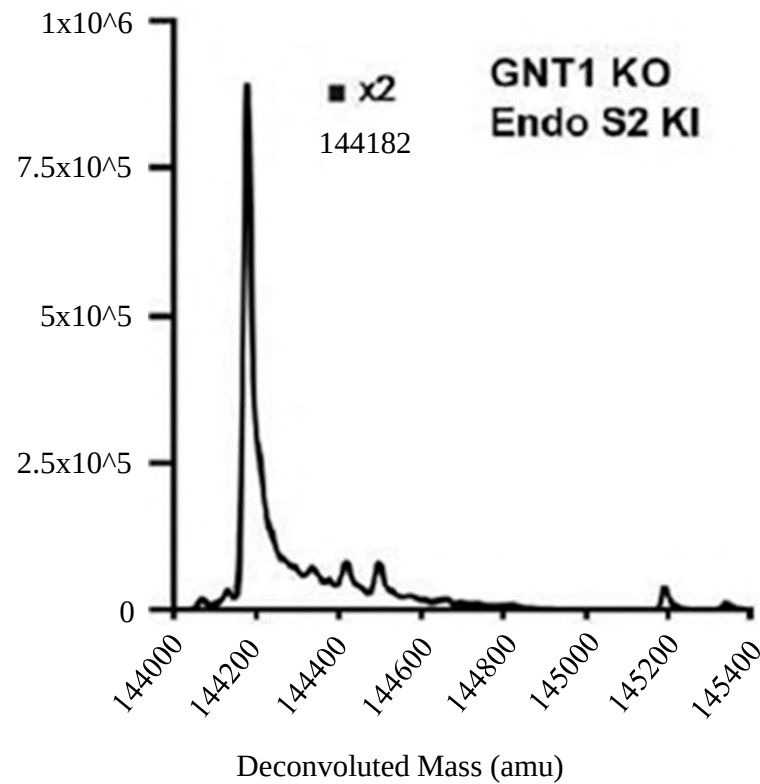


FIG. 13C

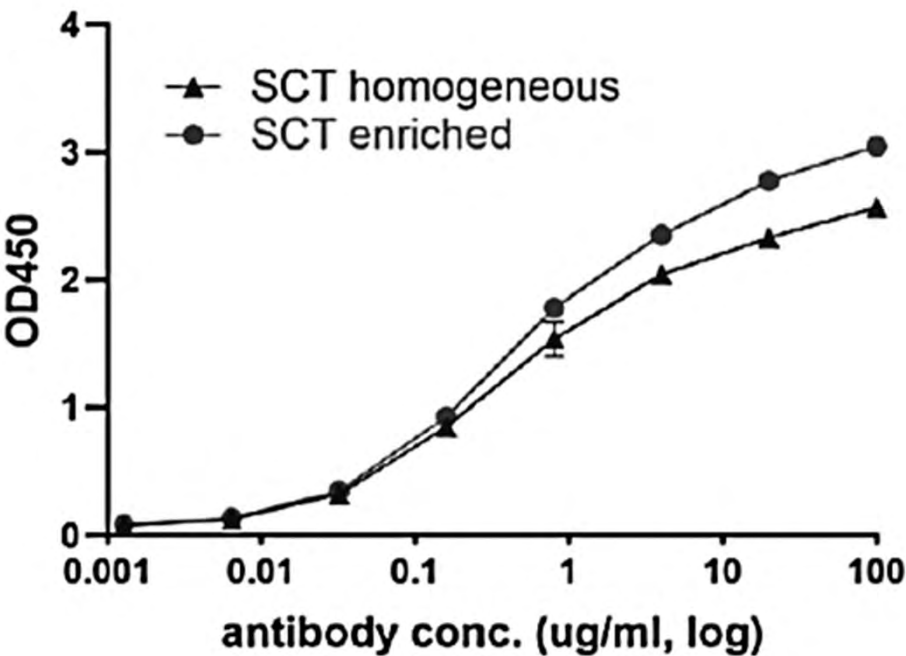


FIG. 14A

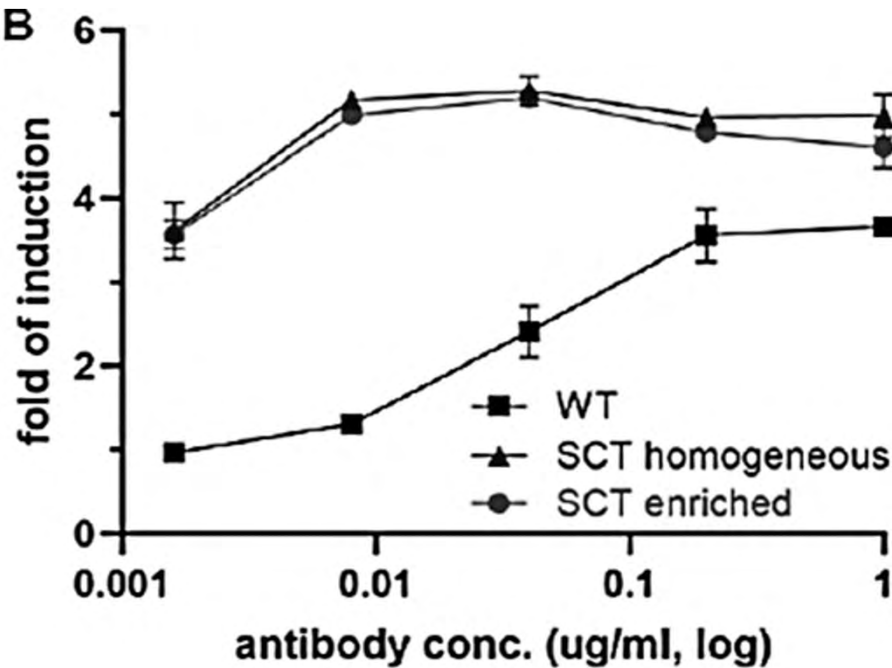


FIG. 14B



DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:	PCT/US2024/034588
International filing date:	19 June 2024 (19.06.2024)
Document type:	Certified copy of priority document
Document details:	Country/Office: US
	Number: 63/639,286
	Filing date: 26 April 2024 (26.04.2024)
Date of receipt at the International Bureau:	13 July 2024 (13.07.2024)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

1322589

THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 12, 2024

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APPLICATION NUMBER: 63/639,286

FILING DATE: *April 26, 2024*

RELATED PCT APPLICATION NUMBER: *PCT/US24/34588*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US63/639,286*



Certified by

Katherine Kelly Vidal

Performing the Functions and Duties of the
Under Secretary of Commerce
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APPLICATION #
63/639,286

RECEIPT DATE / TIME
04/26/2024 03:51:12 PM Z ET

ATTORNEY DOCKET #
A1000-01700

Title of Invention

CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY

Application Information

APPLICATION TYPE Utility - Provisional Application under
35 USC 111(b)

PATENT # -

CONFIRMATION # 3251

FILED BY Shannon Warner

PATENT CENTER # 65266261

FILING DATE -

CUSTOMER # 169053

FIRST NAMED INVENTOR Chi-Huey WONG

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Wade Lin

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01700__20240426__ADS.pdf	8	Application Data Sheet	2224 KB
A1000-01700__20240426__Specification.pdf	11	Specification	10334 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
A1000-	6096AB58EAC2759A0DF706BEB6B68543A8F318DF53D060A7C

01700_20240426_ADS.pdf	0351D57CC21D30A8283E050218CCB662471E52FFCCCF24CB A0F5DA0DEE3A1E57FB10AA4BD74C6C0
A1000- 01700_20240426_Specification. pdf	D52C9D3F0CDD20DB90C67C43C63E43FF3A8DA9313269D0D6 B5B3D98EC96B17D4E1EBED1C4839B3C6A45EB683A9C439CD 330C285BBDB66B1974289739268B483E

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01700
		Application Number	
Title of Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor 1					Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Chi-Huey		WONG		
Residence Information (Select One) ☒ US Residency ☐ Non US Residency ☐ Active US Military Service					
City	Rancho Santa Fe	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1		P.O. Box 8154			
Address 2					
City	Rancho Santa Fe	State/Province	CA		
Postal Code	92067	Country i	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.					Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	169053		
Email Address	ipdocketing@prosylogroup.com	Add Email	Remove Email

Application Information:

Title of the Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		
Attorney Docket Number	A1000-01700	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01700
		Application Number	
Title of Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	169053		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01700
		Application Number	
Title of Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		

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This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01700
		Application Number	
Title of Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01700
	Application Number	
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Title of Invention	CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Cell-based production of Fc-GlcNAc and Fc- α -2,6 sialyl glycan enriched antibody with improved effector functions through glycosylation pathway engineering

ABSTRACT: Glycosylation of antibody plays an important role in Fc-mediated killing of tumor cells and virus-infected cells through effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody dependent cell-mediated phagocytosis (ADCP) and vaccinal effect. Previous studies showed that therapeutical humanized antibodies with α -2,6 sialyl complex type (SCT) glycan attached to Fc-Asn297 exhibited optimal binding to the Fc receptors on effector cells associated with ADCC, ADCP and vaccinal effect. However, the production of antibodies with homogeneous Fc-SCT needs multiple *in vitro* enzymatic and purification steps. In this study, we report two different approaches to shorten the processes to produce SCT-enriched antibodies. First, we expressed a bacterial endoglycosidase in GNT1-KO EXP293 cells to trim all *N*-glycans to mono-GlcNAc glycoforms for *in vitro* transglycosylation to generate homogeneous SCT antibody. Second, we engineered the glycosylation pathway of HEK293 cells through knock-out of the undesired glycosyltransferases and expression of the desired glycosyltransferases to produce SCT enriched antibodies with similar binding affinity to Fc receptors and ADCC activity to homogeneous SCT antibody.

Introduction

Antibody plays an important role in humoral immune response and has diverse plasticity and biological functions. The prototype of antibody is a major component of B cell receptor (BCR) on naive B cells. When the BCRs are activated through pattern recognition receptors (PRRs), the alternative splicing of mRNA helps produce antibodies as secreted forms of IgM or IgD. With the assistance of dendritic cells and T helper cells, the activated B cells could further undergo clonal expansion, somatic hypermutation, and class switch and are differentiated into plasma cells to produce secreted IgG, IgA, or IgE antibodies with high antigen specificity. Once a target cell or pathogen is opsonized by an antibody, it may trigger different downstream immune functions, including phagocytosis, cellular cytotoxicity, vaccinal effect, complement activation etc. These immune cell-based responses required the binding of antibody Fc domain to specific Fc receptors on immune cells. Each subtype or glycoform of antibody can be recognized by several Fc receptors rendering activating or inhibitory signaling to generate different effector functions. For example, the binding of IgG antibody to the Fc γ RIIA receptor on NK cells will activate NK cells to release cytokines to kill target cells, a process called antibody-dependent cellular cytotoxicity (ADCC)¹⁻³ and binding to the Fc γ RIIA receptor on macrophages will activate macrophage to kill the target cell through antibody-dependent cell-mediated phagocytosis (ADCP). In addition, binding of antibody-antigen complex to the Fc γ RIIA receptor on dendritic cells

can facilitate dendritic cell maturation to induce memory CD8 T-cell response⁴.

Glycosylation is a common post- or co-translational modification found on most cell surface proteins. During *N*-glycoprotein synthesis, a high mannose glycan is first synthesized in the ER and then transferred to the growing polypeptide chain followed by chaperone-mediated protein folding and quality control to form glycoproteins. The high-mannose glycoproteins are trimmed by mannosidases and translocated to the Golgi apparatus for addition of more monosaccharides sequentially by different glycosyltransferases before moving to the cell surface. Unlike protein and DNA, glycosylation is template independent, and the sequence of glycans is determined by the repertoire of glycosyltransferases expressed in different cell types. Moreover, the cellular environment and protein accessibility also affect the activity and specificity of glycosyltransferases. Thus, the glycans generated by glycosylation usually exhibit more diversity than other modifications⁵⁻⁷.

Antibodies of each subtype are also glycosylated at different places within the constant region of heavy chain (HC) and these glycans regulate antibody functions⁸. The preBCR assembly is important for B cell development and is critically regulated by the *N*-glycan at N46 on μ HC⁹. The *N*-glycan at N402 on μ HC has been linked to antibody oligomerization and complement activation^{9,10}. Besides, IgG *N*-glycosylation at N297 on γ HC plays a critical role in complement activation and Fc γ receptor activation leading to various effector functions¹¹⁻¹³.

Therefore, it is important to identify optimized glycosylation to improve the efficacy and safety of therapeutic antibodies.

Generally, the production of recombinant antibody is often based on mammalian cell lines, such as NS0, 293T and CHO cells, to produce a mixture of glycoforms with core-fucosylated bi-antennary complex type glycans¹⁴ which are not optimal for binding to the FcγRIIIA receptor, because of the inhibitory function of core-fucosylation¹¹ or the off-target delivery caused by terminal galactosylation¹⁵. Therefore, efforts have been directed toward modification of the Fc-glycan on antibody to improve specific receptor binding and the corresponding effector function. One strategy used was to block the fucosylation pathway in cells. For example, using the cell line deficient in GDP-fucose synthesis could produce antibodies without core-fucosylation, leading to an increase in binding to FcγRIIIA receptor thereby enhancing the ADCC activity^{11, 16}. The emergence of precise gene editing tools such as Zinc Finger Nuclease (ZFN) or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) has facilitated the progress of this strategy¹⁷ and studies on glycosylation pathway engineering in cells to produce therapeutic proteins with improved efficacy have been reported¹⁸⁻¹⁹.

Another strategy to alter protein glycosylation is through *in vitro* glycoengineering by enzymes or chemical ligation. A common approach was endoglycosidase-mediated transglycosylation⁷. Usually, the antibody with high mannose type glycan derived from insect cells²⁰, yeast²¹ or GNT1 knockout (KO) cells was treated with endoglycosidase H or S2 to generate mono GlcNAc- or GlcNAc-Fuc bearing glycoforms. The antibody with either of these two glycoforms is a good acceptor substrate for mutated endoglycosidase (glycosynthase)-mediated transglycosylation⁷ using a homogenous glycan oxazoline as donor substrate to generate a new glycan chain. So far, many glycans have been transferred to antibodies to study the functional impact on receptor binding and effector functions. The fully galactosylated complex type glycan without core-fucose

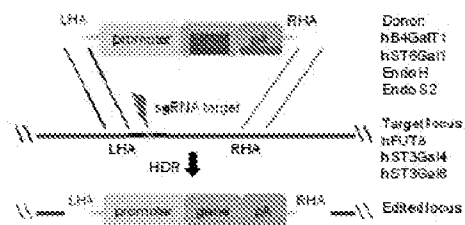


Figure 1. Illustration of CRISPR/Cas9 KO/KI strategies for glycosylation pathway engineering.

revealed that the α2-6 linked SCT provides enhanced binding to FcγRIIIA and FcγRIIA receptors which are associated ADCC, ADCP and vaccinal effect²²⁻²⁴. Although this strategy could produce antibodies as homogenous glycoforms, the process requires multiple reaction and purification steps and the specificity of mutant endoglycosidases is limited. In this report, we used humanized antibody chMC183-70 that targets specific stage embryonic antigen 4 (SSEA4) on various cancer cells as a model to produce and evaluate the Fc-SCT enriched antibody in Fc receptor binding and activation of cellular cytotoxicity.

Result and Discussion

Development of Fc-GlcNAc antibody producing cells by CRISPR-Cas9. Antibodies are generally produced from mammalian cell lines which contain core-fucosylated complex type glycans¹⁴. To generate the Fc-GlcNAc antibody for *in vitro* transglycosylation, antibody glycans need to be trimmed by endoglycosidase and fucosidase to leave only the innermost GlcNAc as an acceptor². We would like to shorten these steps by CRISPR-Cas9 to generate Fc-GlcNAc antibody with commercially available GNT1 KO EXP293 cells. The N-glycan expressed by this cell line was high mannose type which was

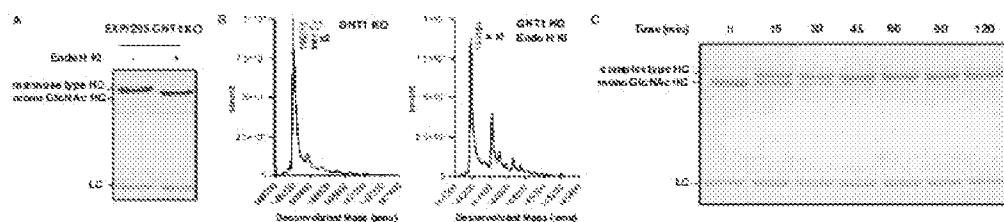


Figure 2. Expression of Endo H in GNT1-knock out cells results in cleavage of N-glycans on antibody. (A) The chMC18370 antibody expressed from engineered and its parental GNT1-KO EXP293 cells were subjected to protein electrophoresis and the difference in molecular weight by band shifting was observed. (B) The chMC18370 antibody expressed by engineered and its parental GNT1-KO EXP293 cells were subjected to IPM analysis to examine the molecular weight and glycan changes. (C) The chMC18370 antibody expressed by engineered GNT1-KO EXP293 cells was subjected to transglycosylation assay and the efficiency of transglycosylation was assessed by protein electrophoresis.

contributes significantly to improve antibody binding to the FcγRIIIA receptor compared to the wild-type antibody²²⁻²⁴. However, it also facilitates the antibody clearance by liver cells through asialoglycoprotein receptor binding. Further studies

preferentially cleaved by Endo H²⁵ to generate Fc-GlcNAc antibody. Thus, we aimed to knock in Endo H in this cell line by CRISPR-Cas9. However, after transfection of CRISPR-Cas9 and donor plasmids, cells started to die with apoptosis and

resulted in reduced cell survival. Fortunately, the survival rate started to rise after several days and returned to normal level again (Fig. S1). We then wondered whether these surviving cells still expressed Endo H to cleave the mannose type *N*-glycans on antibody. The antibodies expressed by the cells were analyzed by electrophoresis and the result showed that the antibody heavy chain (AbHC) expressed by these cells had faster mobility than AbHC from its parental cells (GNT1 KO) and most AbHCs were downshifted (Fig. 2A). This antibody was also subjected to transglycosylation assay and the efficiency was good (Fig. 2C). We then analyzed the glycans on this antibody by intact protein mass analysis, and the results showed that most antibodies contain only GlcNAc on each HC (Fig. 2B and Fig. S2). These results indicate that CRISPR-Cas9-mediated Endo H KI in GNT1 KO cells can serve as a platform to produce Fc-GlcNAc antibody for downstream application.

In addition to Endo H, we also tried to express endoglycosidase S2 (Endo S2) from *Streptococcus pyogenes* in the cells because this enzyme can hydrolyze both high mannose and complex type *N*-glycans to Fc-GlcNAc²⁶. Therefore, the donor vector and CRISPR-Cas9 plasmids were delivered to GNT1 KO cells and the cell survival rate was monitored. Interestingly, we did not see any significant cell death in the GNT1-KO cells after plasmid transfection (Fig. S1). Then the antibody glycans generated by these cells were examined as previously described.

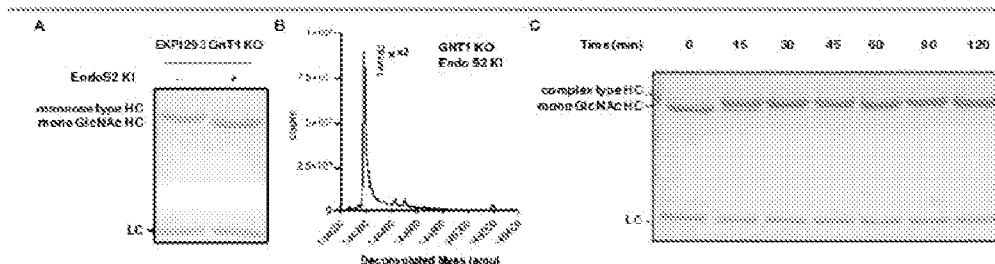


Figure 3. Expression of Endo S2 in GNT1-knock out cells results in cleavage of antibody *N*-glycans to GlcNAc. (A) The chMC18370 antibody glycoforms expressed by engineered and parental GNT1-KO EXP1293 cells were subjected to electrophoresis and a band shifting was observed. (B) The chMC18370 antibody expressed by engineered GNT1-KO EXP1293 cells was subjected to IPM analysis to examine the molecular weight and glycan changes. (C) The chMC18370 antibody expressed by engineered GNT1-KO EXP1293 cells was subjected to transglycosylation and the efficiency was evaluated by protein electrophoresis.

The protein gel showed an obvious downshifted band of AbHC compared with its parental cells (GNT1 KO) (Fig. 3A). Besides, the glycans also can be added onto AbHC through enzymatic transglycosylation (Fig. 3C) and intact protein mass analysis showed a clear signal of Fc-GlcNAc antibody (Fig. 3B.). The glycoform analysis revealed a major Fc-GlcNAc antibody population as well (Fig. S2). Therefore, based on these results, it is concluded that endoglycosidase S2 can be introduced to cells by CRISPR-Cas9 to process the *N*-glycans to Fc-GlcNAc antibody for *in vitro* transglycosylation. In addition, this cell-based method can be used for other glycoproteins, such as influenza hemagglutinin and SARS-CoV-2 spike protein to generate the mono-GlcNAc decorated glycoforms as vaccines to elicit broadly protective immune responses^{27,28}.

Glycosylation pathway engineering in cells to produce Fc-SCT enriched antibody by CRISPR-Cas9. The second

approach to generate antibody with Fc-SCT glycoform was to directly edit the expression of glycosyltransferases in cells. As previously described, the antibody produced from commonly used cell lines usually contained bi-antennary complex type *N*-glycans with core-fucose and terminated mainly with galactose^{14, 16-19, 29}. Therefore, in order to produce the antibody with Fc-SCT, we decided to increase the level of galactosylation and sialylation on the antibody^{19, 30}. In addition, the core-fucosylation, which depleted ADCC³¹, was eliminated by knocking out FUT8³⁰ (Fig. 1). Furthermore, the terminal sialylation with α 2-6 linkage, not the α 2-3 linkage, was introduced as it was found to enhance receptor binding³¹ and improve ADCC and vaccinal effect^{23-26, 32}. Although the mRNA of human α 2-3 sialyltransferases could be detected in 293T and CHO cells^{18, 33}, we did not observe any obvious sialylation on the antibody glycan from these cell lines^{18-19, 29} (Fig. 4, upper). Therefore, we were not concerned about the antibody being glycosylated by the inhibitory α 2-3 sialylation. We therefore used CRISPR-Cas9 to knock out hFUT8 and knock in hB4GalT1 and hST6GalI in 293T cells (Fig. 1). The antibody expressed by this engineered cell line was purified and subjected to intact protein mass analysis of the antibody and the attached glycans. We observed a significant increase of galactosylated and sialylated antibody glycoforms (Fig. 4, lower) compared to the antibody generated by WT cells (Fig. 4, up). Glycoform analysis also revealed a

significant increase in antibody galactosylation and sialylation, with lack of core-fucosylation (Fig. S3).

The sialylation on antibody is α 2-6 linkage. To confirm the sialylation on the antibody was mediated by hST6GalI, the antibody was treated with two different sialidases, *Streptococcus pneumoniae* α 2-3 neuraminidase and *Clostridium perfringens* neuraminidase overnight respectively. The intact protein analysis did not show any obvious difference in the distribution of asialylated and sialylated antibody between the untreated and α 2-3 neuraminidase-treated group (Fig. 5 upper and middle), indicating that the sialylation on antibody was not due to the endogenous α 2-3 sialyltransferases. On the other hand, using neuraminidase which could remove all linkages of sialylation largely eliminated the terminal sialic acids. In addition, the antibody with increased galactosylation generated from cells with knock in of hB4GalT1 only, the terminal sialylation was still

not observed (Fig. S4). These results confirmed that sialylation on the antibody was introduced by hST6GalI and the increase of hB4GalT expression indeed elevated the galactosylation of antibody (Fig. 4). To further explore the capacity of sialylation or galactosylation in cells, we transiently overexpressed hST6GalI or hB4GalT1 in the engineered cells followed by antibody production to see if the galactosylated or sialylated antibody was increased. However, in intact protein mass analysis, we did not observe further elevation of any galactosylated or sialylated antibody after transient overexpression of hST6GalI or hB4GalT1 (Fig. S5). Another study was also attempted to increase the sialylation by elevating the glycosyltransferase expression in CHO cells but it still showed incomplete sialylated glycans³⁹. The sialyltransferase hST6GalI is known as the only human glycosyltransferase which mediates α 2-6 sialylation and preferentially use the α 1-3 branch arm of the biantennary glycan as substrates²⁴. The *in vitro* studies show glycoengineering on antibody glycans by hST6GalI takes a

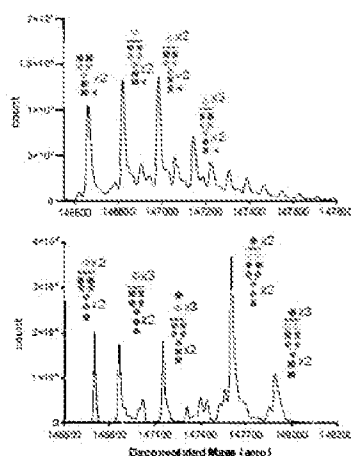


Figure 4. The glycoform composition of chMC81370 antibody. The N-glycans on antibody expressed by WT (upper) and glycosylation pathway engineered 293T cells (lower) were purified by protein A and analyzed by intact protein mass analysis.

long time and difficult to yield fully sialylated antibody glycans^{39,54}. Furthermore, this also could explain why only the antibody with at least one fully galactosylated glycan was sialylated. If the galactose on Glc glycan was not on the α 1-3 branch arm, the hST6GalI may not sialylate it. Therefore, the expression level of glycosyltransferases may not be the bottle neck in this situation rather the substrate preference and enzyme activity are the major factors^{5,7}. Using α 2-6 sialyltransferases from other organisms may have better activity than hST6GalI.⁵⁵

Protein glycosylation varies with expression time. In order to understand if the amount and time of antibody overexpression in cells affected glycosylation, we extended the culture time to 5 days and then collected the medium at day 3 and day 5 to

examine the antibody glycans by intact protein mass analysis. We unexpectedly found that the glycan pattern was somewhat different between these two time points (Fig. S6A). In the IPM analysis, a relatively strong sialylation was observed in the first collection (first three days) as we saw before (Fig. S6A, D0-3) and then sialylation was decreased in the second collection (last two days), accompanied by an increase of terminal gal-

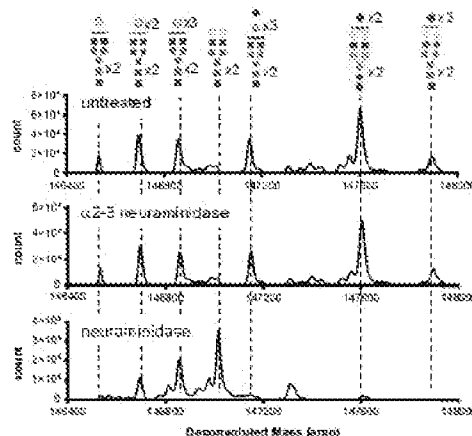


Figure 5. The sialylation on antibody is α 2-6 linkage. The SCT enriched chMC81370 antibody was expressed by glycosylation pathway engineered cells. After incubation with indicated enzyme overnight, the antibody was purified by protein A and analyzed by intact protein mass analysis.

actosylation (Fig. S6A, D4-5), suggesting that the proteins involved in glycosylation may not be sufficient to effectively produce Fo-SCT antibody at later stage. Because the hST6GalI and hB4GalT1 involved in the FC-SCT antibody production were almost artificially and constitutively expressed in the cells, we also examined if endogenous glycosyltransferases faced the same problem of reduced glycosylation. By expressing antibody in FUT8 KO cells, the antibody was almost terminally glycosylated with at least one or more galactoses in the first collection (Fig. S6B, D0-3), but the proportion of galactosylated antibody decreased and even the antibody glycans without any galactoses became the dominant glycoforms in the last two days (Fig. S6B, D4-5). The result showed that short glycosylation after protein overexpression can be observed on endogenous glycosylation products as well. This finding promoted us to dissect the impact of antibody production at different times on glycosylation. We purified antibody every day after plasmid transfection and analyzed the dynamic change of antibody glycans from multiple engineered cells. To our surprise, the antibody with full sialylation could be significantly detected by intact protein mass analysis at the first day, although it became unclear after that (Fig. 6). Overall, the most abundant sialylation level was observed at first day and then started to decrease day by day (Fig. 6 and S4A). Regarding galactosylation, the antibody glycans were capped by at least two galactoses (2-4 galactoses

Figure 6. Antibody expression and time-dependent changes of glycans on antibody. The chMCH1370 antibody expressed in glycosylation pathway engineered cells was collected every day and purified by protein A for intact protein mass analysis.

Fc-SCT enriched antibody showed better binding to the Fcγ receptors than WT antibody. As we can obtain Fc-SCT enriched antibody from glycosylation pathway engineered cells, the next step was to compare the binding of the mixture of antibody glycoforms to Fc receptors with WT antibody³. FcγIIA and FcγIIB are usually expressed simultaneously by the antigen presenting cells, such as dendritic cells and macrophages, and these two receptors work together to modulate immune response³. FcγIIIA is critical for NK cells to mediate ADCC³. Therefore, the binding of antibody to these receptors was

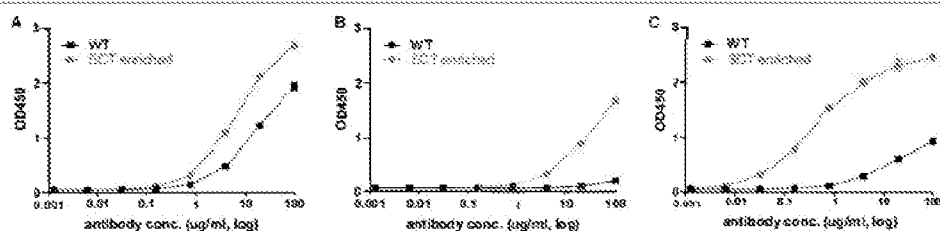


Figure 7. Improved receptor binding of SCT enriched antibody. The Fcγ receptor-coated plate (A, FcγRIIA; B, FcγRIIB; C, FcγRIIA) was incubated with WT antibody (black) or SCT enriched antibody (red) at indicated concentrations. The binding of antibody was measured by anti-human IgG Fc antibody conjugated with HRP. The binding of SCT enriched antibody to tested receptors was greatly improved compared with WT.

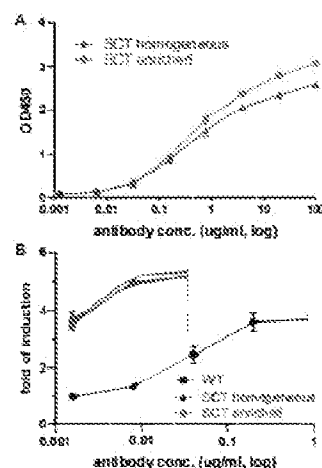


Figure 8. Functional comparison of homogeneous SCT and SCT enriched antibody. (A) The FcγRIIIA-coated plate was incubated with homogeneous SCT antibody (blue) or SCT enriched antibody (red) at indicated concentration. The binding of antibody was measured by anti-human IgG Fc antibody conjugated with HRP. The binding of SCT enriched antibody and homogeneous SCT antibody to FcγRIIIA was similar. (B) Indicated antibody, target cells, and effector cells were co-incubated for 6 hours followed by detection of luciferase activity. SCT enriched and homogeneous antibody SCT showed similar effects on induction of ADCC reporter response and both elicited higher response than WT antibody.

evaluated. We did not study the binding to Fcγ1A here because the glycoforms of complex type *N*-glycans on antibody would not affect the binding^{23,24}. We observed the binding of Fe-SCT-enriched antibodies to FcRα was increased in three tested Fc receptors (Fig. 7). Among these receptors, the increased binding of Fcγ1A and Fcγ1B was consistent with previous studies^{25,26} (Fig. 7A and 7C). Interestingly, the WT antibody showed very weak binding to inhibitory receptor Fcγ2B. Although the

engineered *N*-glycans on AbHC improved binding to FcγIIb, the improvement was less significant than binding to FcγIIa (Fig. 7B). This observation was aligned with the fact that both FcγIIb and α2-6 sialylation were the key factors in intravenous Ig (IVIg) treatment-mediated inflammatory suppression³⁶⁻³⁸. Although the increased binding to the two opposing functional receptors, FcγIIa and FcγIIb, which are typically expressed on the antigen-presenting cells (APCs) simultaneously, appears to be conflicting, the eventual immune response was determined by the expression level and signaling strength of FcγIIa and FcγIIb on effector cells³. The Fc-SCT enriched antibody also exhibited stronger FcγIIa binding ability than the antibody produced from wild type cells (Fig. 7C). This enhancement was mainly contributed by removal of core-fucose¹¹ and addition of galactoses²³.

Fc-SCT enriched antibody and homogenous Fc-SCT antibody are similar in functions. Since the binding of Fc-SCT enriched antibody to FcγIIa was elevated, we compared the binding with the homogenous Fc-SCT antibody. The result showed that Fc-SCT enriched antibody exhibited similar binding avidity to homogenous SCT antibody (Fig. 8A). Then we further evaluated if the Fc-SCT enriched antibody could evoke similar effect as homogenous Fc-SCT antibody in ADCC reporter assay³⁹. At the lowest antibody concentration used for induction, WT antibody did not induce any obvious cell activation, but the homogenous Fc-SCT and Fc-SCT enriched antibodies can trigger cell activation and rapidly reached a maximum at next concentration (Fig. 8B). Even at the higher concentration, the WT antibody can reach a maximum induction, but the activity was still much lower than that of homogenous Fc-SCT or Fc-SCT enriched antibody (Fig. 8B). The overall response induced by homogenous Fc-SCT and Fc-SCT enriched antibodies were also very similar in receptor binding assay (Fig. 8B). Thus, we concluded that the Fc-SCT enriched antibody derived from glycosylation pathway engineered cells could improve antibody effector functions in a similar manner as homogenous Fc-SCT antibody did.

In summary, we have simplified the process in different ways to generate functional Fc-SCT antibodies and evaluated the utility. First, we demonstrated that with endoglycosidase S2 or H expressed in the glycosyltransferase KO cells, the glycoengineered cells can produce antibodies with Fc-GlcNAc and the antibody made by this way can be used for *in vitro* enzymatic transglycosylation (Fig. 2-4). This strategy can be applied to engineer other *N*-glycosylated proteins to produce GlcNAc-decorated glycoproteins as well (Fig. 5). Second, we knocked out the core-fucosyltransferase and constitutively expressed the glycosyltransferases required for the enrichment of Fc-SCT glycan for cell-based production of glycoproteins. The Fc-SCT enriched antibodies generated by these cells (Fig. 6) exhibited similar FcR binding profiles to homogenous Fc-SCT antibody and was able to activate similar effector functions such as ADCC through binding to FcγIIa (Fig. 9). Thus, the cell-based production of Fc-GlcNAc and Fc-SCT enriched antibodies may have the potential advantage of simplifying the production of therapeutic antibodies with improved effector functions, especially ADCC, ADCP and vaccinal effect. Work in progress to

evaluate the comparative activities of Fc-SCT enriched antibody glycoforms and homogenous antibody in animals

ASSOCIATED CONTENT

Supporting Information.

This supporting information is available free of charge via the Internet at <http://pubs.acs.org>, including general methods and materials, IPM analysis of antibody glycan, observation of survival rate, receptor binding of antibody.

AUTHOR INFORMATION

REFERENCES

1. Lu, L. L.; Susacovich, T. J.; Fortune, S. M.; Alter, G., Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol* 2018, 18 (1), 46-61.
2. Ben Mraidem, S.; Benhamou, M.; Monsie, R. C., Understanding Fc Receptor Involvement in Inflammatory Diseases: From Mechanisms to New Therapeutic Tools. *Front Immunol* 2019, 10, 811.
3. Bourmazin, S.; Gupta, A.; Ravetch, J. V., The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* 2020, 20 (10), 633-643.
4. Bourmazin, S.; Corti, D.; Virgin, H. W.; Ravetch, J. V., Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection. *Nature* 2020, 588 (7838), 485-490.
5. Ohtsubo, K.; Marsh, J. D., Glycosylation in cellular mechanisms of health and disease. *Cell* 2006, 128 (5), 855-67.
6. Reilly, C.; Stewart, T. J.; Benfrow, M. B.; Novak, J., Glycosylation in health and disease. *Nat Rev Nephrol* 2019, 15 (6), 346-366.
7. Li, C.; Wang, L. X., Chemotryptic Methods for the Synthesis of Glycoproteins. *Chem Rev* 2018, 118 (17), 8359-8413.
8. Uffellart, R.; Bach, M. P.; Eschbach, C.; Wessling, T.; Roth, M.; Juma, H., N-linked glycosylation selectively regulates autonomous precursor BCR function. *Nat Immunol* 2018, 11 (8), 759-65.
9. Wright, J. F.; Shulman, M. J.; Isenman, D. E.; Painter, R. H., Cl binding by mouse IgM. The effect of abnormal glycosylation at position 402 resulting from a serine to asparagine exchange at residue 406 of the mu-chain. *J Biol Chem* 1990, 265 (18), 10506-13.
10. Mumok, S.; Shulman, M. J., Structural requirements for IgM assembly and cytolytic activity. Effects of mutations in the oligosaccharide acceptor site at Asn402. *J Immunol* 1989, 142 (2), 695-701.

11. Shields, R. L.; Lai, J.; Keck, R.; O'Connell, L. Y.; Hong, K.; Meng, Y. G.; Weikert, S. H.; Presta, L. G., Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular cytotoxicity. *J Biol Chem* **2002**, *277* (30), 26733-40.
12. Duncan, A. R.; Winter, G., The binding site for C1q on IgG. *Nature* **1988**, *332* (6166), 738-40.
13. Kaneko, Y.; Nimmerjahn, F.; Ravetch, J. V., Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* **2006**, *313* (5787), 670-3.
14. Goh, J. B.; Ng, S. K., Impact of host cell line choice on glycan profile. *Crit Rev Biotechnol* **2018**, *38* (6), 851-867.
15. Ashwell, G.; Harford, J., Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* **1982**, *51*, 531-54.
16. Ripka, J.; Adamany, A.; Stanley, P., Two Chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose. *Arch Biochem Biophys* **1986**, *249* (2), 533-45.
17. Chandrasegaran, S.; Carroll, D., Origins of Programmable Nucleases for Genome Engineering. *J Mol Biol* **2016**, *428* (5 Pt B), 963-89.
18. Yang, Z.; Wang, S.; Hallin, A.; Schulz, M. A.; Frodin, M.; Rahman, S. H.; Vestor-Christensen, M. B.; Bohrens, C.; Kristensen, C.; Vakhrushev, S. Y.; Bennett, E. P.; Wandall, H. H.; Clausen, H., Engineered CHO cells for production of diverse, homogeneous glycoproteins. *Nat Biotechnol* **2015**, *33* (8), 842-4.
19. Schulz, M. A.; Tian, W.; Mao, Y.; Van Coillie, J.; Sun, L.; Larsen, J. S.; Chen, Y. H.; Kristensen, C.; Vakhrushev, S. Y.; Clausen, H.; Yang, Z., Glycoengineering design options for IgG1 in CHO cells using precise gene editing. *Glycobiology* **2018**, *28* (7), 542-549.
20. Geisler, C.; Mabashi-Asazuma, H.; Jarvis, D. L., An Overview and History of Glyco-Engineering in Insect Expression Systems. *Methods Mol Biol* **2015**, *1321*, 131-52.
21. Liu, C. P.; Tsai, T. L.; Cheng, T.; Shivatare, V. S.; Wu, C. Y.; Wong, C. H., Glycoengineering of antibody (Herceptin) through yeast expression and in vitro enzymatic glycosylation. *Proc Natl Acad Sci U S A* **2018**, *115* (4), 720-725.
22. Shivatare, S. S.; Huang, L. Y.; Zeng, Y. F.; Liao, J. Y.; You, T. H.; Wang, S. Y.; Cheng, T.; Chiu, C. W.; Chao, P.; Chen, L. T.; Tsai, T. L.; Huang, C. C.; Wu, C. Y.; Lin, N. H.; Wong, C. H., Development of glycosyltransferases with broad glycan specificity for the efficient glyco-remodeling of antibodies. *Chem Commun (Camb)* **2018**, *54* (48), 6161-6164.
23. Shivatare, V. S.; Chuang, P. K.; Tseng, T. H.; Zeng, Y. F.; Huang, H. W.; Veerajanyepulu, G.; Wu, H. C.; Wong, C. H., Study on antibody Fc-glycosylation for optimal effector functions. *Chem Commun (Camb)* **2023**, *59* (37), 5555-5558.
24. Lin, C. W.; Tsai, M. H.; Li, S. T.; Tsai, T. L.; Chu, K. C.; Liu, Y. C.; Lai, M. Y.; Wu, C. Y.; Tseng, Y. C.; Shivatare, S. S.; Wang, C. H.; Chao, P.; Wang, S. Y.; Shih, H. W.; Zeng, Y. F.; You, T. H.; Liao, J. Y.; Tu, Y. C.; Lin, Y. S.; Chuang, H. Y.; Chen, C. L.; Tsai, C. S.; Huang, C. C.; Lin, N. H.; Ma, C.; Wong, C. H., A common glycan structure on immunoglobulin G for enhancement of effector functions. *Proceedings of the National Academy of Sciences of the United States of America* **2015**, *112* (34), 10611-6.
25. Reeves, P. J.; Callewaert, N.; Contreras, R.; Khorana, H. G., Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line. *Proc Natl Acad Sci U S A* **2002**, *99* (21), 13419-24.
26. Sjogren, J.; Struwe, W. B.; Cosgrave, E. F.; Rudd, P. M.; Siervander, M.; Althorn, M.; Hollands, A.; Nizet, V.; Collin, M., Endo82 is a unique and conserved enzyme of serotype M49 group A Streptococcus that hydrolyses N-linked glycans on IgG and alpha1-acid glycoprotein. *Biochem J* **2013**, *455* (1), 107-18.
27. Huang, H. Y.; Liao, H. Y.; Chen, X.; Wang, S. W.; Cheng, C. W.; Shahed-Al-Mahmud, M.; Liu, Y. M.; Mohapatra, A.; Chen, T. H.; Lo, J. M.; Wu, Y. M.; Ma, H. H.; Chang, Y. H.; Tsai, H. Y.; Chou, Y. C.; Hsueh, Y. P.; Tsai, C. Y.; Huang, P. Y.; Chang, S. Y.; Chao, T. L.; Kao, H. C.; Tsai, Y. M.; Chen, Y. H.; Wu, C. Y.; Jan, J. T.; Cheng, T. R.; Lin, K. I.; Ma, C.; Wong, C. H., Vaccination with SARS-CoV-2 spike protein lacking glycan shields elicits enhanced protective responses in animal models. *Sci Transl Med* **2022**, *14* (639), eabm0899.
28. Chen, J. R.; Yu, Y. H.; Tseng, Y. C.; Chiang, W. L.; Chiang, M. F.; Ko, Y. A.; Chiu, Y. K.; Ma, H. H.; Wu, C. Y.; Jan, J. T.; Lin, K. I.; Ma, C.; Wong, C. H., Vaccination of monoglycosylated hemagglutinin induces cross-strain protection against influenza virus infections. *Proc Natl Acad Sci U S A* **2014**, *111* (7), 2476-81.
29. Nguyen, N. T. B.; Lin, J.; Tay, S. J.; Mariati, Yeo, J.; Nguyen-Khuong, T.; Yang, Y., Multiplexed engineering glycosyltransferase genes in CHO cells via targeted integration for producing antibodies with diverse complex-type N-glycans. *Sci Rep* **2021**, *11* (1), 12969.
30. Miyoshi, E.; Noda, K.; Yamaguchi, Y.; Inoue, S.; Ikeda, Y.; Wang, W.; Ko, J. H.; Uozumi, N.; Li, W.; Taniguchi, N., The alpha1-6-fucosyltransferase gene and its biological significance. *Biochimica et biophysica acta* **1999**, *1473* (1), 9-20.
31. Thomann, M.; Schlothauer, T.; Dashivets, T.; Malik, S.; Avenal, C.; Bulau, P.; Ruger, P.; Reusch, D., In vitro glycoengineering of IgG1 and its effect on Fc receptor binding and ADCC activity. *PLoS One* **2015**, *10* (8), e0134949.
32. Branstetter, E.; Duff, R. J.; Kuhns, S.; Padaki, R., Fc glycan sialylation of biotherapeutic monoclonal antibodies has limited impact on antibody-dependent cellular cytotoxicity. *FEBS Open Bio* **2021**, *11* (1), 2943-2949.
33. Qi, F.; Isaji, T.; Duan, C.; Yang, J.; Wang, Y.; Fukuda, T.; Gu, J., ST3GAL3, ST3GAL4, and ST3GAL6 differ in their regulation of biological functions via the specificities for the alpha2,3-sialylation of target proteins. *FASEB J* **2020**, *34* (1), 881-897.
34. Barb, A. W.; Brady, E. K.; Prestegard, J. H., Branch-specific sialylation of IgG-Fc glycans by ST6Gal-I. *Biochemistry* **2009**, *48* (41), 9705-7.
35. Geissner, A.; Baumann, L.; Morley, T. J.; Wong, A. K. O.; Sim, L.; Rich, J. R.; So, P. P. L.; Dullaghan, E. M.; Lessard, E.; Iqbal, U.; Moreno, M.; Wakarchuk, W. W.; Withers, S. G., 7-Fluorosialyl Glycosides Are Hydrolysis Resistant but Readily Assembled by Sialyltransferases Providing Easy Access to More Metabolically Stable Glycoproteins. *ACS Cent Sci* **2021**, *7* (2), 345-354.
36. Samuelsson, A.; Towers, T. L.; Ravetch, J. V., Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* **2001**, *291* (5503), 484-6.
37. Anthony, R. M.; Nimmerjahn, F.; Ashline, D. J.; Reinhold, V. N.; Paulson, J. C.; Ravetch, J. V., Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* **2008**, *320* (5874), 373-6.
38. Schwab, L.; Mihai, S.; Seeling, M.; Kasperkiewicz, M.; Ludwig, R. J.; Nimmerjahn, F., Broad requirement for terminal sialic acid residues and FcγRIIIb for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. *Eur J Immunol* **2014**, *44* (5), 1444-53.
39. Parekh, B. S.; Berger, E.; Sibley, S.; Cahya, S.; Xiao, L.; LaCerte, M. A.; Vaillancourt, P.; Wooden, S.; Gately, D., Development and validation of an antibody-dependent cell-mediated cytotoxicity-reporter gene assay. *Mabs* **2012**, *4* (3), 310-8.

Supplementary Information

Methods and Material Cell Culture

HEK 293T cells (ATCC) were cultured in the DMEM (Thermo Fisher) supplied with 10% FBS (Thermo Fisher) at 37°C with 5% CO₂ in the cell incubator. EXPI293F and EXPI293F GNT1 KO cells (Thermo Fisher) were cultured in the EXPI293 expression medium (Thermo Fisher) at 37°C with 8% CO₂ in the cell incubator.

Plasmid

The plasmids for CRISPR/Cas9 expression were constructed as kit manual (Thermo Fisher). Briefly, the validated sgRNA sequence targeting glycosyltransferase¹ was synthesized (Integrated DNA Technologies) and cloned into the vector for Cas9 and sgRNA expression. The synthetic codon-optimized gene insert (Integrated DNA Technologies), flanked by homologous arm of target gene at sgRNA target site, was cloned into empty vector as the donor plasmid.

Transfection

Transfection of 293T cells and EXPI293F cells were mediated by TransIT-293 (Mirus Bio) or expi293fectamine (Thermo Fisher) following the reagent manual.

Antibody production

The plasmids for antibody chMC81370 were transfected into cells followed by incubation. At the time of harvest, the culture medium was collected and then subjected to protein A sepharose beads (GE HealthCare) column to purify the antibody.

Protein gel analysis

The protein was heated at 95 °C for 5 mins in the LDS sample buffer supplied with 2-Mercaptoethanol and then subjected to gel electrophoresis with 12% SDS-PAGE. The protein on gel was stained by coomassie blue buffer (ApexBio).

Enzymatic transglycosylation assay

The antibody was incubated with SCT glycan-oxazoline and Endo S2 mutant in Tris buffer at 37 °C for the time indicated² before harvest for purification or gel electrophoresis.

Intact protein mass analysis

The samples were diluted with LCMS grade water at a concentration between 5–10 μM and analyzed by 6230 TOF LC/MS with a Dual AJS ESI ion source (Agilent Technologies) with PLRP-S 1000 Å 5 μm column (Agilent Technologies). Solvent A was 0.1% Formic Acid in H₂O and Solvent B was 0.1% Formic Acid in ACN.

Glycosidase treatment

The proteins were incubated with alpha-2-3 neuraminidase, neuraminidase, or PNGase F (New England Biolabs) in the supplied buffer at 37 °C overnight followed by purification or gel electrophoresis.

ELISA binding assay

The recombinant soluble FcγRIIIA (R&D) was coated with 50 ng/well in bicarbonate/carbonate coating buffer (50 mM, pH: 10) at 4 °C overnight before blocking the well by 5% BSA in TPBS (0.05% Tween 20 in PBS) at 4 °C overnight. The antibody was added into wells with final concentration started at 100 μg/ml with 5x series dilution and incubated at room temperature for 1 hour followed by incubation with HRP-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch) for another 1 hour at room temperature. Finally, the TMB substrate (Bethyl Laboratories) was added to react with HRP at RT prior to stopping the reaction by H₂SO₄. The absorbance at 450 nm was detected by SpectraMax M5 spectrum reader (Molecular Device). The wells were washed by TPBS 3–5 times between each step.

ADCC reporter assay

ADCC reporter assay (Promega) was performed as kit manual. Briefly, the SSEA4 expressing target cells SK-OV3³ was seeded into 96-well plate followed by addition of antibody with final concentration started at 1 μg/ml with 5x series dilution. Then the FcγRIIIA expressing effector cells (effector: target cell ratio, 6:1) was added and incubated with target cells in the incubator at 37 °C for 6 hrs. The plate was placed at room temperature for 15 min prior to adding the luciferase substrate. After 5 min incubation, the luminescence was measured by SpectraMax M5 spectrum reader. The induction fold was calculated by RLU (induced-background)/RLU (no antibody control-background).

Glycoform analysis

The FASP (filter-aided sample preparation) method⁴ was used for in-solution protease digestion. Briefly, 5 μg of protein samples were loaded onto filter units (Microcon YM-30) and mixed with 100 μl of 8 M urea in 0.1 M Tris-HCl pH 8.5 (UB) each for protein denaturation. The filter units were centrifuged with 14,000 x g for ~30 min until the solution was removed, followed by the addition of 100 μl of 25 mM DTT in UB each for protein reduction, and incubated at room temperature for 10 min. After centrifugation with 14,000 x g for ~30 min to remove the DTT solution, 100 μl of 50 mM Iodoacetamide in UB was added to each sample for alkylation, and the filter units were incubated in the dark for 10 min, followed by centrifugation to remove Iodoacetamide solution. To each filter unit, 100 μl of UB was added again. After another centrifugation to remove the solution, 100 μl of 50 mM ammonium bicarbonate in water (ABC) was added to change the buffer system to ABC for in-solution protease digestion. After centrifugation to remove the solution, 40 μl of ABC with 0.1 μg Trypsin/Lys-C Mix (Promega, sequencing grade) was added to each sample and incubated in a wet chamber at 37 °C overnight. The digested peptides in samples were spun to collection tubes, and an additional rinse with 40 μl ABC for each sample was performed. The filter units were centrifuged at 14,000 x g for 10 min to collect the flow-through, which contains digested peptides. Samples were acidified with FA to a final concentration of 0.1%. The peptide samples were dried in a SpeedVac evaporator, and stored at -20 °C until analysis with LC-MS/MS.

Samples were analyzed using the LC-ESI-MS method on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an EASY-nLC 1200 system (Thermo, San Jose, CA, US) and an EASY-spray source (Thermo, San Jose, CA, US). A 5 μ l digestion solution was injected at a flow rate of 1 μ l/min onto an easy column (C18, 0.075 mm x 150 mm, ID 3 μ m; Thermo Scientific). Chromatographic separation utilized 0.1% formic acid in water as mobile phase A and 0.1% formic acid in 80% acetonitrile as mobile phase B, operated at a flow rate of 300 nl/min. The gradient employed was from 5% buffer B at 2 min to 60% buffer B at 55 min. For full-scan MS, the conditions included a mass range of m/z 375-1800 (AGC target 5E5) with a lock mass, resolution set at 60,000 at m/z 200, and a maximum injection time of 50 ms. MS/MS was conducted in top speed mode with 3 s cycles using both CID and HCD, with a dynamic exclusion duration of 60 s and a 10 ppm tolerance around the selected precursor and its isotopes. The electrospray voltage was maintained at 1.8 kV, and the capillary temperature was set to 275°C.

Byonic software version 4.2.10 (Protein Metric) was used for the identification of summary formulas of glycans associated with glycopeptides. The glycan database contained 132 entries, and the parameters used were: precursor mass tolerance of 10 ppm, fragment mass tolerance of 0.5 Da, maximum missed cleavages set to 5, and cysteine carbamidomethylation and methionine oxidation considered."

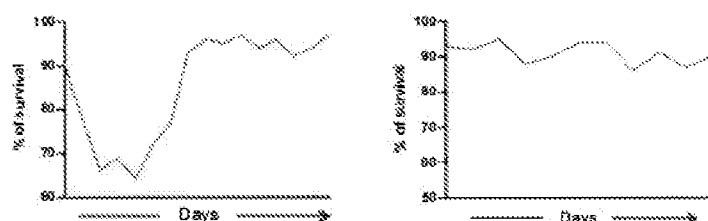


Figure S1. The survival curve of the GNT1-KO EXP1293F cells after CRISPR/Cas9 and Endo H (left) or Endo S2 (right) donor plasmid cotransfection.

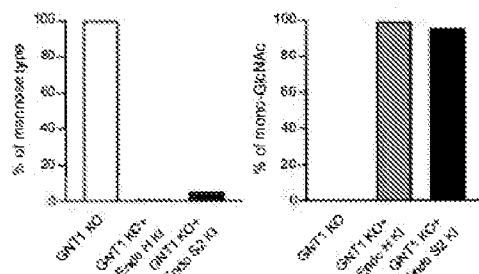


Figure S2. The glycoform analysis of chMCR1370 antibody expressed by GNT1 KO and engineered GNT1 KO EXP1293 cells.

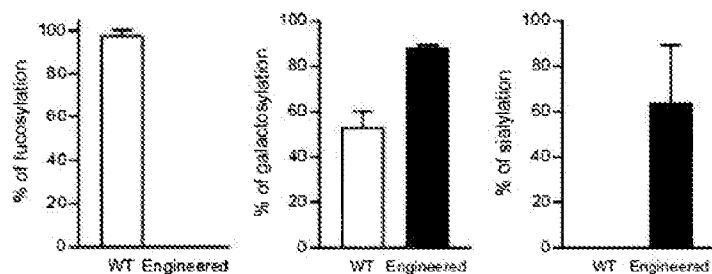


Figure S3. The glycoform analysis of chMC81370 antibody expressed by WT or engineered 293T cells.

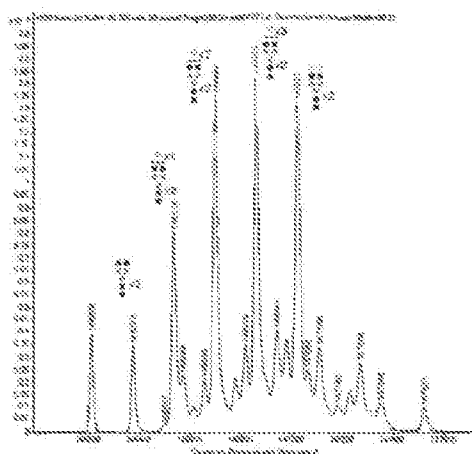


Figure S4. The IPM analysis of chMC81370 antibody expressed by FUT 8KO/B4GalT1 KI 293T cells

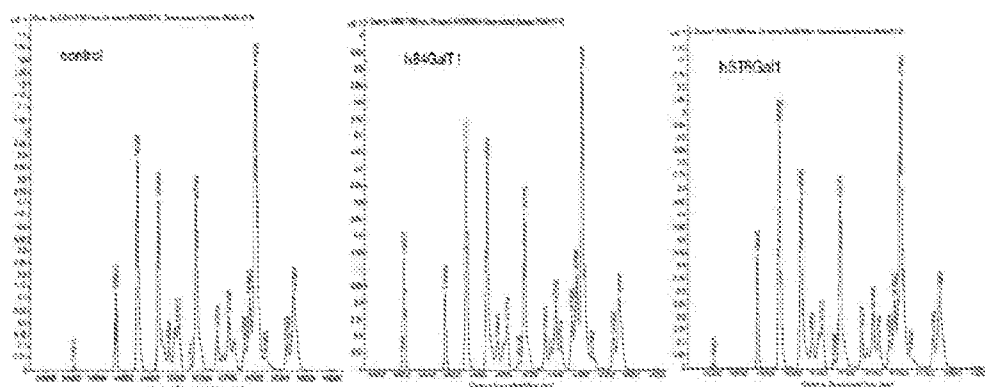


Figure S5. The IPM analysis of chMC81370 antibody expressed by untreated, hB4GalT1 transiently overexpressed, and hST6GalT1 transiently overexpressed, engineered 293T cells.

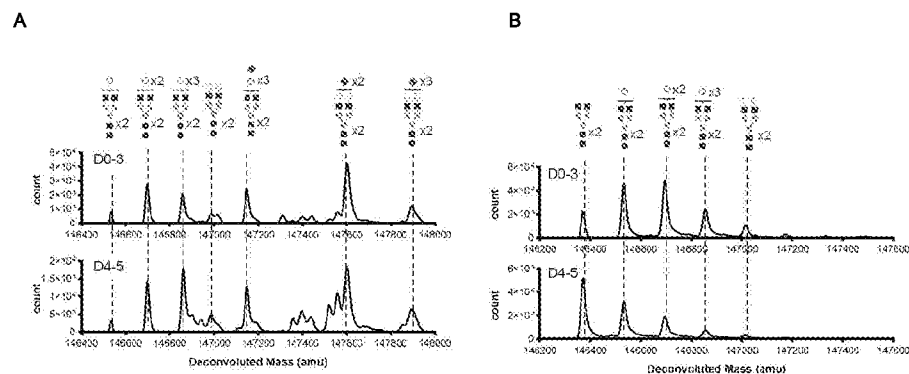


Figure S6. The IPM analysis of chMC81370 antibody expressed by multiple engineered (A) and FUT8 KO (B) cells at different collection time point.

Reference

1. Narimatsu, Y.; Joshi, H. J.; Yang, Z.; Gomes, C.; Chen, Y. H.; Lorenzetti, F. C.; Furukawa, S.; Schjoldager, K. T.; Hansen, L.; Clausen, H.; Bennett, E. P.; Wandall, H. H., A validated gRNA library for CRISPR/Cas9 targeting of the human glycosyltransferase genome. *Glycobiology* **2018**, *28* (5), 295-305.
2. Shivatare, V. S.; Chuang, P. K.; Tseng, T. H.; Zeng, Y. F.; Huang, H. W.; Veeranjanyulu, G.; Wu, H. C.; Wong, C. H., Study on antibody Fc-glycosylation for optimal effector functions. *Chem Commun (Camb)* **2023**, *59* (37), 5555-5558.
3. Lou, Y. W.; Wang, P. Y.; Yeh, S. C.; Chuang, P. K.; Li, S. T.; Wu, C. Y.; Khoo, K. H.; Hsiao, M.; Hsu, T. L.; Wong, C. H., Stage-specific embryonic antigen-4 as a potential therapeutic target in glioblastoma multiforme and other cancers. *Proc Natl Acad Sci U S A* **2014**, *111* (7), 2482-7.
4. Wisniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M., Universal sample preparation method for proteome analysis. *Nat Methods* **2009**, *6* (5), 359-62.



DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:	PCT/US2024/034588
International filing date:	19 June 2024 (19.06.2024)
Document type:	Certified copy of priority document
Document details:	Country/Office: US
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Date of receipt at the International Bureau:	30 August 2024 (30.08.2024)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

1328949

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 29, 2024

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 63/581,389

FILING DATE: *September 08, 2023*

RELATED PCT APPLICATION NUMBER: *PCT/US24/34588*

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US63/581,389*



Certified by

Katherine Kelly Vidal

Performing the Functions and Duties of the
Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

Targeting Human Viruses with Broadly Protective Low-sugar Vaccines

Chi-Huey Wong



Major Outbreaks That Cause Pandemics

Flu Pandemics

- 1918 Spanish Flu (H1N1, 50m deaths)
- 1957 Asian Flu (H2N2, 2m deaths)
- 1968 Hong Kong Flu (H3N2, 1m deaths)
- 2009 Swine Flu (H1N1, 0.6m deaths)

Other Major Pandemics:

Smallpox (1500 BCE, >500m deaths, 1980 eradicated)

Black Death (1346-53, 200m deaths)

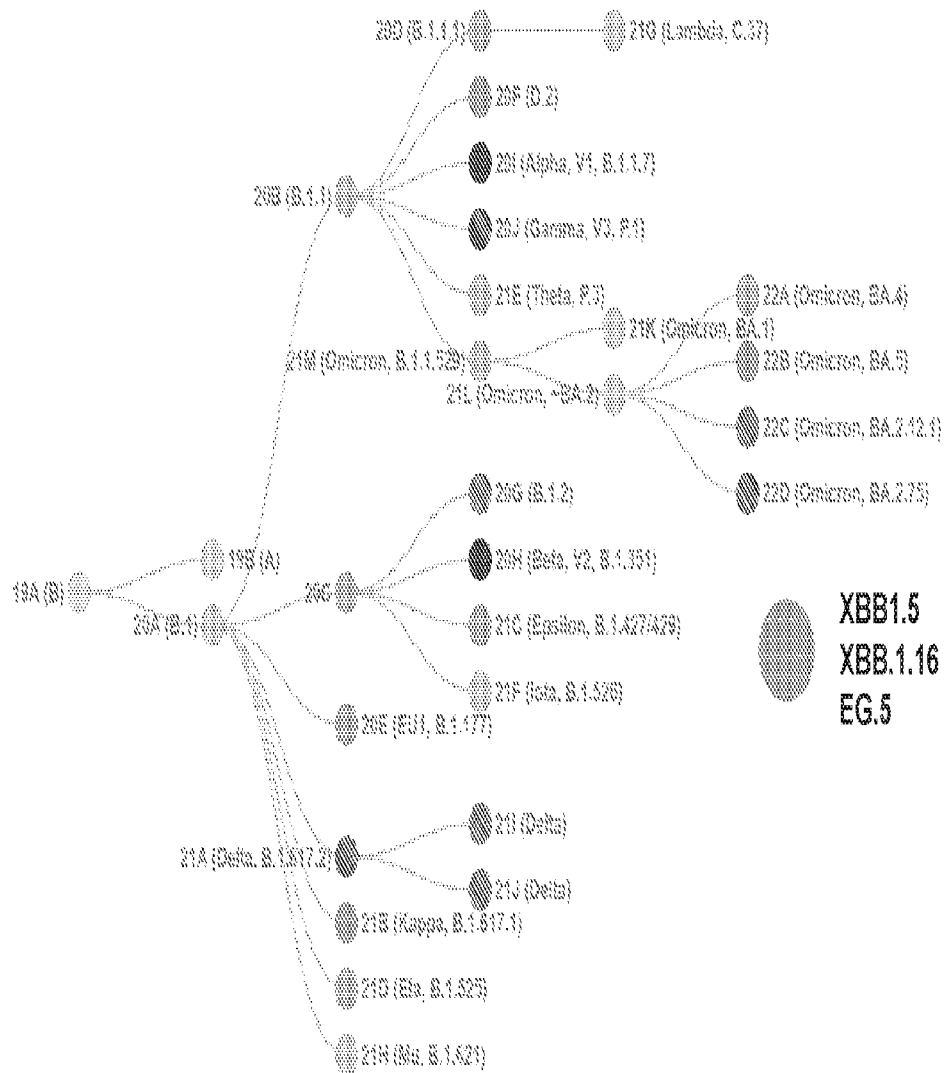
Cholera (1817, 7th 1961, >1m deaths)

HIV/AIDS (1976-, 36m deaths)

SARS Outbreaks

- SARS Outbreak: 2002&2003 (8,096 cases/774 deaths)
- MERS Outbreak: 2012 (2,494 cases/858 deaths)
- SARS-CoV-2 Pandemic: 2019 (>700m cases, >7m deaths)

Evolution of SARS-CoV-2 (GISAID)

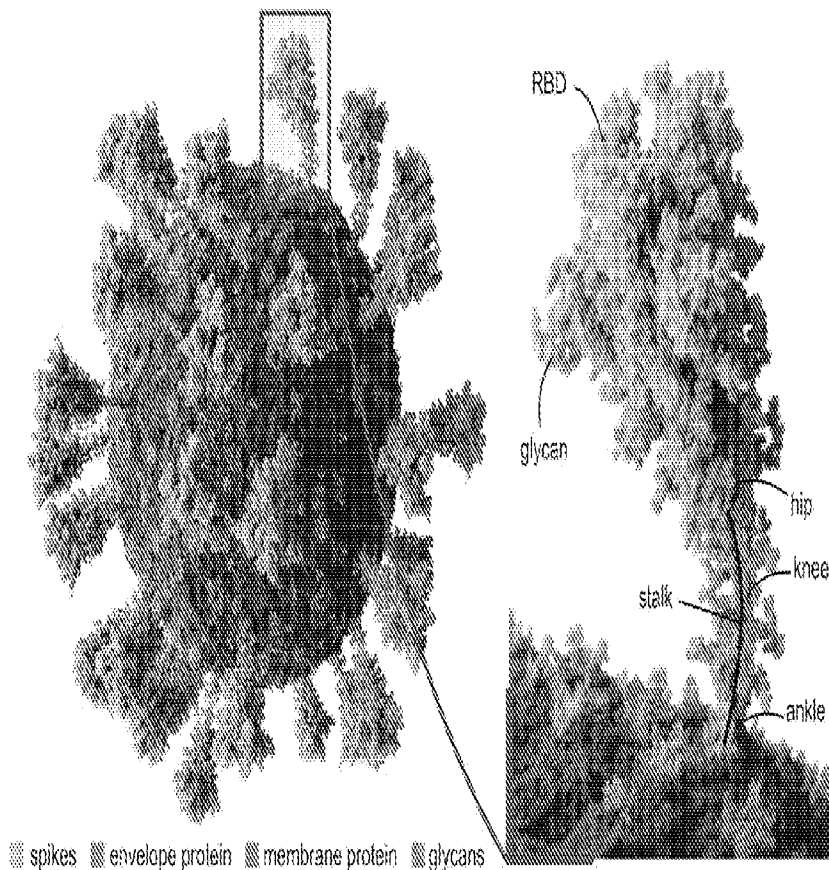


2021 October: alpha (20%), beta (0.7%), gamma (2%), delta (54%), omicron (0.01%).

2021 November: delta >98%. December: omicron (70%), delta (30%). Now: EG.5

Atomic Model of SARS-CoV-2

Since 2019, over 750-million cases, 7-million deaths



RNA Virus (26-31 kb):

1) Virus size: 80-120 nm.

spike: 20 nm length, 74 spikes per virus.

2) Easy to mutate.

3) Human viruses depend on host-made sugars on the surface to facilitate infection and escape immune response

https://commons.m.wikimedia.org/wiki/File:Coronavirus_SARS-CoV-2.png

Analysis of 15 Million S Protein Sequences: All 18 Conserved Epitopes are Shielded by Glycans Except E18

Ψ: N-glycan ♀: O-glycan

NTD Epitopes

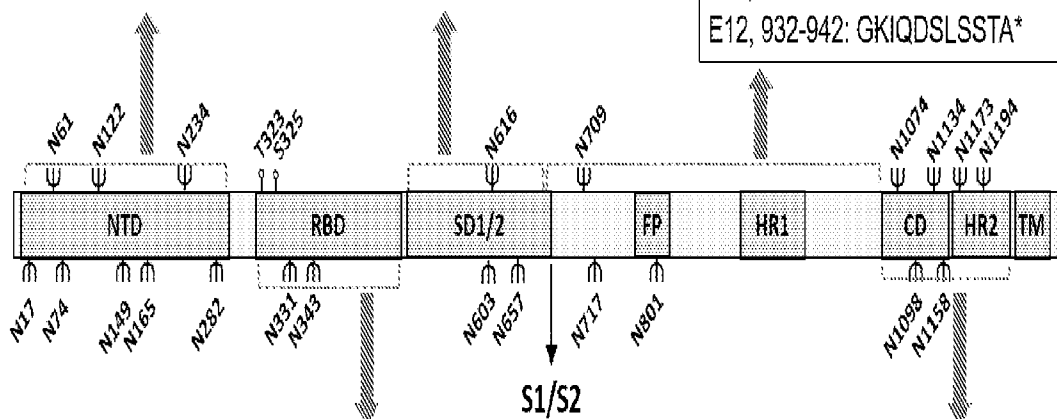
E1, 161-173: SSAN[N]CTFEYVSQ*
E2, 177-187: MDLEGKQGNFK

SD1/2 Epitopes

E6, 618-628: TEVPVAIHADQ*
E7, 634-646: RVYSTGSNVFQTR*

S2 Epitopes

E8, 682-691: RRARSVASQS*
E9, 808-817: DPSKPSKRSE*
E10, 833-844: FIKQYGDCLGDI*
E11, 918-929: ENQKLIANQFNS**
E12, 932-942: GKIQDSLSTA*



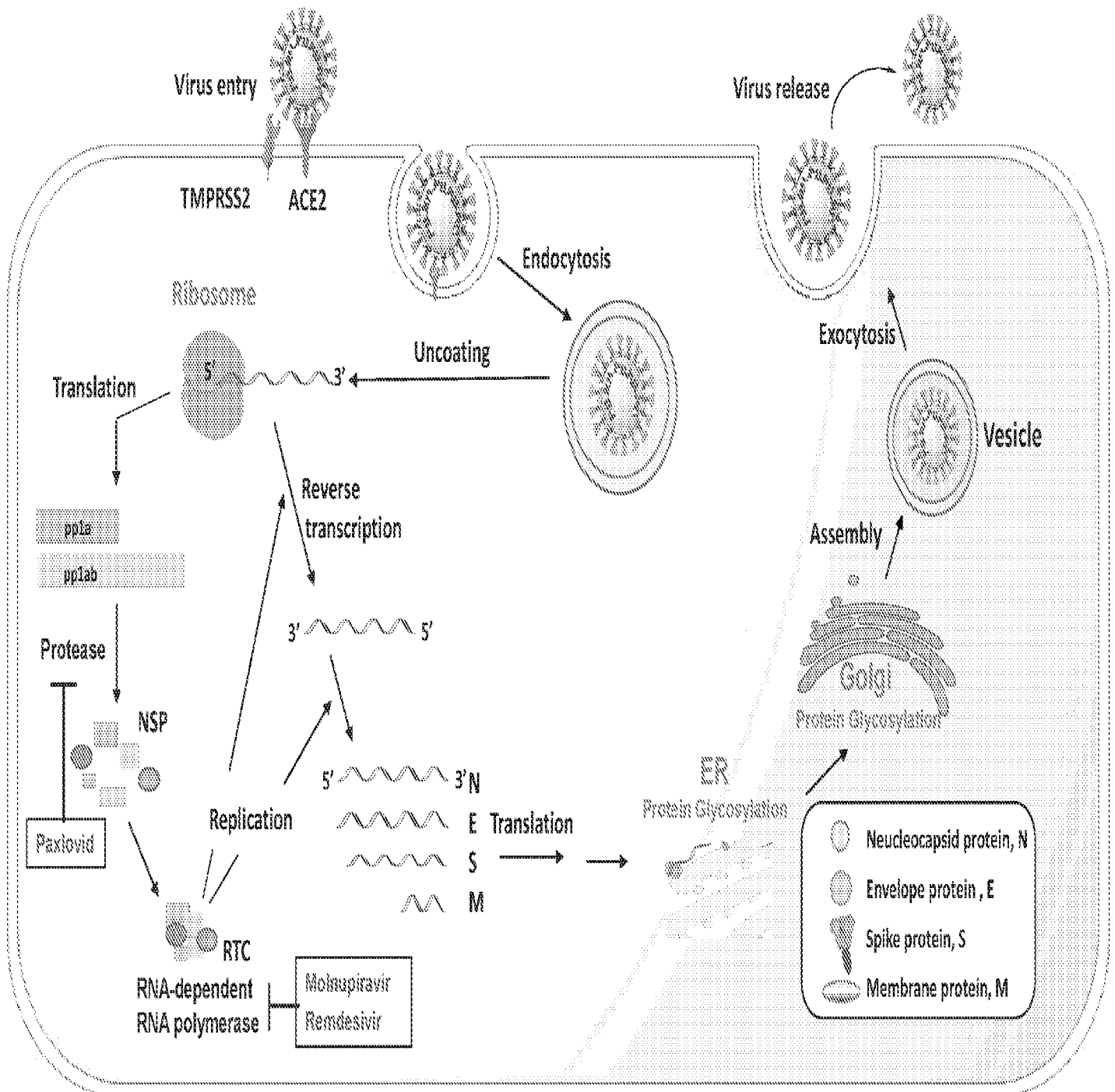
RBD Epitopes

E3, 323-335: [T]E[S]IVRFP[N]ITNL**
E4, 462-476: KPFERDISTEIQAG**
E5, 526-538: GPKKSTNLVKNKC**

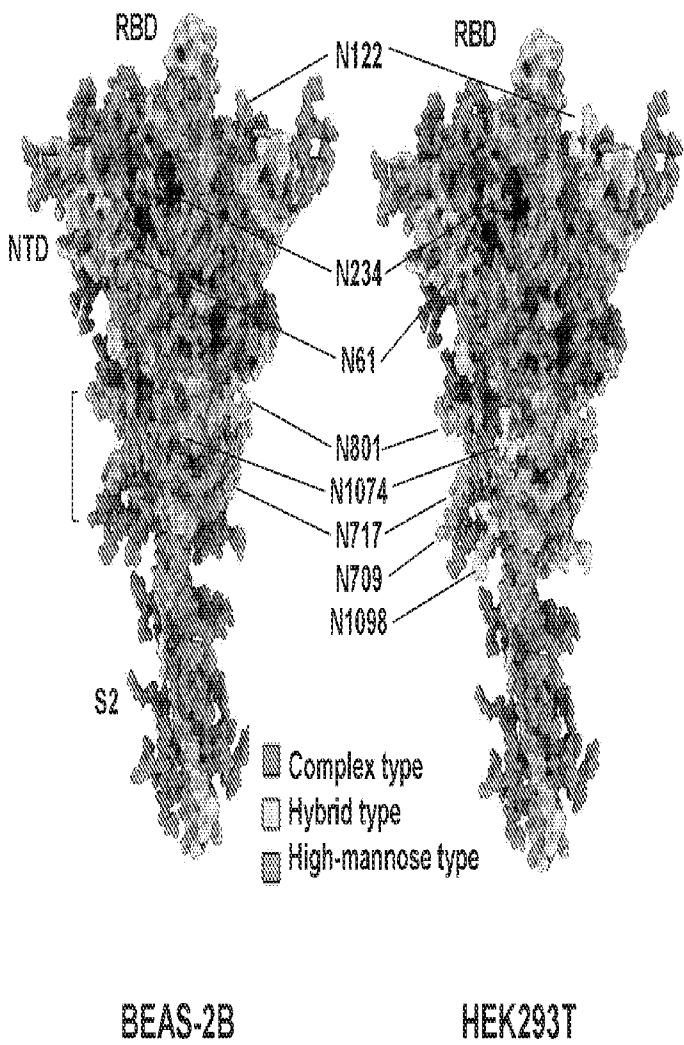
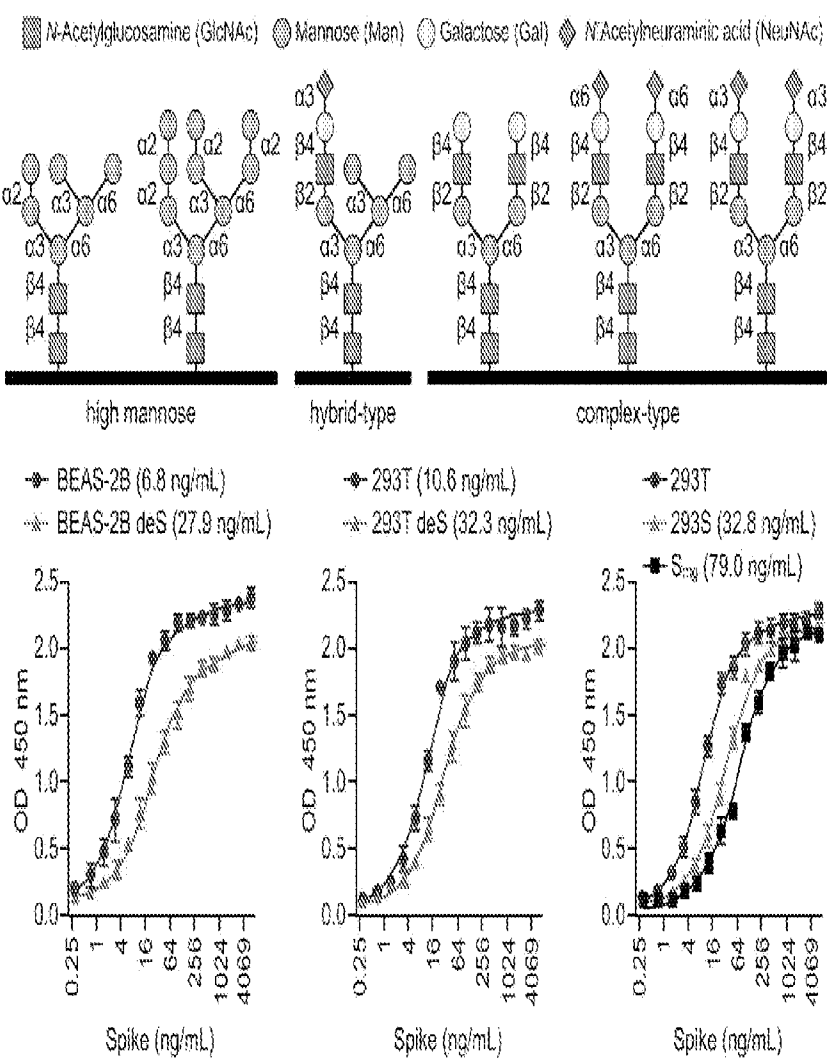
CD & HR2 (S2 Stem) Epitopes

E13, 1125-1138: NCDVVIGIV[N]INTVY**
E14, 1143-1161: PELDSFKEELDKYFK[N]HTS**
E15, 1160-1174: TSPDVLGDIGINA*
E16, 1177-1190: VNIQKEIDRLNEVA**
E17, 1192-1201: NL[N]JESLIDLQ**
E18, 1203-1213: LGKYEQYIKWP**

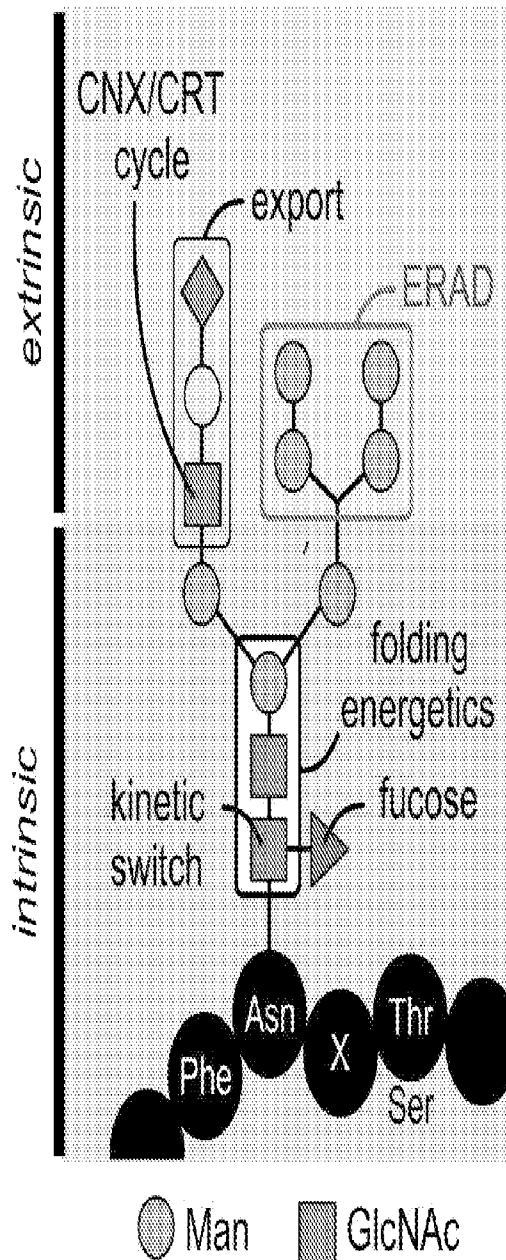
SARS-CoV-2 Life Cycle: Glycosylation is Host Dependent



Glycosylation is Cell Dependent: S Protein from Two Cell Lines



Glycosylation Affects Glycoprotein Folding and Function



The Core Triose Man-GlcNAc-GlcNAc

- Conserved from yeast to human
- Contributes 3 kcal/mol to protein folding in hCD2ad:
the innermost GlcNAc for 2 kcal/mol;
Man-GlcNAc for 1.1 kcal/mol; the rest for function.

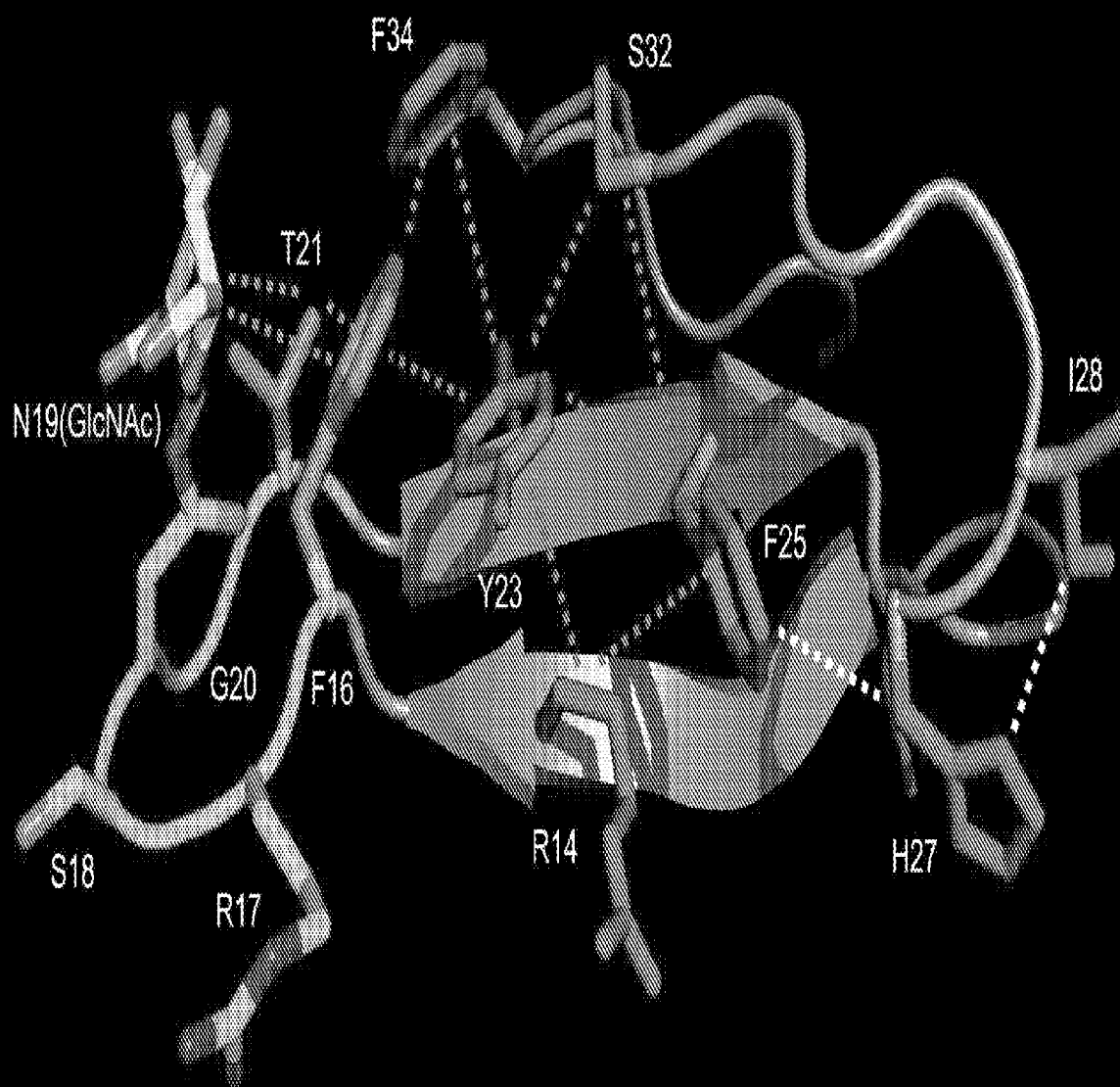
NMR of PinWW Variants: Chemical Shift Changes

* interactions within the
Enhanced Aromatic Sequon

* interactions tightened
upon glycosylation ("zipper")

* interactions unchanged
upon glycosylation

Aromatic Sequon for N-Glycosylation: Phe-x(x)-Asn-x-Thr(Ser)



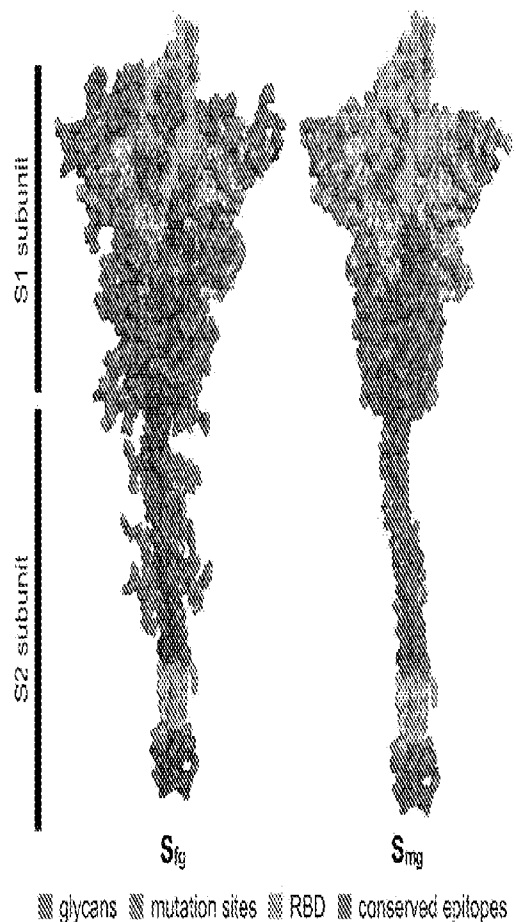
Low-Sugar Universal COVID Vaccine Design

Low-Sugar Universal Vaccine:

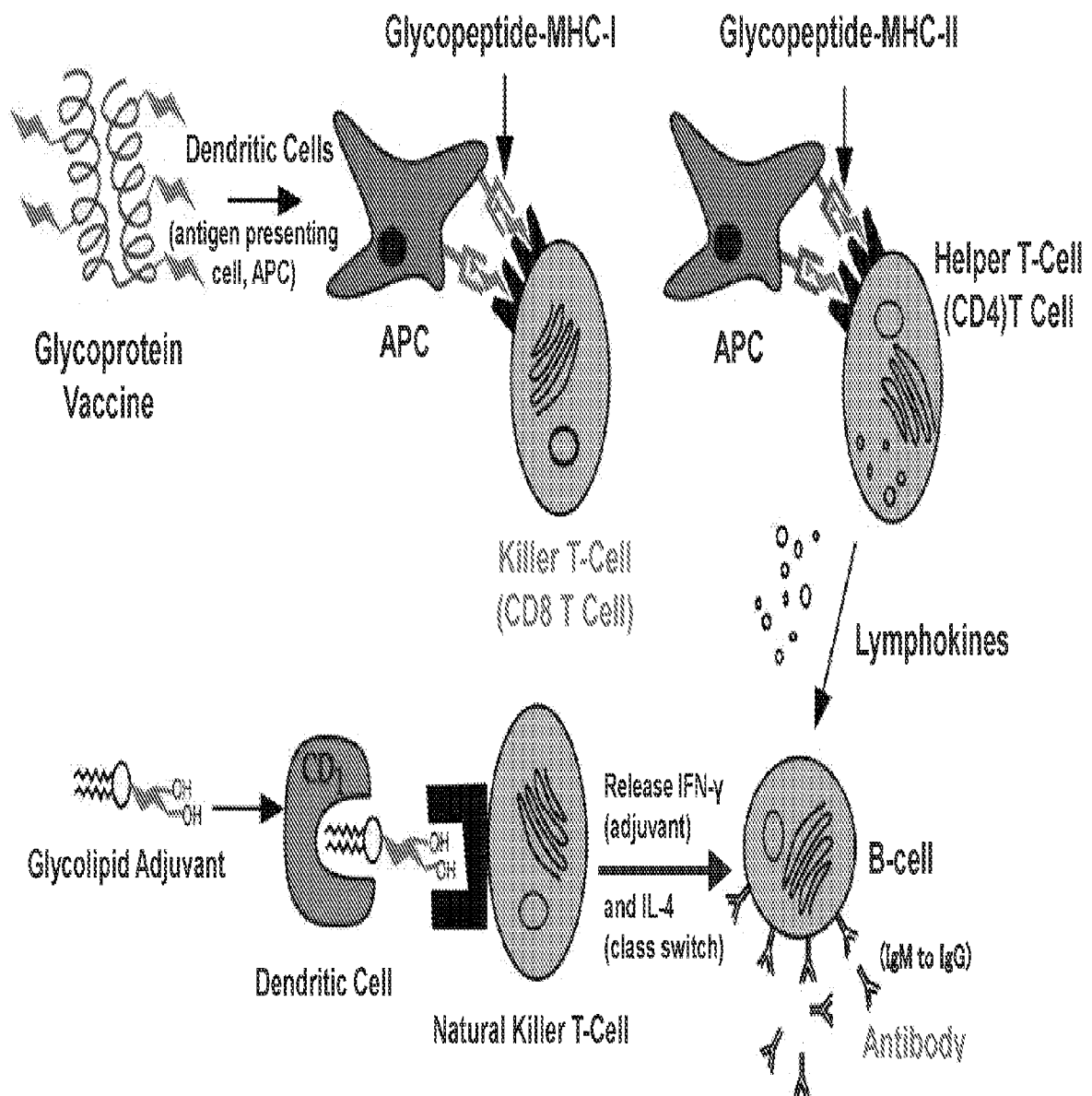
deletion of sugar coat exposes conserved epitopes and elicits enhanced and broadly protective immune response.

SARS-CoV-2 Vaccines:

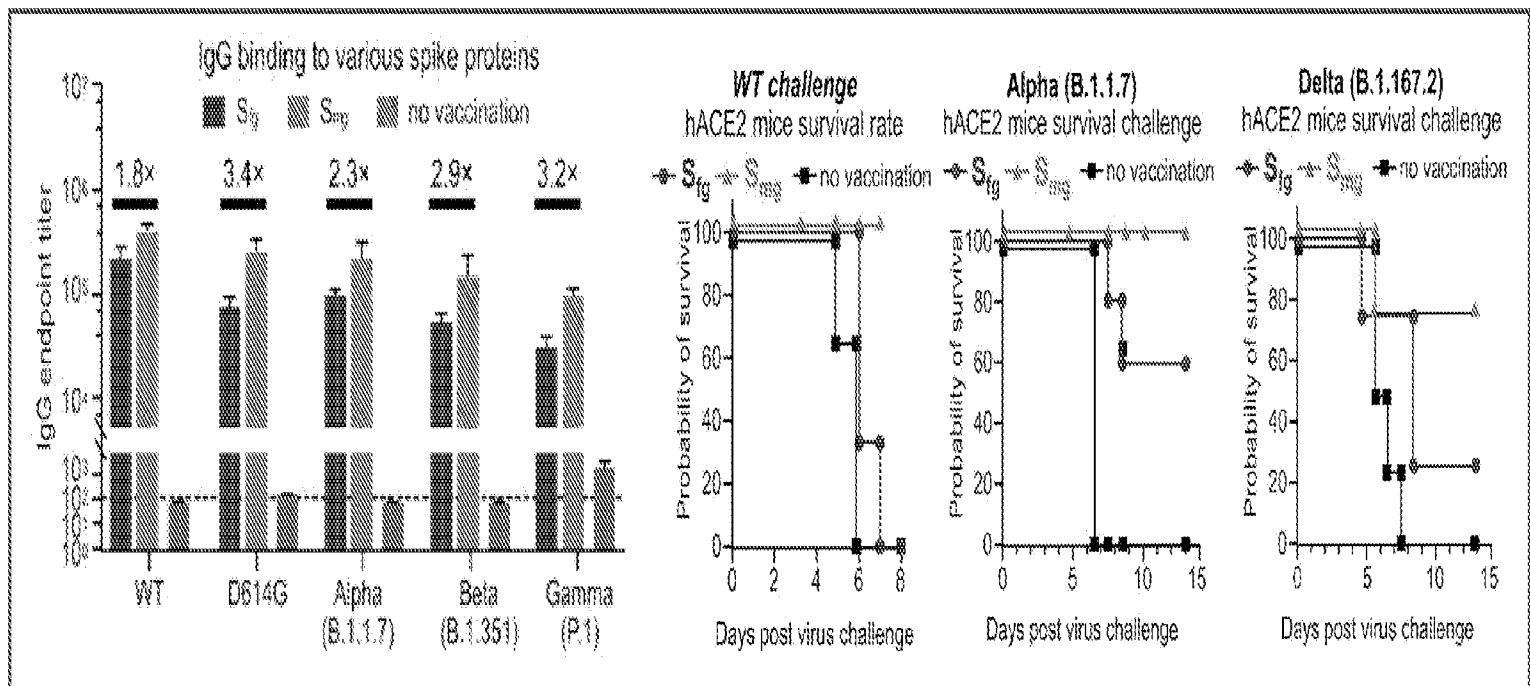
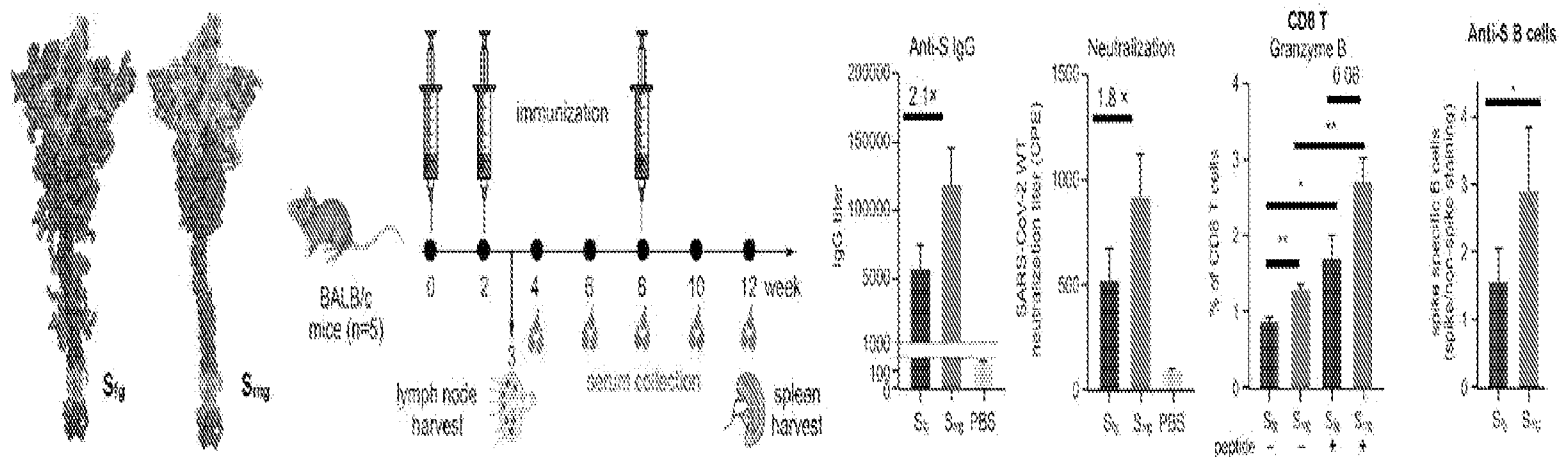
30 used in humans, 10 approved by WHO, >350 in development



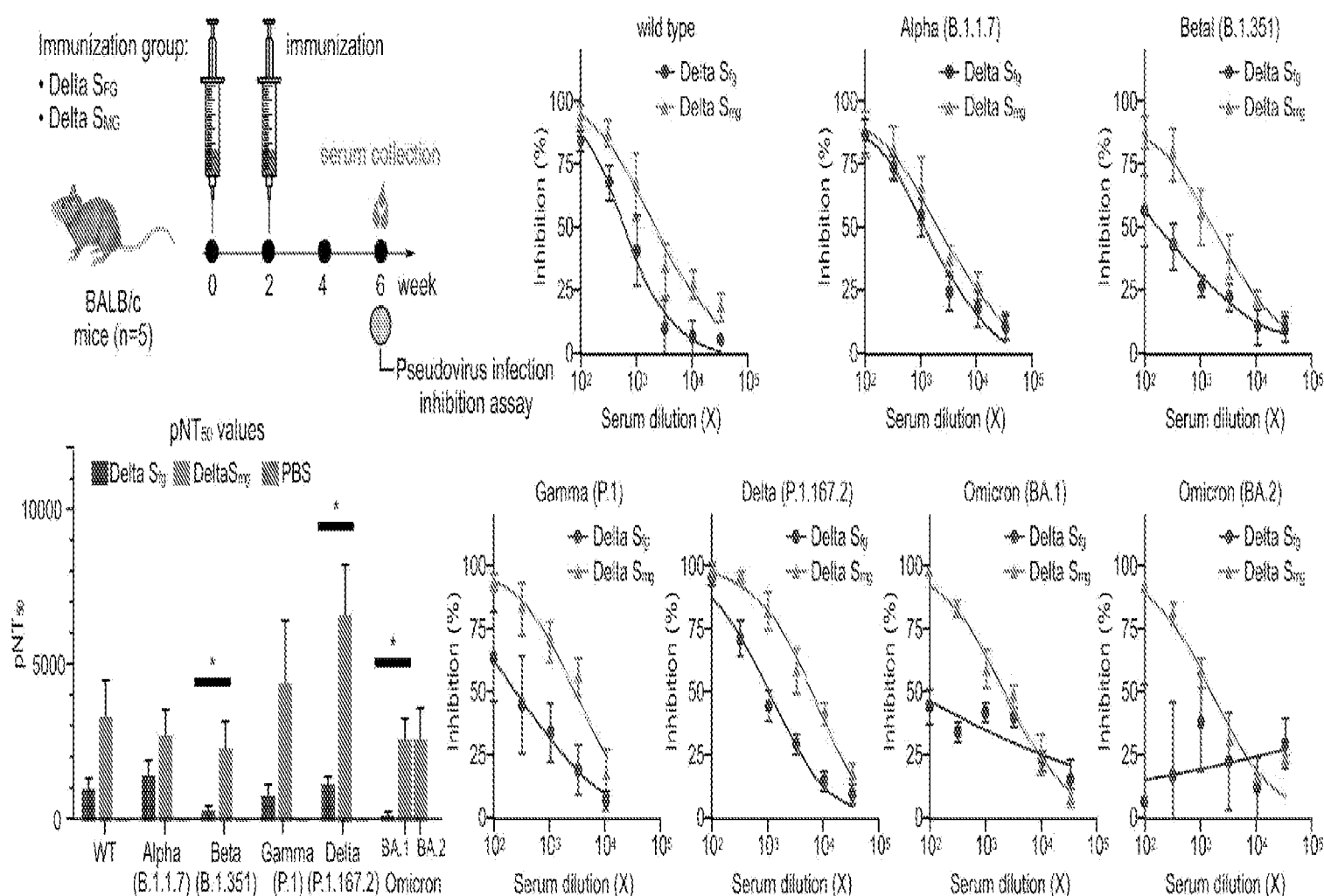
Adaptive Immune Response and Class Switch



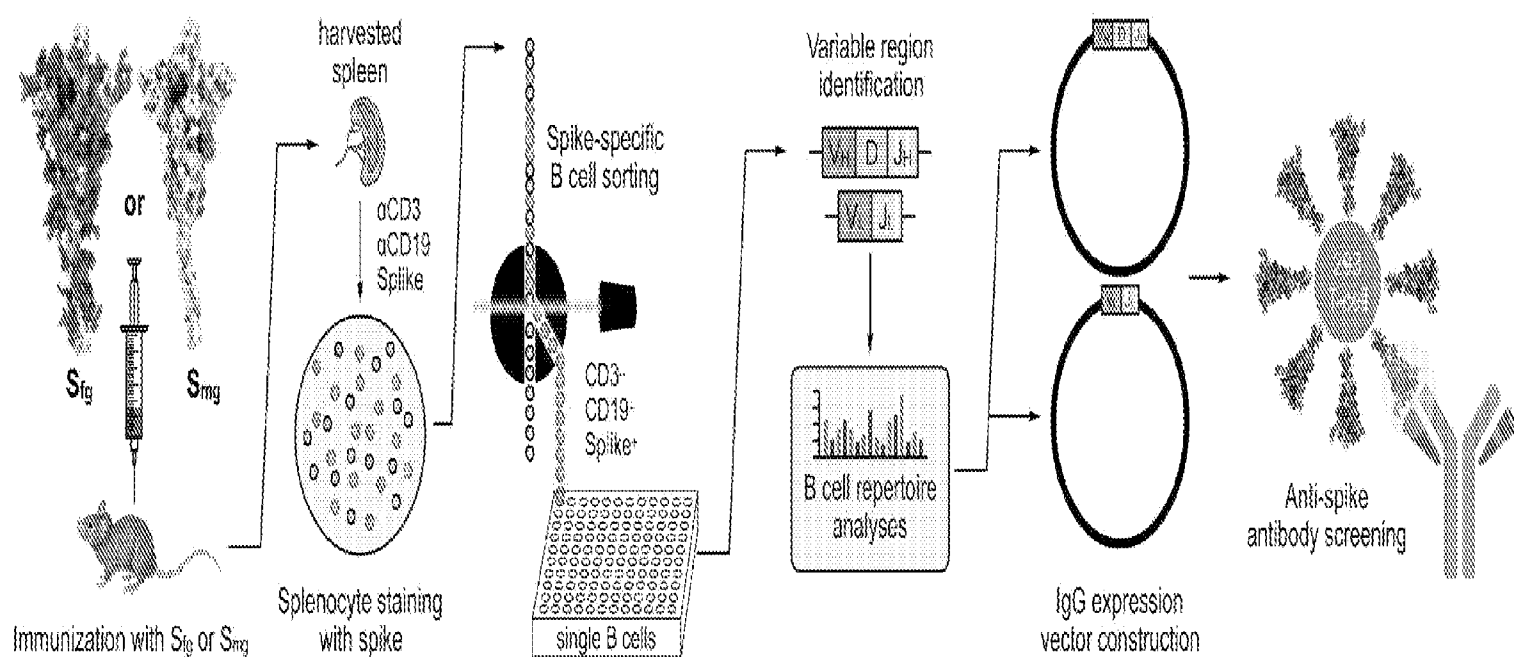
S_{mg} Vaccination Elicits Enhanced and Broadly Protective Antibody and CD8⁺ T Cell Responses



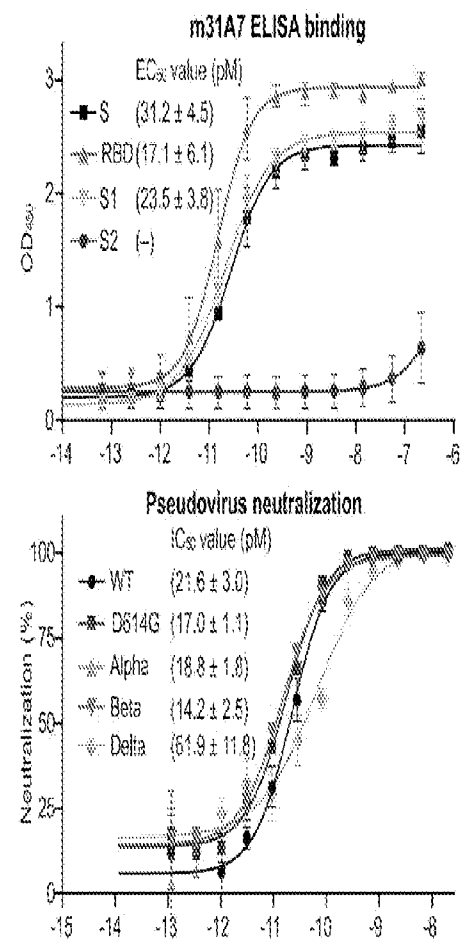
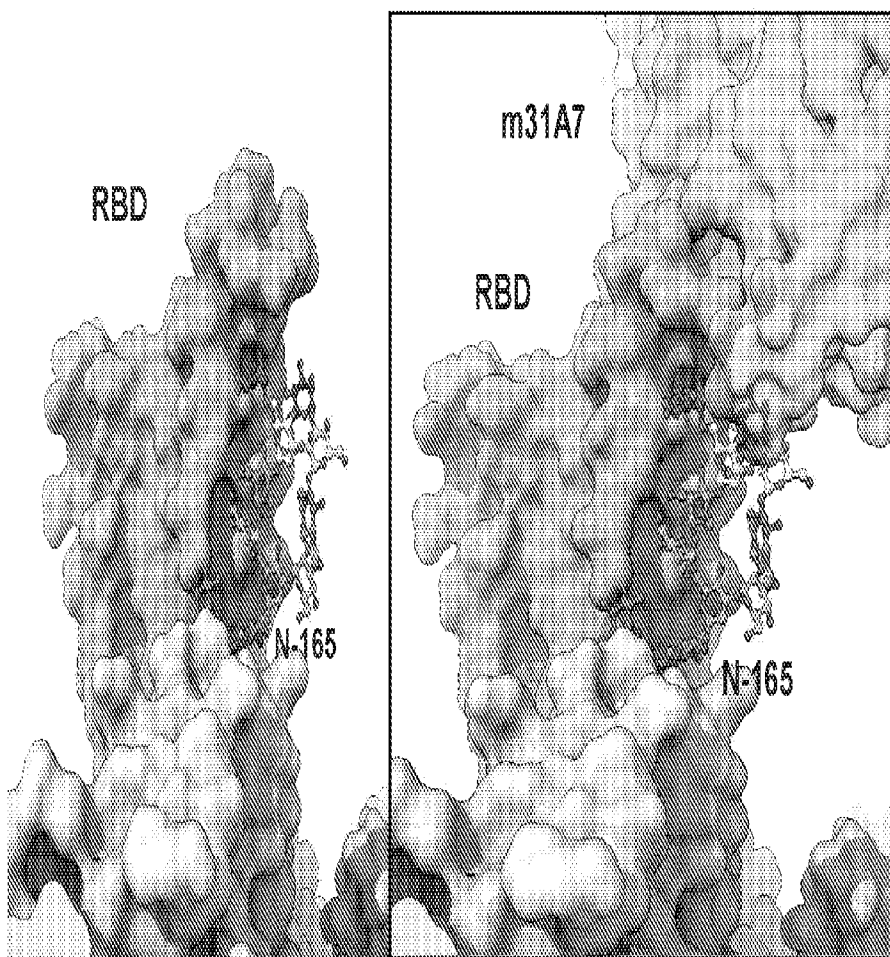
Delta Smg Vaccine is Broadly Protective against Alpha, Beta, Gamma, Delta and Omicron Variants in Pseudovirus Neutralization Assay



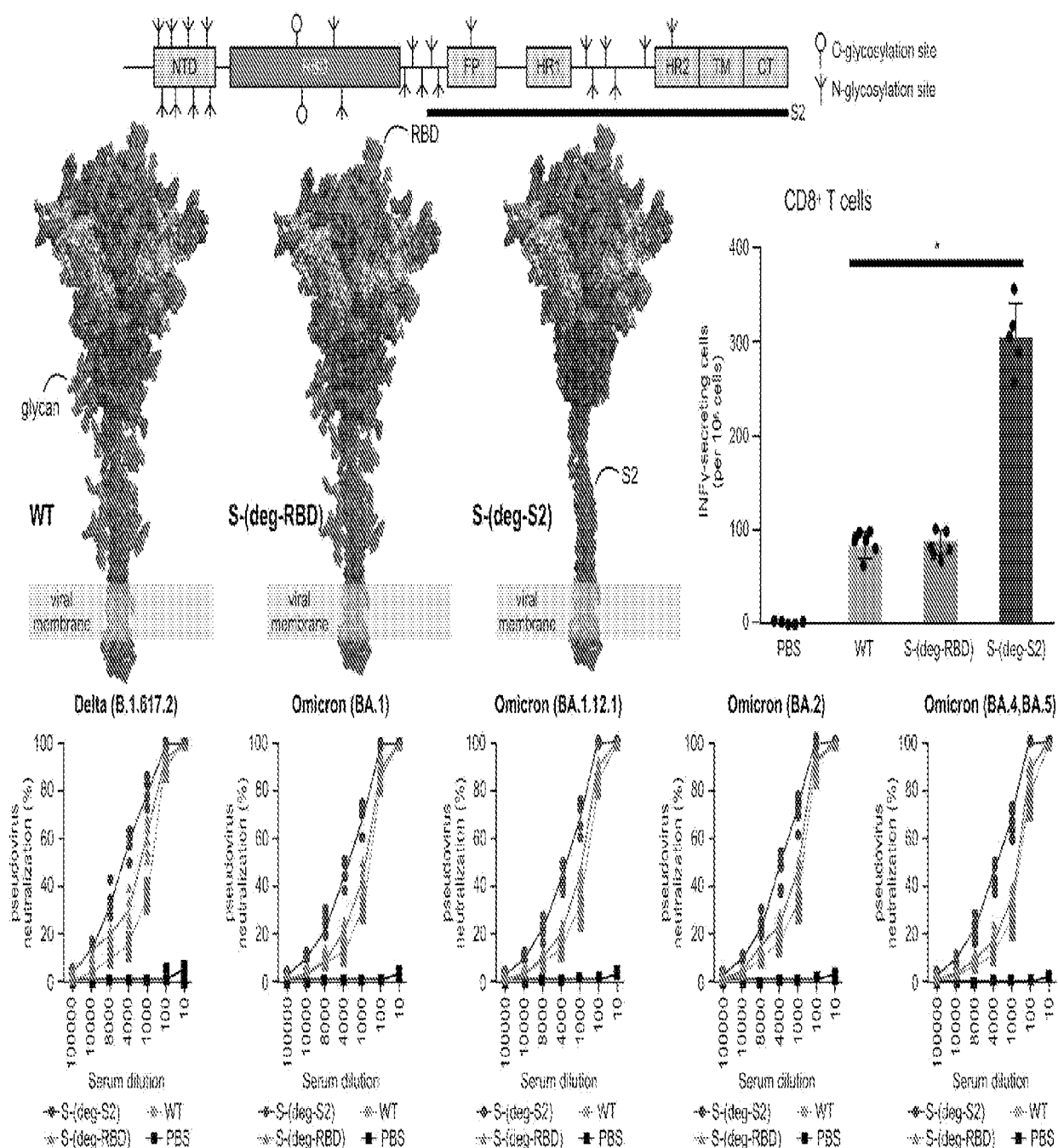
Sfg-Specific Single B Cell Sorting and Humanized Monoclonal Antibody Cloning in Mice Immunized by S_{mg}



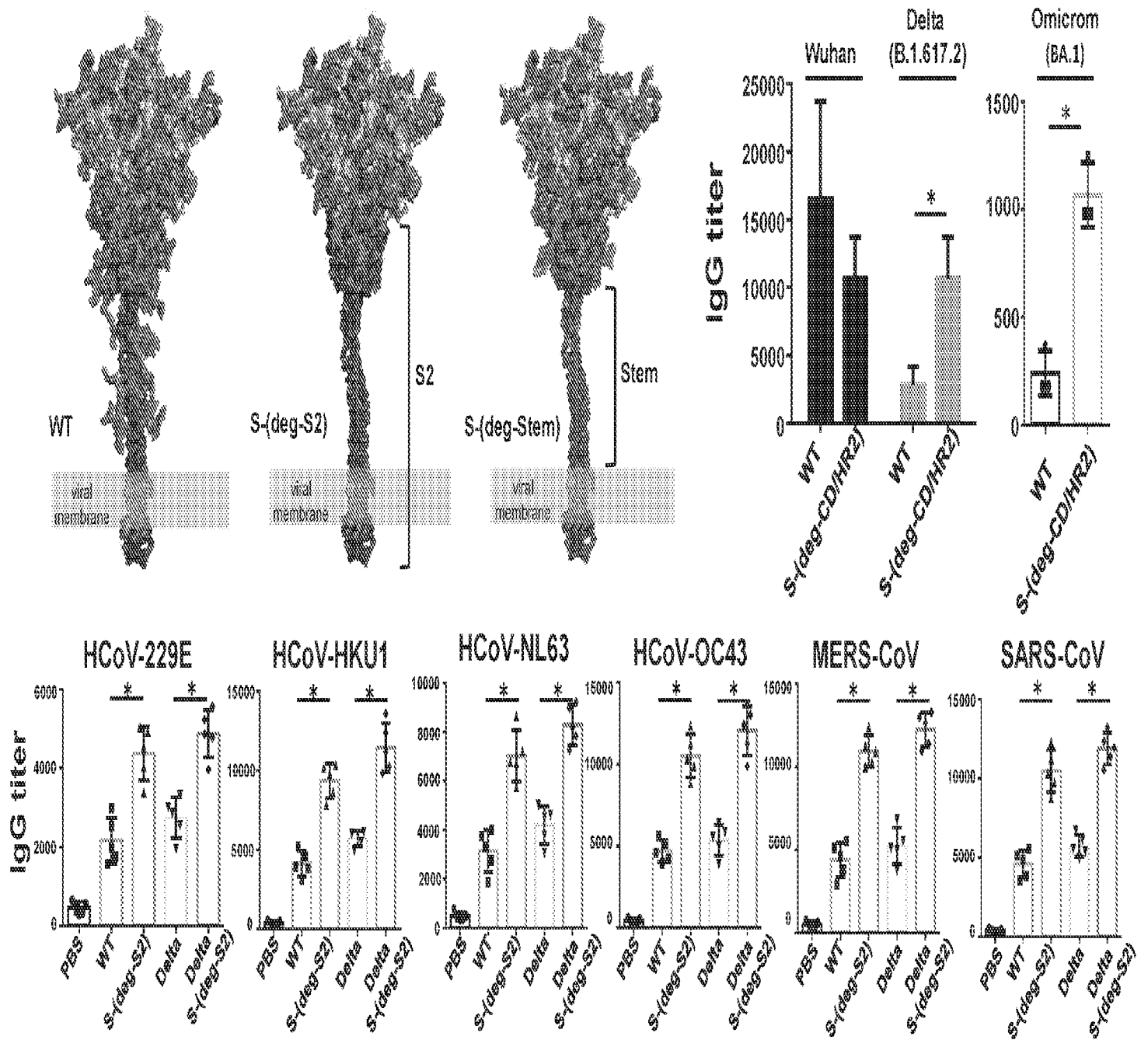
Monoclonal Antibody m31A7 from S_{mg} Immunized Mice: Cryo-EM Structure and pM Neutralization Activity



Spike mRNA Vaccine with Deletion of 9 Glycosites in S2 is Broadly Protective

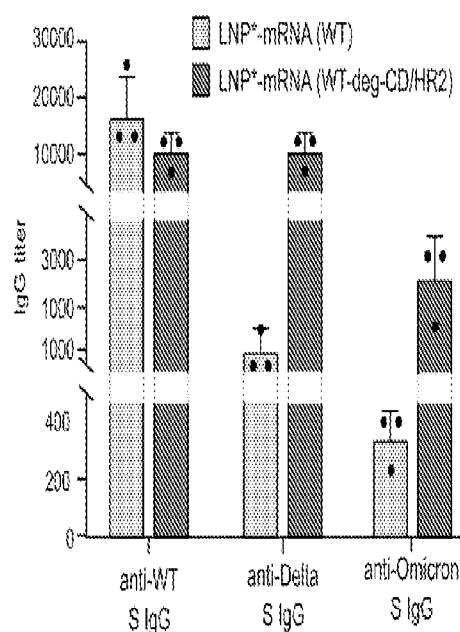
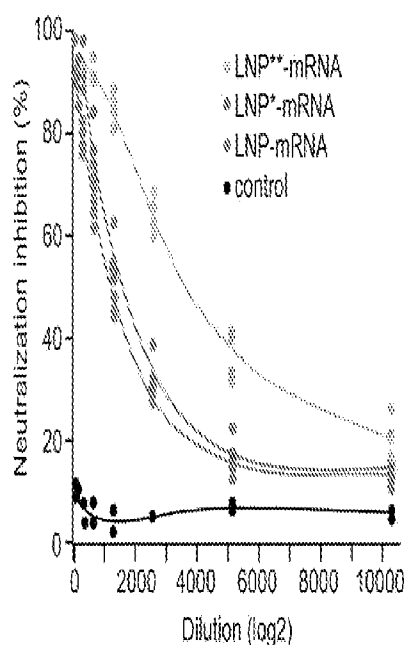
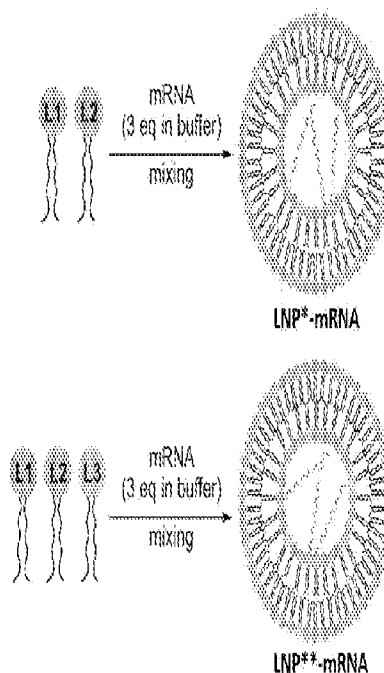
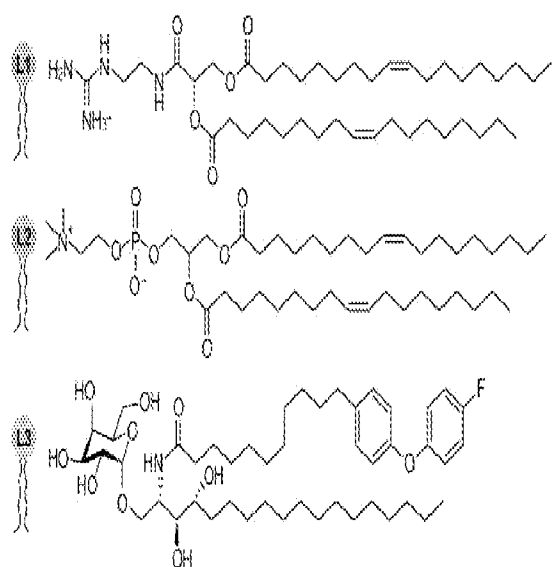


A Broadly Protective S mRNA Vaccine with Deletion of 6 Glycosites in the Stem

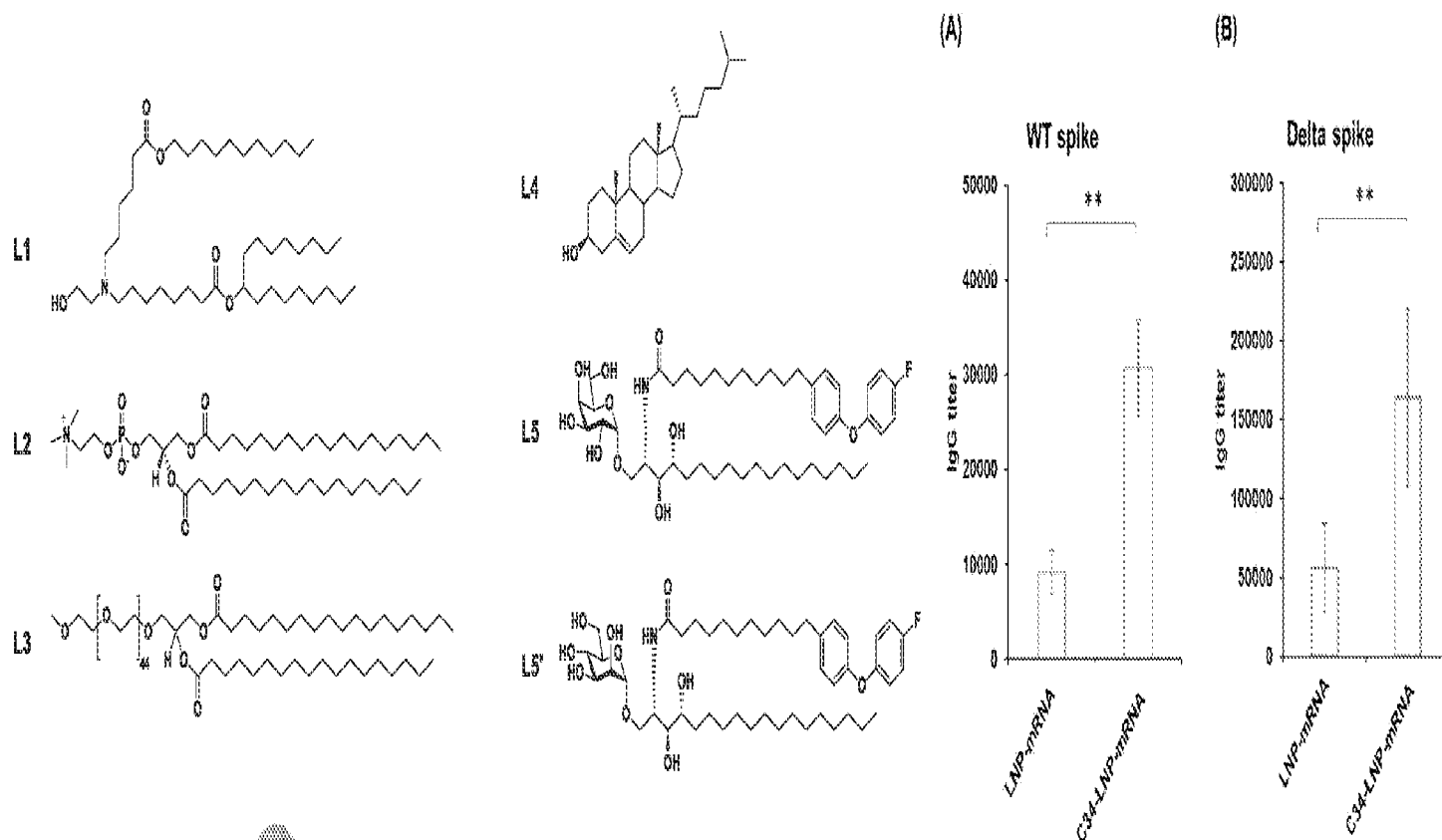


Lipid Nanoparticle (LNP) for Selective mRNA Delivery to Dendritic Cell

L3 is a glycolipid targeting dendritic cells

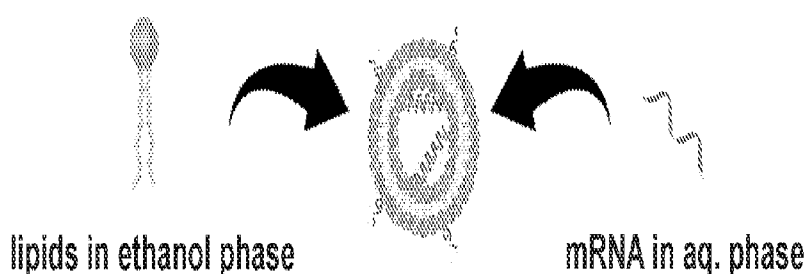


LNP-mRNA Formulation with Dendritic Cell Targeting Glycolipid (L5 or L5') Enhances Immune Response



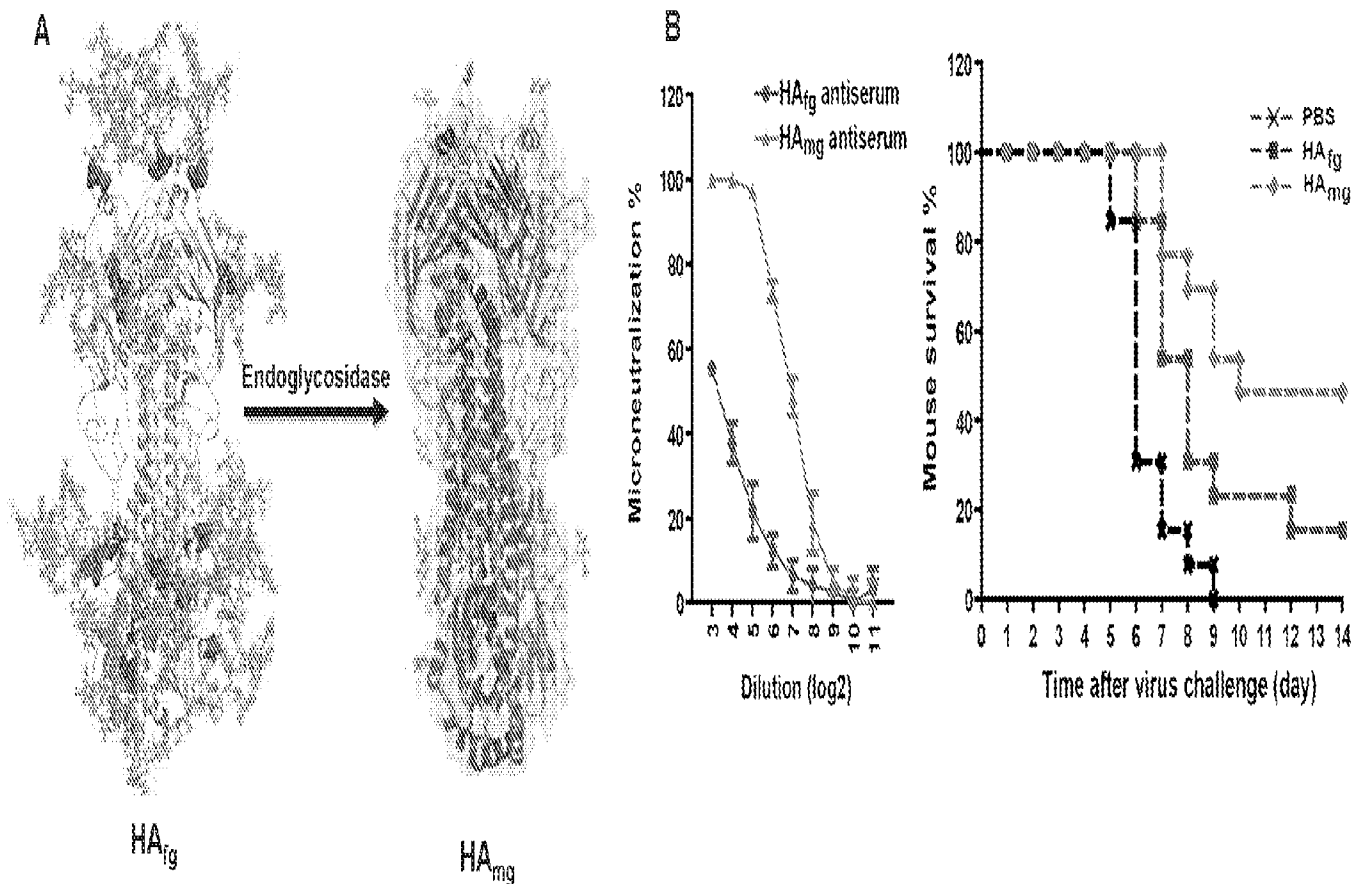
lipids mixing ratio
L1 : L2 : L3 : L4 : (L5 or L5')
= 45 : 10 : 1.5 : 23.5 : 20

Lipids/mRNA = 1 : 3 (w/w) mix at room temp.



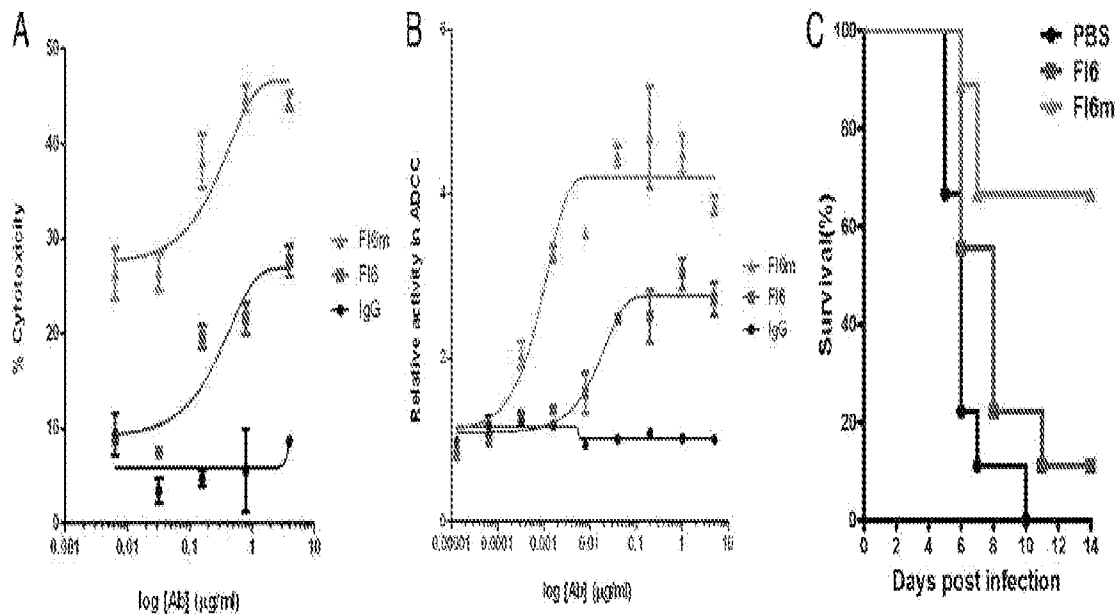
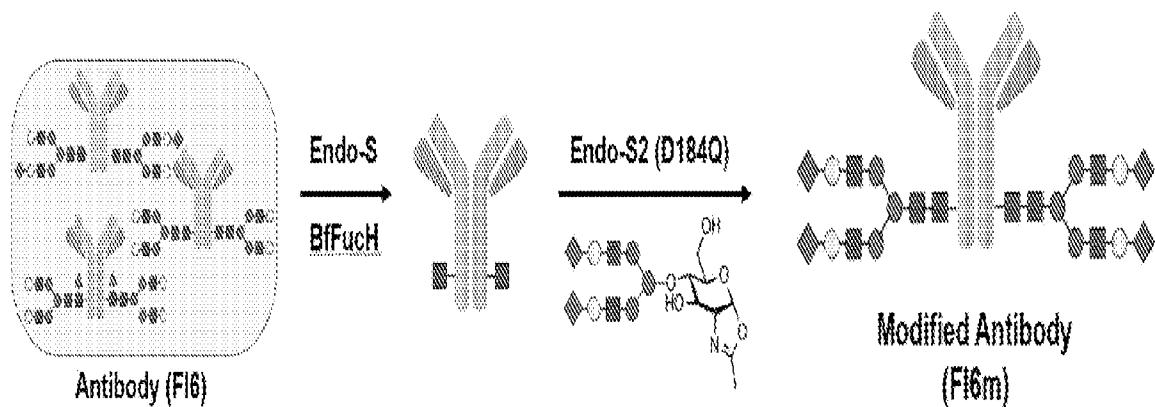
Universal Vaccine: Consensus HA Sequence + Glycodeletion

mono-GlcNAc HA induced more CD4- and CD8-T cell responses to the conserved stem



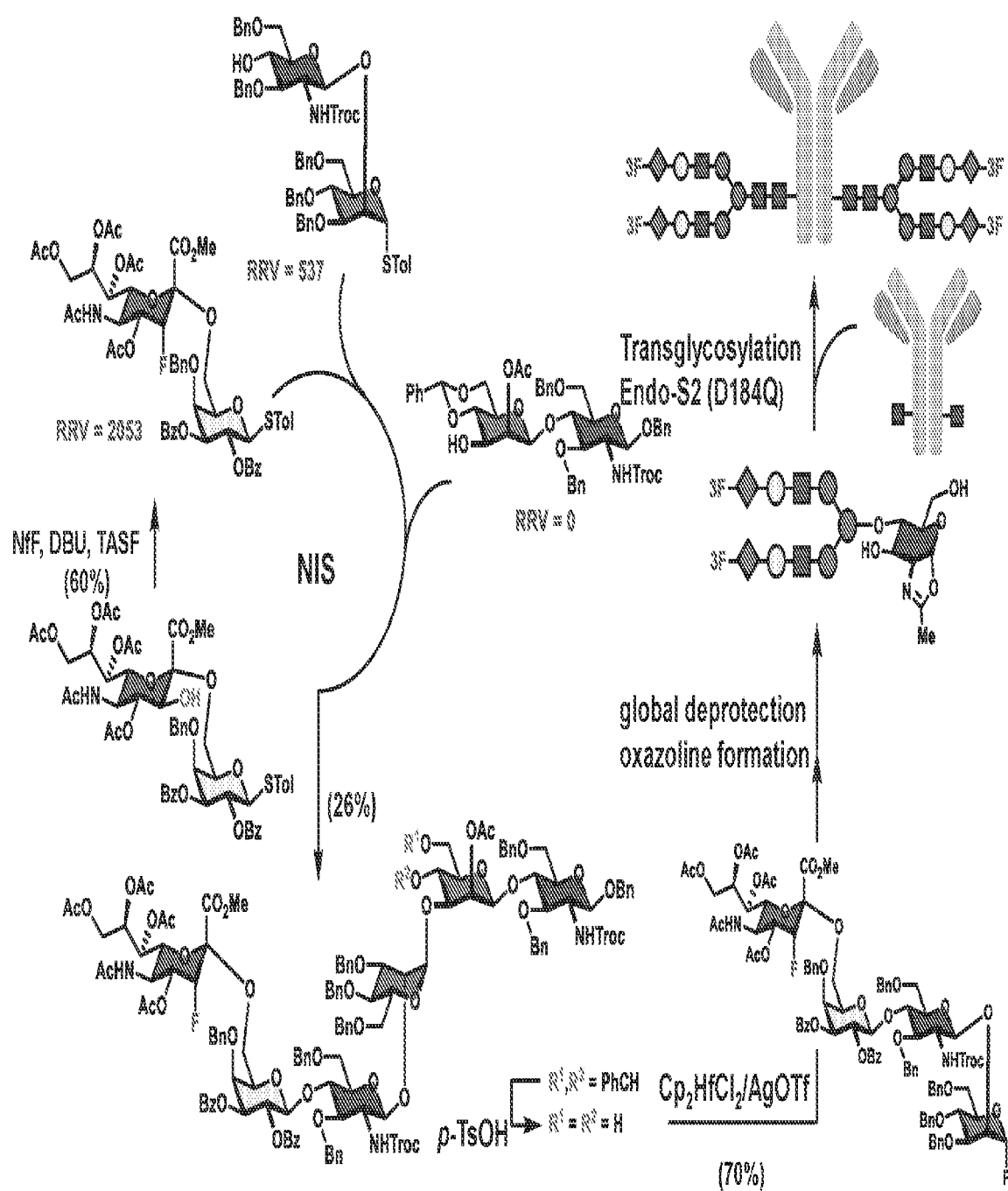
A) Fully glycosylated HA (HA_{fg}) and mono-GlcNAc HA (HA_{mg}) trimers
B) (left) Neutralization of H5N1 (right) Vaccine protection against H5N1.

Glycoengineering of Universal anti-Influenza Antibody FI6



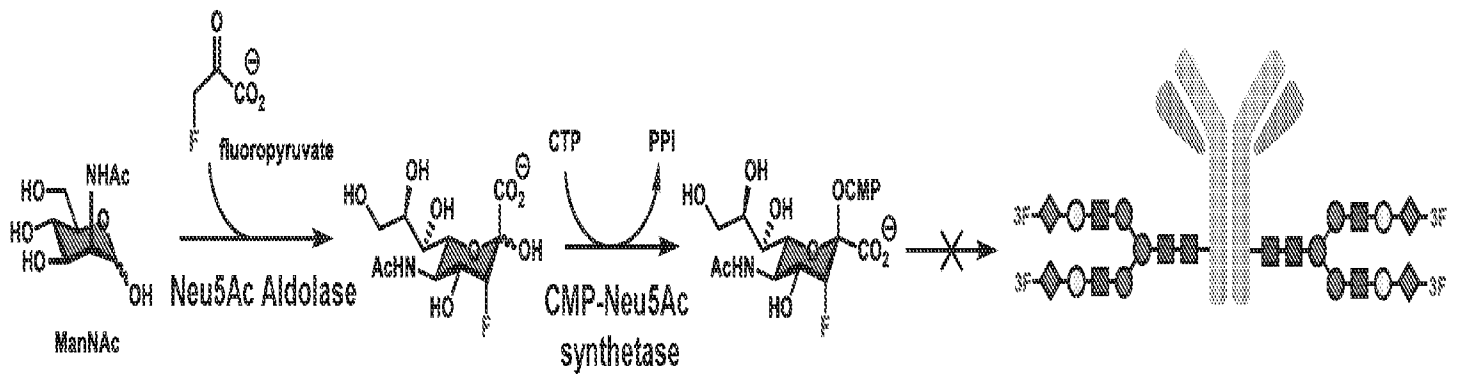
(A),(B) Cytotoxicity of HA-expressing HEK293T cells incubated with PBMC or NK cells and antibody.
 (C) Challenge study: lethal dose (10 MLD₅₀) infection of mice with H1N1 (A/California/07/09).

Programmable One-pot Synthesis of 3F-Sialyl Glycan

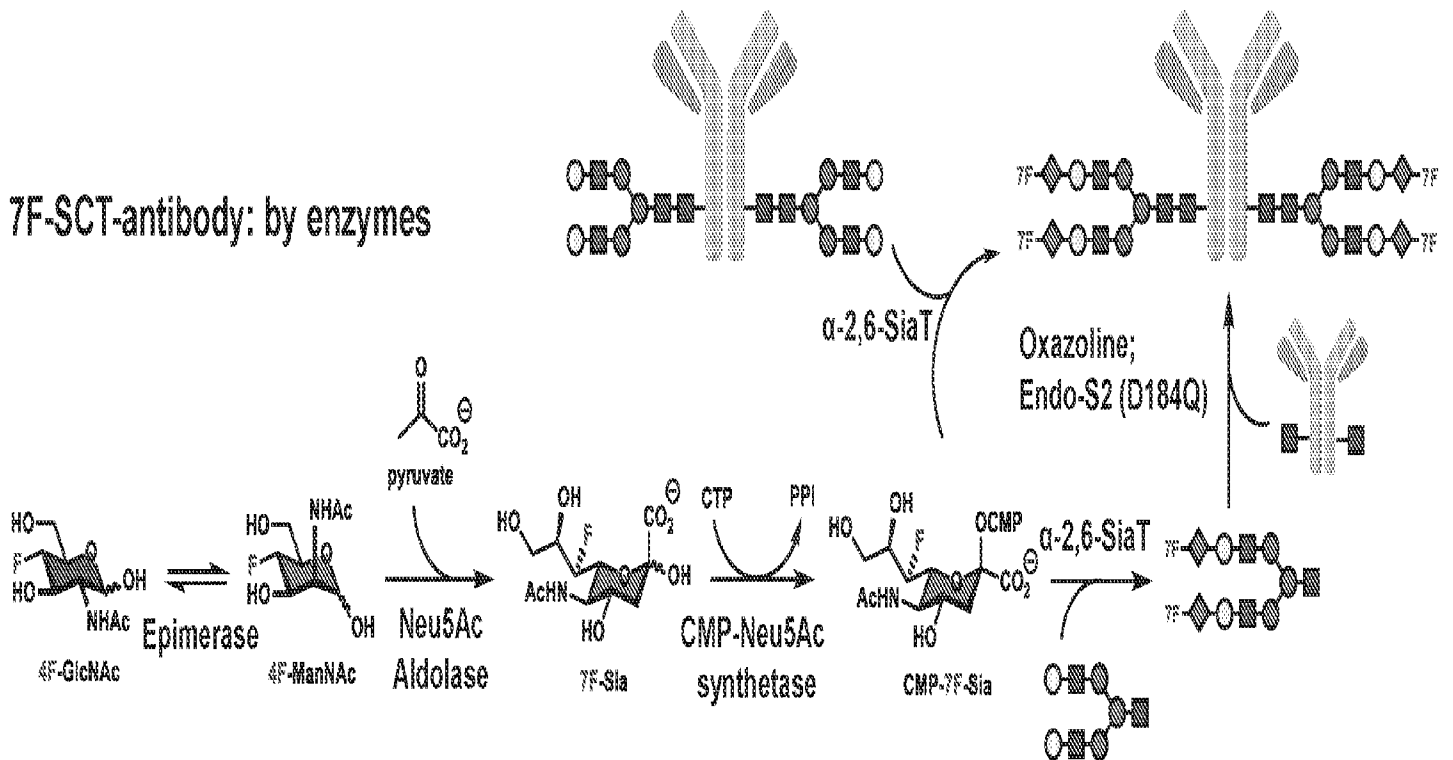


Enzymatic Synthesis of Homogeneous Antibody with 7F-SCT

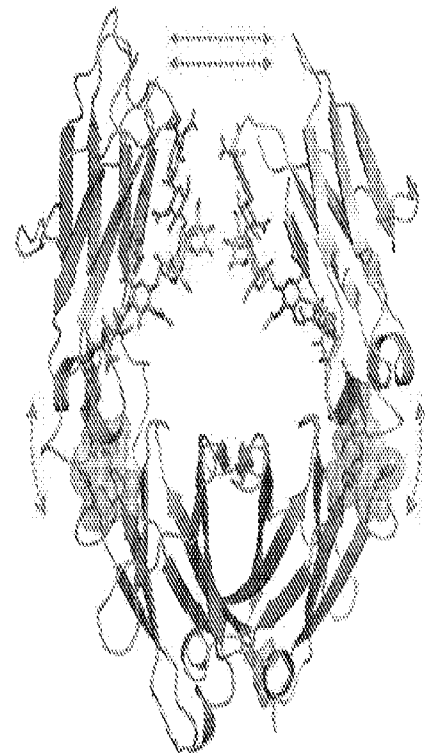
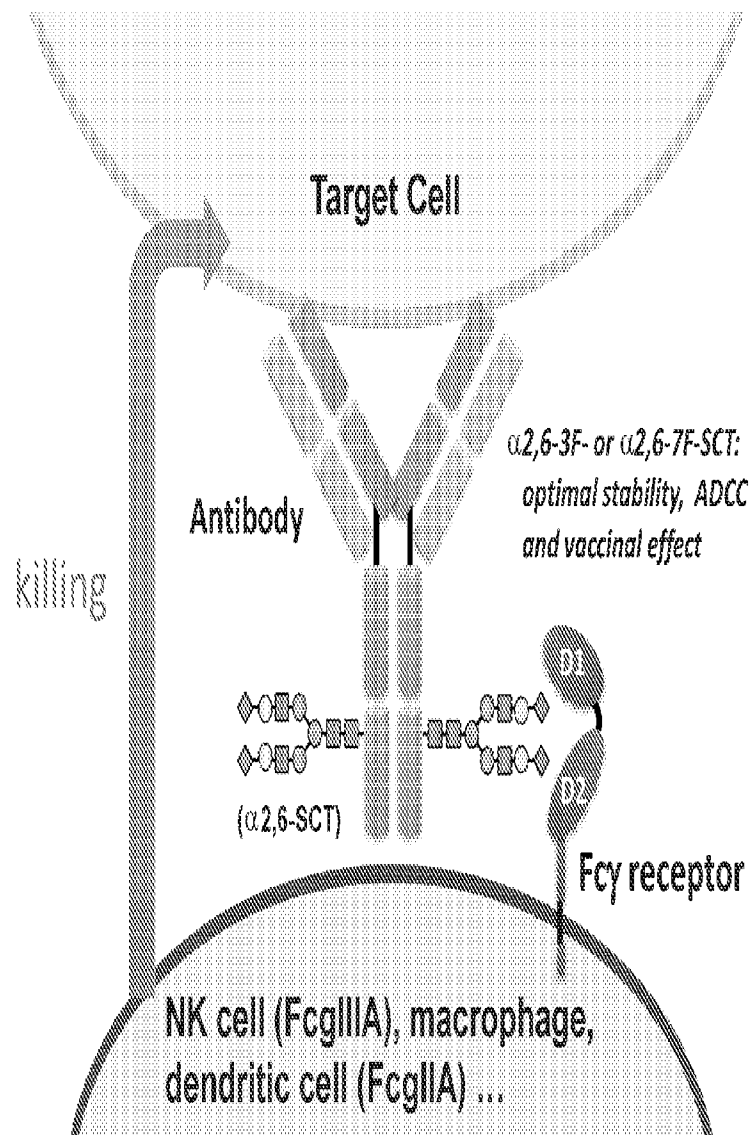
3F-SCT-antibody: CMP-3F-Neu5Ac is not a substrate for sialyltransferase



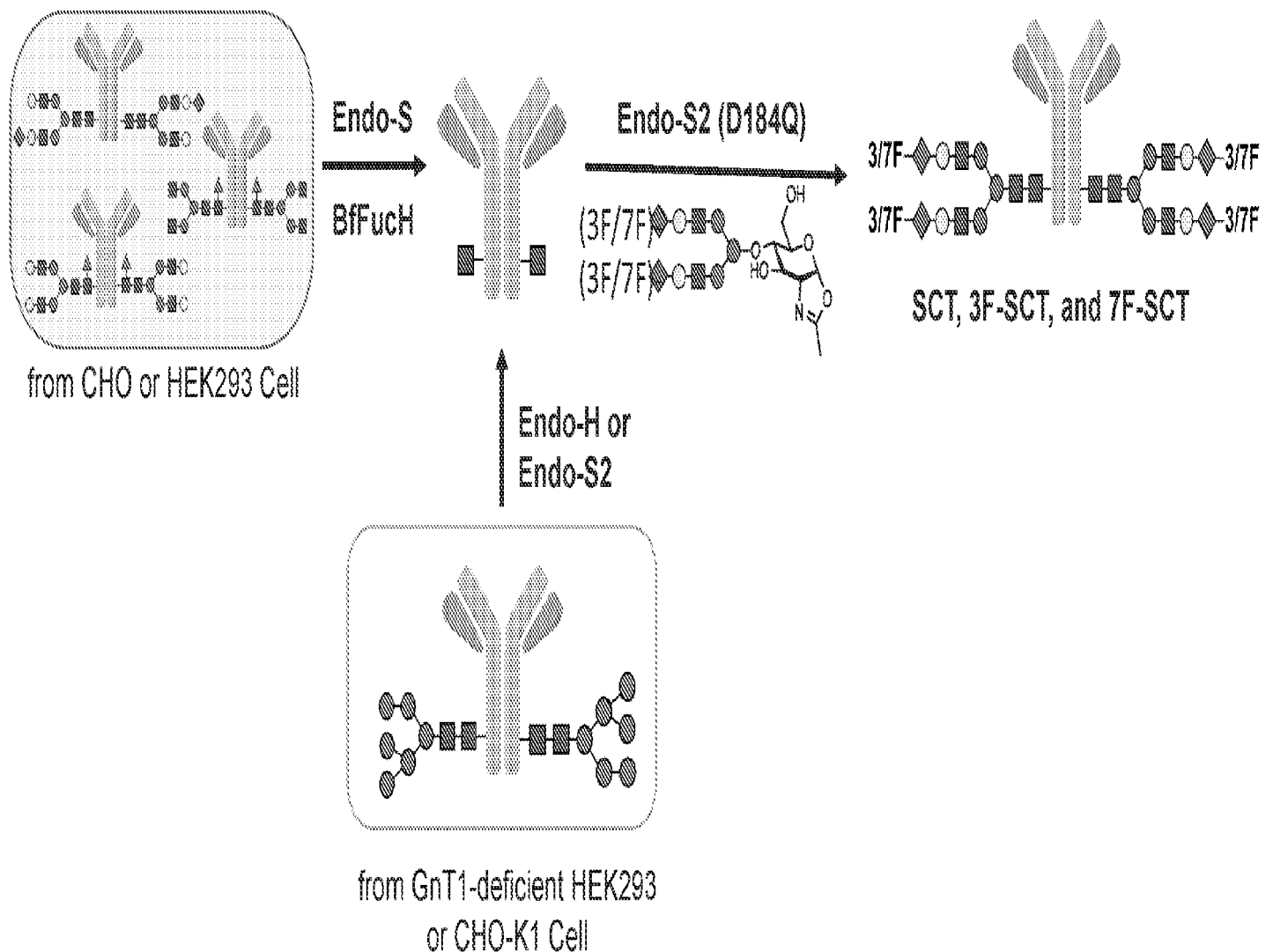
7F-SCT-antibody: by enzymes



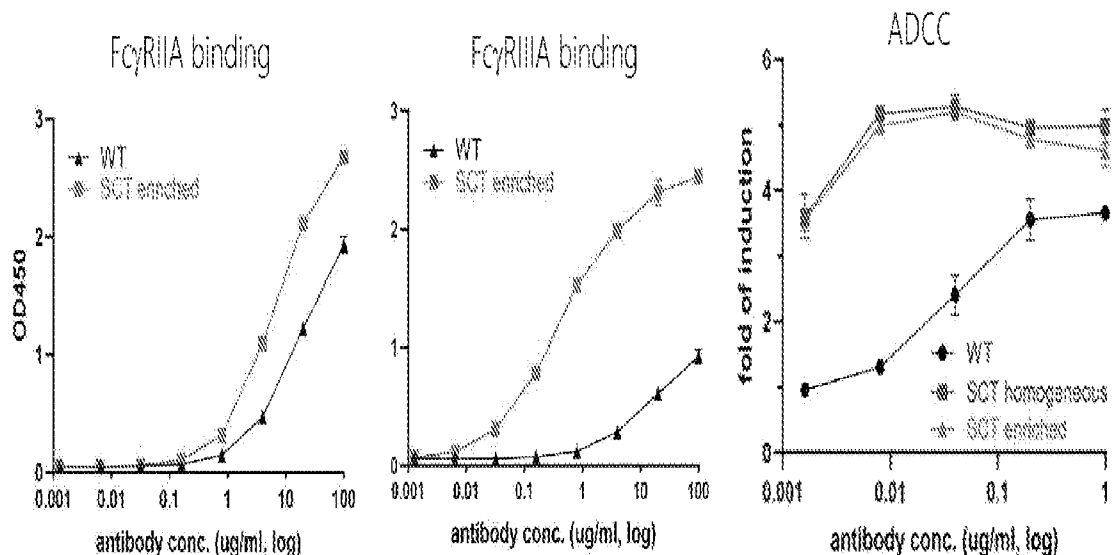
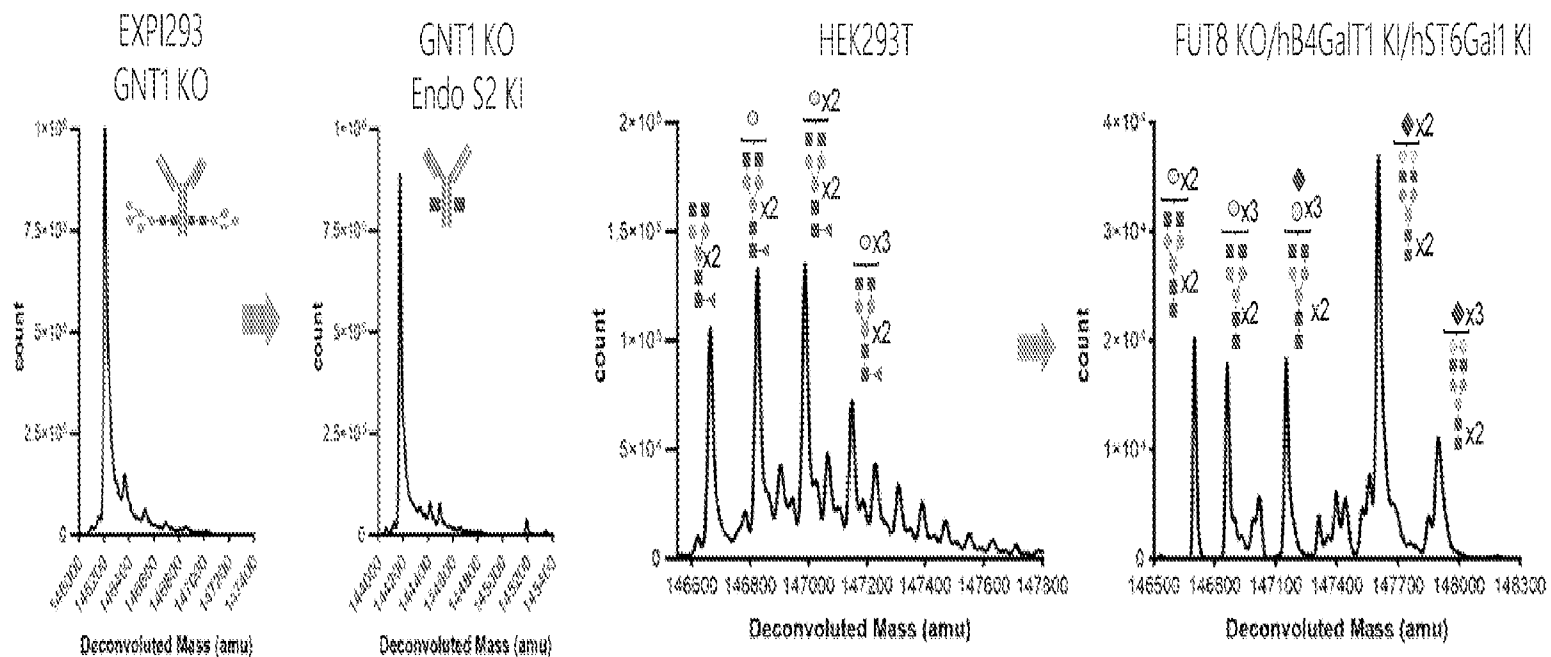
Homogeneous Antibody with Fc-glycan Optimized for Effector Function



Homogeneous Antibody with α -2,6-Sialyl Complex-Type (SCT) Glycan and 3F-SCT, 7F-SCT Glycoforms



Engineering Glycosylation Pathway in HEK293 Cells to Produce Desired Glycoforms





UNITED STATES
PATENT AND TRADEMARK OFFICE

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
63/581,389

RECEIPT DATE / TIME
09/08/2023 02:46:33 PM ET

ATTORNEY DOCKET #
A1000-01100P2

Title of Invention

TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES

Application Information

APPLICATION TYPE Utility - Provisional Application under
35 USC 111(b)

PATENT # -

CONFIRMATION # 6062

FILED BY Shannon Warner

PATENT CENTER # 62763685

FILING DATE -

CUSTOMER # 169053

FIRST NAMED
INVENTOR Chi-Huey Wong

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Kay Yang

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
A1000-01100P2_ADS_20230908.pdf	8	Application Data Sheet	2224 KB
A1000-01100P2_Specification_20230908_FINAL.pdf	26	Specification	13018 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
A1000-	467ECFB0E4171E0F80F5BA8267FEF15C4FA3E0451146604FC6

01100P2_ADS_20230908.pdf	8760402456B49517213959D3BB6483D33287492207C2242C4B4 F27D8381AA60DE12EC926BFE0A7
A1000- 01100P2_Specification_202309 08_FINAL.pdf	F4BD3B521EC974AB955C3A92AA754AD7588471E1EBD863CF B44BC68CCC532678FB5F3B054876C03F69042EA93D564E2F8 D3C425DC16C87DB8CA1E78693CEEC4F

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor 1					Remove		
Legal Name							
Prefix	Given Name		Middle Name		Family Name		Suffix
	Chi-Huey				Wong		
Residence Information (Select One)							☒ US Residency ☐ Non US Residency ☐ Active US Military Service
City	Rancho Santa Fe		State/Province	CA	Country of Residence	US	
Mailing Address of Inventor:							
Address 1		P.O. Box 8154					
Address 2							
City	Rancho Santa Fe			State/Province	CA		
Postal Code	92067			Country i	US		
All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.							Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).			
☐ An Address is being provided for the correspondence Information of this application.			
Customer Number	169053		
Email Address	ipdocketing@prosytagroup.com		Add Email Remove Email

Application Information:

Title of the Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		
Attorney Docket Number	A1000-01100P2	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ US Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	169053		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			Add

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	A1000-01100P2
	Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.				
Applicant 1				Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.				
Clear				
☐ Assignee	☐ Legal Representative under 35 U.S.C. 117		☐ Joint Inventor	
☐ Person to whom the inventor is obligated to assign.		☐ Person who shows sufficient proprietary interest		
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:				
Name of the Deceased or Legally Incapacitated Inventor:				
If the Applicant is an Organization check here. ☐				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Applicant:				
Address 1				
Address 2				
City		State/Province		
Country		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Applicant Data may be generated within this form by selecting the Add button.				Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

[Remove](#)If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

[Add](#)**Signature:**[Remove](#)

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Kay Yang/			Date (YYYY-MM-DD)	2023-09-08
First Name	Kay	Last Name	Yang	Registration Number	61072

Additional Signature may be generated within this form by selecting the Add button.

[Add](#)

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	A1000-01100P2
		Application Number	
Title of Invention	TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES		

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

WADE LIN
PROSYLA GROUP PC
530 TECHNOLOGY DR., SUITE 100
IRVINE, CA 92618
UNITED STATES OF AMERICA

PCT

NOTIFICATION OF TRANSMITTAL OF THE
INTERNATIONAL SEARCH REPORT AND THE
WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing (day/month/year)	24 October 2024 (24.10.2024)
Applicant's or agent's file reference A1000-01700PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)
Applicant	ROCK BIOMEDICAL INC.

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

How? Directly to the International Bureau preferably through ePCT, or on paper to:
The International Bureau of WIPO, 34, chemin des Colombettes, 1211 Geneva 20, Switzerland

For more detailed instructions, see PCT Applicant's Guide, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the PCT Applicant's Guide, National Chapters.

Within **22 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45bis.1). The procedure for requesting supplementary international search is described in the PCT Applicant's Guide, International Phase, paragraphs 8.006-8.032.

Name and mailing address of the ISA/US COMMISSIONER FOR PATENTS MAIL STOP PCT, ATTN: ISA/US P.O. Box 1450 Alexandria, VA 22313-1450 UNITED STATES OF AMERICA	Authorized officer TAINA MATOS
Facsimile No. 571-273-8300	Telephone No. 571-272-4300

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference A1000-01700PCT		FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)	(Earliest) Priority Date (day/month/year) 08 September 2023 (08.09.2023)
Applicant ROCK BIOMEDICAL INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. 1

☒ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034588

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 27
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-12 are drawn to cells expressing sialylated glycoproteins.

Group II: claims 13-20 are drawn to methods for glycoengineering recombinant glycoproteins.

Group III: claims 21-26 are drawn to recombinant glycoproteins.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase, are not present in Groups II or III; the special technical features of Group II, method for glycoengineering a recombinant glycoprotein, are not present in Groups I and III; and the special technical features of Group III, enriched recombinant glycoproteins, are not present in Groups I or II.

Additionally, even if Groups I-III were considered to share the technical features of a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2021/0207106 A1 to The General Hospital Corporation discloses a cell for expressing a sialylated glycoprotein (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; wherein the catalytic domain of sialyltransferase catalyzes sialylation of a glycoprotein; and a second fusion polypeptide having an antibody heavy chain CH2 region, an antibody heavy chain CH3 region, and a catalytic domain of galactosyltransferase, wherein the catalytic domain of galactosyltransferase catalyzes galactosylation of a glycoprotein, Para. [0016]) wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

galactosyltransferase catalytic peptide (this disclosure provides a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of sialyltransferase or the catalytic domain of sialyltransferase, Para. [0103]; a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of galactosyltransferase or the catalytic domain of galactosyltransferase, Para. [0104]), wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; a first fusion polypeptide having ...a catalytic domain of sialyltransferase ...a second fusion polypeptide having ...a catalytic domain of galactosyltransferase, Para. [0016]).

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-12**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034588

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 9/10* (2024.01); *C12P 21/00* (2024.01); *C07K 19/00* (2024.01); *C12N 15/52* (2024.01); *C12N 5/10* (2024.01); *C07K 16/24* (2024.01); *C12N 15/62* (2024.01); *C12N 15/63* (2024.01); *C12N 1/15* (2024.01); *C12N 1/19* (2024.01); *C12N 1/21* (2024.01)

CPC: *C12N 9/1051*; *C12N 15/52*; *C12N 5/10*; *C12P 21/005*; *C07K 2319/00*; *C07K 2319/03*; *C07K 2319/30*; *C12N 9/1081*; *C12Y 204/01038*; *C12Y 204/99001*; *C07K 16/241*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2022/229854 A1 (NOVARTIS AG) 03 November 2022 (03.11.2022)	
Y	entire document	1-4, 7-11
Y	entire document	5, 6, 12
Y	US 2006/0073542 A1 (BAYER et al.) 06 April 2006 (06.04.2006)	
Y	entire document	5, 6
A	US 2021/0207106 A1 (THE GENERAL HOSPITAL CORPORATION) 08 July 2021 (08.07.2021)	
A	entire document	1-12
Y	YANG et al. "Glycoproteomic Characterization of FUT8 Knock-Out CHO Cells Reveals Roles of FUT8 in the Glycosylation," <i>Frontiers in Chemistry</i> , 28 October 2021 (28.10.2021), Vol. 9, No. 755238, Pgs. 1-9. entire document	
Y		12

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 August 2024 (29.08.2024)

Date of mailing of the international search report

24 October 2024 (24.10.2024)

Name and mailing address of the ISA/US

**COMMISSIONER FOR PATENTS
MAIL STOP PCT, ATTN: ISA/US
P.O. Box 1450
Alexandria, VA 22313-1450 UNITED STATES OF AMERICA**

Authorized officer

TAINA MATOS

Facsimile No. **571-273-8300**

Telephone No. **571-272-4300**

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

WADE LIN
PROSYLA GROUP PC
530 TECHNOLOGY DR., SUITE 100
IRVINE, CA 92618
UNITED STATES OF AMERICA

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Applicant's or agent's file reference A1000-01700PCT		Date of mailing (day/month/year) 24 October 2024 (24.10.2024)
FOR FURTHER ACTION See paragraph 2 below		
International application No. PCT/US2024/034588	International filing date (day/month/year) 19 June 2024 (19.06.2024)	Priority date (day/month/year) 08 September 2023 (08.09.2023)
International Patent Classification (IPC) or both national classification and IPC IPC: See continuation sheet CPC: See continuation sheet		
Applicant ROCK BIOMEDICAL INC.		

1. This opinion contains indications relating to the following items:

- | | | |
|-------------------------------------|--------------|---|
| ☒ | Box No. I | Basis of the opinion |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☒ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US COMMISSIONER FOR PATENTS MAIL STOP PCT, ATTN: ISA/US P.O. Box 1450 Alexandria, VA 22313-1450 UNITED STATES OF AMERICA	Authorized officer TAINA MATOS
Facsimile No. 571-273-8300	Telephone No. 571-272-4300

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2024/034588

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).
3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2024/034588

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
☒ claims Nos. See claims listed below

because:

- ☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 27 are so unclear that no meaningful opinion could be formed (*specify*):

Claim 27 is a multiple dependent claim not drafted in accordance with the second and third sentences of Rule 6.4(a).

- ☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

- ☒ no international search report has been established for said claims Nos. 13-27

- ☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:
- ☐ furnish a sequence listing complying with WIPO Standard ST.26, and such listing was not available to the International Searching Authority in the form, language and manner acceptable to it.
 - ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a).
 - ☐ See Supplemental Box for further details.

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees.
 - ☐ paid additional fees under protest and, where applicable, the protest fee.
 - ☐ paid additional fees under protest but the applicable protest fee was not paid.
 - ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-12 are drawn to cells expressing sialylated glycoproteins.

Group II: claims 13-20 are drawn to methods for glycoengineering recombinant glycoproteins.

Group III: claims 21-26 are drawn to recombinant glycoproteins.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase, are not present in Groups II or III; the special technical features of Group II, method for glycoengineering a recombinant glycoprotein, are not present in Groups I and III; and the special technical features of Group III, enriched recombinant glycoproteins, are not present in Groups I or II.

Additionally, even if Groups I-III were considered to share the technical features of a cell for expressing a sialylated glycoprotein wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2021/0207106 A1 to The General Hospital Corporation discloses a cell for expressing a sialylated glycoprotein (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; wherein the catalytic domain of sialyltransferase catalyzes sialylation of a glycoprotein; and a second fusion polypeptide having an antibody heavy chain CH2 region, an antibody heavy chain CH3 region, and a catalytic domain of galactosyltransferase, wherein the catalytic domain of galactosyltransferase catalyzes galactosylation of a glycoprotein, Para. [0016]) wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide (this disclosure provides a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of sialyltransferase or the catalytic domain of sialyltransferase, Para. [0103]; a fusion protein or a peptide comprising or consisting of the enzymatic luminal domain of galactosyltransferase or the catalytic domain of galactosyltransferase, Para. [0104]), wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity (a cell having the vector as described herein, and the vector optionally expresses the fusion polypeptide as described herein, Para. [0015]; a first fusion polypeptide having ...

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2024/034588

Box No. IV Lack of unity of invention

a catalytic domain of sialyltransferase ...a second fusion polypeptide having ...a catalytic domain of galactosyltransferase, Para. [0016]).

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

4. Consequently, this opinion has been established in respect of the following parts of the international application:

☐

all parts.

☒

the parts relating to claims Nos. 1-12

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5, 6, 12	YES
	Claims	1-4, 7-11	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	None	NO

2. Citations and explanations:

----- EXPLANATION -----

Claims 1-4 and 7-11 lack novelty under PCT Article 33(2) as being anticipated by WO 2022/229854 to Novartis Ag (hereinafter, "Novartis").

Regarding Claim 1, Novartis discloses a cell (cell, Abstract) wherein the cell constitutively and/or controllably expresses an exogenous sialyltransferase catalytic peptide and an exogenous galactosyltransferase catalytic peptide (vectors which had the α 2,6-sialyltransferase under the control of a strong promoter while expression of the β 1,4-galactosyltransferase and the CMP-sialic acid transporter is controlled by a moderate promoter, Pg. 2, Lns. 21-23), wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are translated in close proximity (the alpha-2,6-sialyltransferase is expressed by a first expression cassette and the beta-1,4- galactosyltransferase and the CMP-sialic acid transporter are both expressed by a second expression cassette ... all three glycosylation enzymes are expressed by the same expression cassette ... coding sequences are expressed in one single open reading frame, Pg. 12, Lns. 25-32). Further, in regards to the limitation relating to "for expressing a sialylated glycoprotein," a recitation of the intended use of the claimed invention must result in a structural difference between the claimed invention and the prior art in order to distinguish the claimed invention from the prior art. If the prior art is capable of performing the intended use, it meets the claim.

Regarding Claim 2, Novartis discloses the cell of claim 1, wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are expressed in a single transcript (the alpha-2,6-sialyltransferase is expressed by a first expression cassette and the beta-1,4- galactosyltransferase and the CMP-sialic acid transporter are both expressed by a second expression cassette ... all three glycosylation enzymes are expressed by the same expression cassette ... coding sequences are expressed in one single open reading frame, Pg. 12, Lns. 25-32).

Regarding Claim 3, Novartis discloses the cell of claim 1, comprising a first nucleic acid configured to express the exogenous sialyltransferase catalytic peptide (first exogenous promoter operatively linked to an endogenous coding sequence for an alpha-2,6-sialyltransferase, Pg. 16, Lns. 15-16) and a second nucleic acid configured to express the exogenous galactosyltransferase catalytic peptide (a second exogenous promoter operatively linked to an endogenous coding sequence for a beta-1,4-galactosyltransferase, Pg. 16, Lns. 17-18), wherein the first nucleic and the second nucleic acid are transcriptionally controlled by the same promoter (the same promoters as used for the exogenous nucleic acids, Pg. 16, Ln. 22; the first promoter is ...simian virus 40 (SV40) promoter ...the second promoter ...simian virus 40 (SV40) promoter, Pg. 35, Lns. 7-17).

Regarding Claim 4, Novartis discloses the cell of claim 3, wherein the first nucleic acid and the second nucleic acid are connected to each other via a connecting nucleic acid, which is configured to encode a ribosomal shifting peptide (wherein two or more glycosylation enzymes are expressed by the same expression cassette, the expression cassette may further comprise an internal ribosome entry site (IRES) or a coding sequence for a 2A element between the coding sequences for the different glycosylation enzymes, Pg. 12, Lns. 26-30).

Box No. V

Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

Regarding Claim 7, Novartis discloses the cell of claim 3, wherein the first nucleic acid is derived from an ST6Gal 1 gene or a PspST gene (a first exogenous expression cassette comprising ...ST6Gal1, Pg. 33, Lns. 5-7).

Regarding Claim 8, Novartis discloses the cell of claim 3, wherein the second nucleic acid is derived from an B4GALT1 gene (a second exogenous expression cassette comprising ...B4GALT1, Pg. 33, Lns. 8-10).

Regarding Claim 9, Novartis discloses the cell of claim 3, wherein the promoter is a constitutive promoter or an activable promoter (the term "promoter" refers to a nucleic acid sequence which is located upstream (5') of the nucleic acid sequence which is to be expressed and controls expression of the sequence ...constitutive, Pg. 5, Lns. 20-22).

Regarding Claim 10, Novartis discloses the cell of claim 1, wherein the sialyltransferase is a beta-galactoside alpha-2,6-sialyltransferase 1 (a first exogenous expression cassette comprising ...ST6Gal1, Pg. 33, Lns. 5-7).

Regarding Claim 11, Novartis discloses the cell of claim 1, wherein the galactosyltransferase catalytic peptide is a beta-1,4- galactosyltransferase 1 (a second exogenous expression cassette comprising ...B4GALT1, Pg. 33, Lns. 8-10).

Claims 5 and 6 lack an inventive step under PCT Article 33(3) as being obvious over WO 2022/229854 to Novartis Ag (hereinafter, "Novartis") in view of US 2006/0073542 to Bayer et al. (hereinafter, "Bayer").

Regarding Claim 5, Novartis discloses the cell of claim 3. Novartis fails to explicitly disclose wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are co-configured to be expressed as a fusion protein.

Bayer is in the field of recombinant glycosyltransferase fusion proteins having a desired level of expression (Abstract) and teaches wherein the exogenous sialyltransferase catalytic peptide and the exogenous galactosyltransferase catalytic peptide are co-configured to be expressed as a fusion protein (recombinant glycosyltransferase fusion proteins having high expression levels ...fusion proteins ... are not limited to sequences of two different and distinct glycosyltransferases, Para. [0012]; a combination of recombinant sialyltransferase fusion protein and a galactosyltransferase, which may ...be part of a fusion protein, Para. [0173]).

It would have been obvious to one of ordinary skill in the art before the priority date to modify Novartis with the teaching of Bayer for the purpose of efficient production of novel glycosyltransferases that are expressed at a high level in cells and/or have high enzymatic activity (Bayer, Para. [0011]).

Regarding Claim 6, modified Novartis discloses the cell of claim 5. Novartis fails to explicitly disclose wherein the fusion protein comprises a first portion having the sialyltransferase catalytic peptide domain and a second portion having the galactosyltransferase catalytic peptide domain, wherein the first portion and the second portion are connected to each other via a cleavable linker, further wherein the cleavable linker is configured to be cleavable post-translation of the fusion protein, thereby upon cleavage releasing the sialyltransferase catalytic peptide and the galactosyltransferase catalytic peptide as separate proteins.

Bayer teaches wherein a fusion protein comprises a first portion having a sialyltransferase catalytic peptide domain and a second portion having a galactosyltransferase catalytic peptide domain (recombinant glycosyltransferase fusion proteins having high expression levels ...fusion proteins ... are not limited to sequences of two different and distinct glycosyltransferases, Para. [0012]; a combination of recombinant sialyltransferase fusion protein and a galactosyltransferase, which may ...be part of a fusion protein, Para. [0173]), wherein a first portion and the second portion are connected to each other via a cleavable linker, further wherein the cleavable linker is configured to be cleavable post-translation of the fusion protein, thereby upon cleavage releasing the sialyltransferase catalytic peptide and the galactosyltransferase catalytic peptide as separate proteins ([t]he recombinant glycosyltransferase fusion proteins of ... encode at least a subsequence of a functional domain of a first glycosyltransferase joined, directly or through a peptide linker, Para. [0012]; For certain applications, it may be desirable to cleave the ...accessory enzyme amino acids from the fusion protein, Para. [0158]).

Box No. V

Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

It would have been obvious to one of ordinary skill in the art before the priority date to modify Novartis with the teaching of Bayer for the purpose of efficient production of novel glycosyltransferases that are expressed at a high level in cells and/or have high enzymatic activity (Bayer, Para. [0011]).

Claim 12 lacks an inventive step under PCT Article 33(3) as being obvious over WO 2022/229854 to Novartis Ag (hereinafter, "Novartis") in view of YANG et al. "Glycoproteomic Characterization of FUT8 Knock-Out CHO Cells Reveals Roles of FUT8 in the Glycosylation," Frontiers in Chemistry, 28 October to Yang et al. (hereinafter, "Yang").

Regarding Claim 12, Novartis discloses the cell of claim 1. Novartis fails to explicitly disclose wherein the cell is deficient in fucosyltransferase activity.

Yang is in the field of FUT8 knockout CHO cell lines (Abstract) and teaches wherein a cell is deficient in fucosyltransferase activity (stable FUT8KO and wild-type (WT) CHO cells were adapted to suspension cell culture status in CD-CHO media, Pg. 2, right-hand column, second paragraph).

It would have been obvious to one of ordinary skill in the art before the priority date to modify Novartis with the teaching of Yang for the purpose of silencing α -1,6-fucosylation on antibodies expressed in CHO cells (Yang, Pg. 2, right-hand column, second paragraph).

Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2024/034588

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 18 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claim 18 is indefinite for the following reason:

With regards to claim 18, the claim includes a plurality of trademarks (Humira, Amjevntia...) which characterize the commercial origin, rather than the properties therein relevant to the invention, which is indefinite. Herein, claim 18 is best interpreted without the trademarks.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US2024/034588

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of IPC: *C12N 9/10* (2024.01); *C12P 21/00* (2024.01); *C07K 19/00* (2024.01); *C12N 15/52* (2024.01); *C12N 5/10* (2024.01); *C07K 16/24* (2024.01); *C12N 15/62* (2024.01); *C12N 15/63* (2024.01); *C12N 1/15* (2024.01); *C12N 1/19* (2024.01); *C12N 1/21* (2024.01)

Continuation of CPC: *C12N 9/1051*; *C12N 15/52*; *C12N 5/10*; *C12P 21/005*; *C07K 2319/00*; *C07K 2319/03*; *C07K 2319/30*; *C12N 9/1081*; *C12Y 204/01038*; *C12Y 204/99001*; *C07K 16/241*



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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
63/639,286	04/26/2024		120	A1000-01700		

169053
Prosyla Group PC
530 Technology Dr. Suite 100
Irvine, CA 92618

CONFIRMATION NO. 3251
FILING RECEIPT



00000000/2081130

Date Mailed: 05/03/2024

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt identifying the requested changes, preferably by including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Chi-Huey WONG, Rancho Santa Fe, CA;

Applicant(s)

Rock BioMedical Inc., Taipei City, TAIWAN;

Power of Attorney:

ZONG WEI LIN -L1383

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 05/02/2024

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 63/639,286**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

CELL-BASED PRODUCTION OF GLYCAN-ENRICHED ANTIBODY

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No**PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES**

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
63/581,389	09/08/2023		120	A1000-01100P2		

169053
Prosyla Group PC
530 Technology Dr. Suite 100
Irvine, CA 92618

CONFIRMATION NO. 6062
FILING RECEIPT



0000000061863728

Date Mailed: 09/14/2023

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

Chi-Huey Wong, Rancho Santa Fe, CA;

Applicant(s)

Chi-Huey Wong, Rancho Santa Fe, CA;

Power of Attorney:

KAY YANG -61072

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

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If Required, Foreign Filing License Granted: 09/13/2023

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 63/581,389**

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

TARGETING HUMAN VIRUSES WITH BROADLY PROTECTIVE LOW-SUGAR VACCINES

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

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Cell-based production of Fc-GlcNAc and Fc-alpha-2,6 sialyl glycan enriched antibody with improved effector functions through glycosylation pathway engineering

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ABSTRACT: Glycosylation of antibody plays an important role in Fc-mediated killing of tumor cells and virus-infected cells through effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody dependent cell-mediated phagocytosis (ADCP) and vaccinal effect. Previous studies showed that therapeutical humanized antibodies with α 2-6 sialyl complex type (SCT) glycan attached to Fc-Asn297 exhibited optimal binding to the Fc receptors on effector cells associated with ADCC, ADCP and vaccinal effect. However, the production of antibodies with homogeneous Fc-SCT needs multiple *in vitro* enzymatic and purification steps. In this study, we report two different approaches to shorten the processes to produce SCT-enriched antibodies. First, we expressed a bacterial endoglycosidase in GNT1-KO EXP1293 cells to trim all *N*-glycans to mono-GlcNAc glycoforms for *in vitro* transglycosylation to generate homogeneous SCT antibody. Second, we engineered the glycosylation pathway of HEK293 cells through knock-out of the undesired glycosyltransferases and expression of the desired glycosyltransferases to produce SCT enriched antibodies with similar binding affinity to Fc receptors and ADCC activity to homogenous SCT antibody.

Introduction

Antibody plays an important role in humoral immune response and has diverse plasticity and biological functions. The prototype of antibody is a major component of B cell receptor (BCR) on naive B cells. When the BCRs are activated through pattern recognition receptors (PRRs), the alternative splicing of mRNA helps produce antibodies as secreted forms of IgM or IgD. With the assistance of dendritic cells and T helper cells, the activated B cells could further undergo clonal expansion, somatic hypermutation, and class switch and are differentiated into plasma cells to produce secreted IgG, IgA, or IgE antibodies with high antigen specificity. Once a target cell or pathogen is opsonized by an antibody, it may trigger different downstream immune functions, including phagocytosis, cellular cytotoxicity, vaccinal effect, complement activation etc. These immune cell-based responses required the binding of antibody Fc domain to specific Fc receptors on immune cells. Each subtype or glycoform of antibody can be recognized by several Fc receptors rendering activating or inhibitory signaling to generate different effector functions. For example, the binding of IgG antibody to the Fc γ IIIA receptor on NK cells will activate NK cells to release cytokines to kill target cells, a process called antibody-dependent cellular cytotoxicity (ADCC)¹⁻³ and binding to the Fc γ IIA receptor on macrophages will activate macrophage to kill the target cell through antibody-dependent cell-mediated phagocytosis (ADCP). In addition, binding of antibody-antigen complex to the Fc γ IIA receptor on dendritic cells

can facilitate dendritic cell maturation to induce memory CD8 T-cell response⁴.

Glycosylation is a common post- or co-translational modification found on most cell surface proteins. During *N*-glycoprotein synthesis, a high mannose glycan is first synthesized in the ER and then transferred to the growing polypeptide chain followed by chaperone-mediated protein folding and quality control to form glycoproteins. The high-mannose glycoproteins are trimmed by mannosidases and translocated to the Golgi apparatus for addition of more monosaccharides sequentially by different glycosyltransferases before moving to the cell surface. Unlike protein and DNA, glycosylation is template independent, and the sequence of glycans is determined by the repertoire of glycotransferases expressed in different cell types. Moreover, the cellular environment and protein accessibility also affect the activity and specificity of glycosyltransferases. Thus, the glycans generated by glycosylation usually exhibit more diversity than other modifications⁵⁻⁷.

Antibodies of each subtype are also glycosylated at different places within the constant region of heavy chain (HC) and these glycans regulate antibody functions¹. The preBCR assembly is important for B cell development and is critically regulated by the *N*-glycan at N46 on μ HC⁸. The *N*-glycan at N402 on μ HC has been linked to antibody oligomerization and complement activation⁹⁻¹⁰. Besides, IgG *N*-glycosylation at N297 on γ HC plays a critical role in complement activation and Fc γ receptor activation leading to various effector functions¹¹⁻¹³.

Therefore, it is important to identify optimized glycosylation to improve the efficacy and safety of therapeutic antibodies.

Generally, the production of recombinant antibody is often based on mammalian cell lines, such as NS0, 293T and CHO cells, to produce a mixture of glycoforms with core-fucosylated bi-antennary complex type glycans¹⁴ which are not optimal for binding to the FcγRIIIA receptor, because of the inhibitory function of core-fucosylation¹¹ or the off-target delivery caused by terminal galactosylation¹⁵. Therefore, efforts have been directed toward modification of the Fc-glycan on antibody to improve specific receptor binding and the corresponding effector function. One strategy used was to block the fucosylation pathway in cells. For example, using the cell line deficient in GDP-fucose synthesis could produce antibodies without core-fucosylation, leading to an increase in binding to FcγRIIIA receptor thereby enhancing the ADCC activity^{11, 16}. The emergence of precise gene editing tools such as Zinc Finger Nuclease (ZFN) or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/ CRISPR-associated protein 9 (Cas9) has facilitated the progress of this strategy¹⁷ and studies on glycosylation pathway engineering in cells to produce therapeutic proteins with improved efficacy have been reported¹⁸⁻¹⁹.

Another strategy to alter protein glycosylation is through *in vitro* glycoengineering by enzymes or chemical ligation. A common approach was endoglycosidase-mediated transglycosylation⁷. Usually, the antibody with high mannose type glycan derived from insect cells²⁰, yeast²¹ or GNT1 knockout (KO) cells was treated with endoglycosidase H or S2 to generate mono GlcNAc- or GlcNAc-Fuc bearing glycoforms. The antibody with either of these two glycoforms is a good acceptor substrate for mutated endoglycosidase (glycosynthase)-mediated transglycosylation⁷ using a homogenous glycan oxazoline as donor substrate to generate a new glycan chain. So far, many glycans have been transferred to antibodies to study the functional impact on receptor binding and effector functions. The fully galactosylated complex type glycan without core-fucose

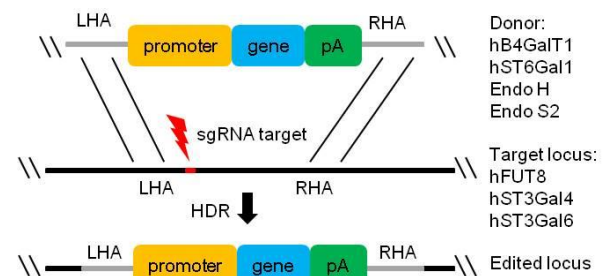


Figure 1. Illustration of CRISPR/Cas9 KO/KI strategies for glycosylation pathway engineering.

revealed that the α 2-6 linked SCT provides enhanced binding to FcγRIIIA and FcγRIIA receptors which are associated ADCC, ADCP and vaccinal effect²³⁻²⁴. Although this strategy could produce antibodies as homogenous glycoforms, the process requires multiple reaction and purification steps and the specificity of mutant endoglycosidases is limited. In this report, we used humanized antibody chMC183-70 that targets specific stage embryonic antigen 4 (SSEA4) on various cancer cells as a model to produce and evaluate the Fc-SCT enriched antibody in Fc receptor binding and activation of cellular cytotoxicity.

Result and Discussion

Development of Fc-GlcNAc antibody producing cells by CRISPR-Cas9. Antibodies are generally produced from mammalian cell lines which contain core-fucosylated complex type glycans¹⁴. To generate the Fc-GlcNAc antibody for *in vitro* transglycosylation, antibody glycans need to be trimmed by endoglycosidase and fucosidase to leave only the innermost GlcNAc as an acceptor⁷. We would like to shorten these steps by CRISPR-Cas9 to generate Fc-GlcNAc antibody with commercially available GNT1 KO EXP1293F cells. The N-glycan expressed by this cell line was high mannose type which was

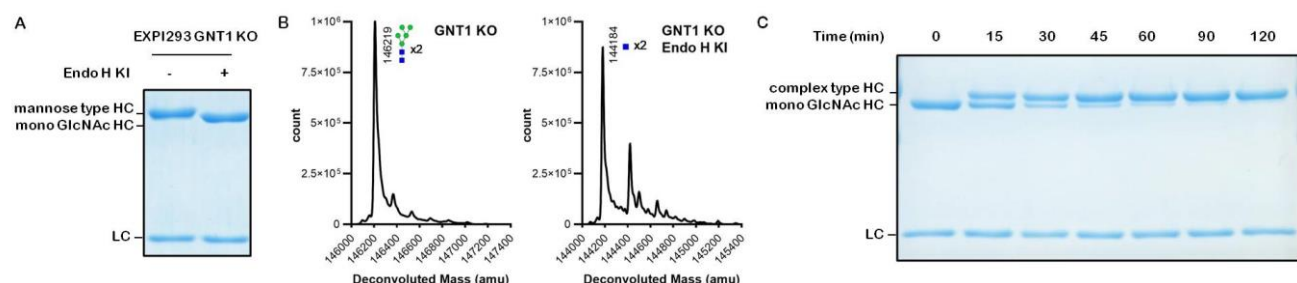


Figure 2. Expression of Endo H in GNT1-knock out cells results in cleavage of N-glycans on antibody. (A) The chMC18370 antibody expressed from engineered and its parental GNT1-KO EXP1293 cells were subjected to protein electrophoresis and the difference in molecular weight by band shifting was observed. (B) The chMC18370 antibody expressed by engineered and its parental GNT1-KO EXP1293 cells were subjected to IPM analysis to examine the molecular weight and glycan changes. (C) The chMC18370 antibody expressed by engineered GNT1-KO EXP1293 cells was subjected to transglycosylation assay and the efficiency of transglycosylation was assessed by protein electrophoresis.

contributes significantly to improve antibody binding to the FcγRIIIA receptor compared to the wild-type antibody²²⁻²³. However, it also facilitates the antibody clearance by liver cells through asialoglycoprotein receptor binding. Further studies

preferentially cleaved by Endo H²⁵ to generate Fc-GlcNAc antibody. Thus, we aimed to knock in Endo H in this cell line by CRISPR-Cas9. However, after transfection of CRISPR-Cas9 and donor plasmids, cells started to die with apoptosis and

resulted in reduced cell survival. Fortunately, the survival rate started to rise after several days and returned to normal level again (Fig. S1). We then wondered whether these surviving cells still expressed Endo H to cleave the mannose type *N*-glycans on antibody. The antibodies expressed by the cells were analyzed by electrophoresis and the result showed that the antibody heavy chain (AbHC) expressed by these cells had faster mobility than AbHC from its parental cells (GNT1 KO) and most AbHCs were downshifted (Fig. 2A). This antibody was also subjected to transglycosylation assay and the efficiency was good (Fig. 2C). We then analyzed the glycans on this antibody by intact protein mass analysis, and the results showed that most antibodies contain only GlcNAc on each HC (Fig. 2B and Fig. S2). These results indicate that CRISPR-Cas9-mediated Endo H KI in GNT1 KO cells can serve as a platform to produce Fc-GlcNAc antibody for downstream application.

In addition to Endo H, we also tried to express endoglycosidase S2 (Endo S2) from *Streptococcus pyogenes* in the cells because this enzyme can hydrolyze both high mannose and complex type *N*-glycans to Fc-GlcNAc²⁶. Therefore, the donor vector and CRISPR-Cas9 plasmids were delivered to GNT1 KO cells and the cell survival rate was monitored. Interestingly, we did not see any significant cell death in the GNT1-KO cells after plasmid transfection (Fig. S1). Then the antibody glycans generated by these cells were examined as previously described.

approach to generate antibody with Fc-SCT glycoform was to directly edit the expression of glycosyltransferases in cells. As previously described, the antibody produced from commonly used cell lines usually contained bi-antennary complex type *N*-glycans with core-fucose and terminated mainly with galactose^{14, 18-19, 29}. Therefore, in order to produce the antibody with Fc-SCT, we decided to increase the level of galactosylation and sialylation on the antibody^{19, 29}. In addition, the core-fucosylation, which depleted ADCC¹¹, was eliminated by knocking out FUT8³⁰ (Fig. 1). Furthermore, the terminal sialylation with α 2-6 linkage, not the α 2-3 linkage, was introduced as it was found to enhance receptor binding³¹ and improve ADCC and vaccinal effect^{23-24, 32}. Although the mRNA of human α 2-3 sialyltransferases could be detected in 293T and CHO cells^{18, 33}, we did not observe any obvious sialylation on the antibody glycan from these cell lines^{18-19, 29} (Fig. 4, upper). Therefore, we were not concerned about the antibody being glycosylated by the inhibitory α 2-3 sialylation. We therefore used CRISPR-Cas9 to knock out hFUT8 and knock in hB4GalT1 and hST6Gal1 in 293T cells (Fig. 1). The antibody expressed by this engineered cell line was purified and subjected to intact protein mass analysis of the antibody and the attached glycans. We observed a significant increase of galactosylated and sialylated antibody glycoforms (Fig. 4, lower) compared to the antibody generated by WT cells (Fig. 4, up). Glycoform analysis also revealed a

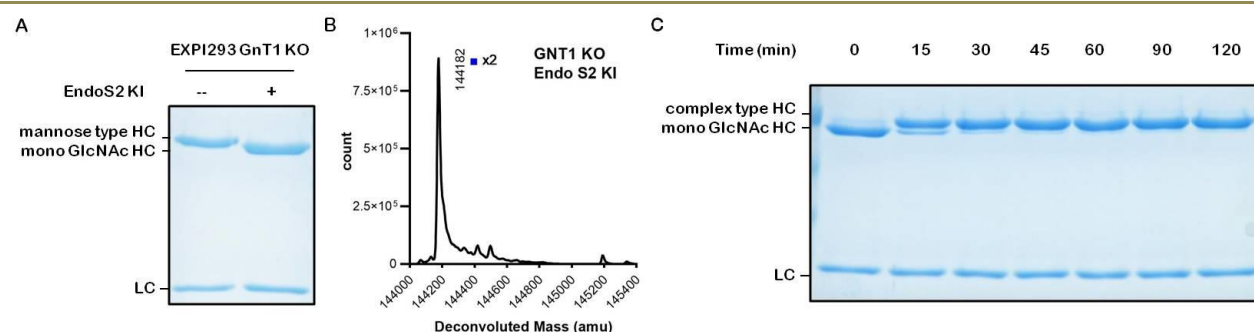


Figure 3. Expression of Endo S2 in GNT1-knock out cells results in cleavage of antibody *N*-glycans to GlcNAc. (A) The chMC18370 antibody glycoforms expressed by engineered and parental GNT1-KO EXPI293 cells were subjected to electrophoresis and a band shifting was observed. (B) The chMC81370 antibody expressed by engineered GNT1-KO EXPI293 cells was subjected to IPM analysis to examine the molecular weight and glycan changes. (C) The chMC81370 antibody expressed by engineered GNT1-KO EXPI293 cells was subjected to transglycosylation and the efficiency was evaluated by protein electrophoresis.

The protein gel showed an obvious downshifted band of AbHC compared with its parental cells (GNT1 KO) (Fig. 3A). Besides, the glycans also can be added onto AbHC through enzymatic transglycosylation (Fig. 3C) and intact protein mass analysis showed a clear signal of Fc-GlcNAc antibody (Fig. 3B.). The glycoform analysis revealed a major Fc-GlcNAc antibody population as well (Fig. S2). Therefore, based on these results, it is concluded that endoglycosidase S2 can be introduced to cells by CRISPR-Cas9 to process the *N*-glycans to Fc-GlcNAc antibody for *in vitro* transglycosylation. In addition, this cell-based method can be used for other glycoproteins, such as influenza hemagglutinin and SARS-CoV-2 spike protein to generate the mono-GlcNAc decorated glycoforms as vaccines to elicit broadly protective immune responses²⁷⁻²⁸.

Glycosylation pathway engineering in cells to produce Fc-SCT enriched antibody by CRISPR-Cas9. The second

significant increase in antibody galactosylation and sialylation, with lack of core-fucosylation (Fig. S3).

The sialylation on antibody is α 2-6 linkage. To confirm the sialylation on the antibody was mediated by hST6Gal1, the antibody was treated with two different sialidases, *Streptococcus pneumonia* α 2-3 neuraminidase and *Clostridium perfringens* neuraminidase overnight respectively. The intact protein analysis did not show any obvious difference in the distribution of asialylated and sialylated antibody between the untreated and α 2-3 neuraminidase-treated group (Fig. 5 upper and middle), indicating that the sialylation on antibody was not due to the endogenous α 2-3 sialyltransferases. On the other hand, using neuraminidase which could remove all linkages of sialylation largely eliminated the terminal sialic acids. In addition, the antibody with increased galactosylation generated from cells with knock in of hB4GalT1 only, the terminal sialylation was still

not observed (Fig. S4). These results confirmed that sialylation on the antibody was introduced by hST6Gal1 and the increase of hB4Gal1 expression indeed elevated the galactosylation of antibody (Fig. 4). To further explore the capacity of sialylation or galactosylation in cells, we transiently overexpressed hST6Gal1 or hB4GalT1 in the engineered cells followed by antibody production to see if the galactosylated or sialylated antibody was increased. However, in intact protein mass analysis, we did not observe further elevation of any galactosylated or sialylated antibody after transient overexpression of hST6Gal1 or hB4GalT1 (Fig. S5). Another study was also attempted to increase the sialylation by elevating the glycosyltransferase expression in CHO cells but it still showed incomplete sialylated glycans²⁹. The sialyltransferase hST6Gal1 is known as the only human glycosyltransferase which mediates α 2-6 sialylation and preferentially use the α 1-3 branch arm of the biantennary glycan as substrates³⁴. The *in vitro* studies show glycoengineering on antibody glycans by hST6Gal1 takes a

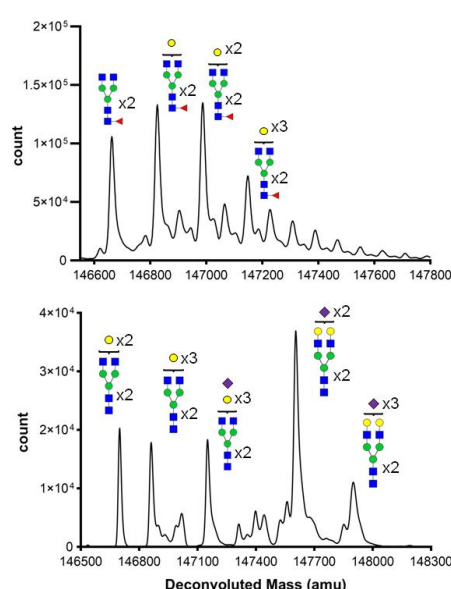


Figure 4. The glycoform composition of chMC81370 antibody. The N-glycans on antibody expressed by WT (upper) and glycosylation pathway engineered 293T cells (lower) were purified by protein A and analyzed by intact protein mass analysis.

long time and difficult to yield fully sialylated antibody glycans^{31, 34}. Furthermore, this also could explain why only the antibody with at least one fully galactosylated glycan was sialylated. If the galactose on G1 glycan was not on the α 1-3 branch arm, the hST6Gal1 may not sialylate it. Therefore, the expression level of glycosyltransferases may not be the bottle neck in this situation rather the substrate preference and enzyme activity are the major factors⁵⁻⁷. Using α 2-6 sialyltransferases from other organisms may have better activity than hST6Gal1.³⁵

Protein glycosylation varies with expression time. In order to understand if the amount and time of antibody overexpression in cells affected glycosylation, we extended the culture time to 5 days and then collected the medium at day 3 and day 5 to

examine the antibody glycans by intact protein mass analysis. We unexpectedly found that the glycan pattern was somewhat different between these two time points (Fig. S6A). In the IPM analysis, a relatively strong sialylation was observed in the first collection (first three days) as we saw before (Fig. S6A, D0-3) and then sialylation was decreased in the second collection (last two days), accompanied by an increase of terminal gal-

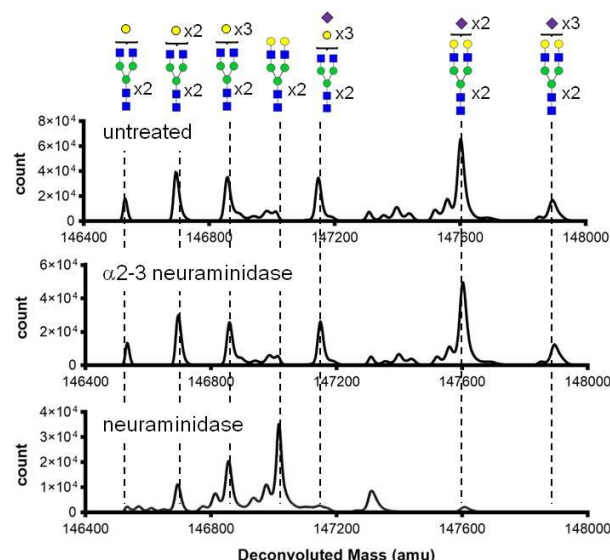


Figure 5. The sialylation on antibody is α 2-6 linkage. The SCT enriched chMC81370 antibody was expressed by glycosylation pathway engineered cells. After incubation with indicated enzyme overnight, the antibody was purified by protein A and analyzed by intact protein mass analysis.

actosylation (Fig. S6A, D4-5), suggesting that the proteins involved in glycosylation may not be sufficient to effectively produce Fc-SCT antibody at later stage. Because the hST6Gal1 and hB4GalT1 involved in the FC-SCT antibody production were almost artificially and constitutively expressed in the cells, we also examined if endogenous glycosyltransferases faced the same problem of reduced glycosylation. By expressing antibody in FUT8 KO cells, the antibody was almost terminally glycosylated with at least one or more galactoses in the first collection (Fig. S6B, D0-3), but the proportion of galactosylated antibody decreased and even the antibody glycans without any galactoses became the dominant glycoforms in the last two days ((Fig. S6B, D4-5). The result showed that short glycosylation after protein overexpression can be observed on endogenous glycosylation products as well. This finding promoted us to dissect the impact of antibody production at different times on glycosylation. We purified antibody every day after plasmid transfection and analyzed the dynamic change of antibody glycans from multiple engineered cells. To our surprise, the antibody with full sialylation could be significantly detected by intact protein mass analysis at the first day, although it became unclear after that (Fig. 6). Overall, the most abundant sialylation level was observed at first day and then started to decrease day by day (Fig. 6 and S4A). Regarding galactosylation, the antibody glycans were capped by at least two galactoses (2~4 galactoses

engineered *N*-glycans on AbHC improved binding to FcγIIB, the improvement was less significant than binding to FcγIIA (Fig. 7B). This observation was aligned with the fact that both FcγIIB and α2-6 sialylation were the key factors in intravenous Ig (IVIg) treatment-mediated inflammatory suppression³⁶⁻³⁸. Although the increased binding to the two opposing functional receptors, FcγIIA and FcγIIB, which are typically expressed on the antigen-presenting cells (APCs) simultaneously, appears to be conflicting, the eventual immune response was determined by the expression level and signaling strength of FcγIIA and FcγIIB on effector cells³. The Fc-SCT enriched antibody also exhibited stronger FcγIIIA binding ability than the antibody produced from wild type cells (Fig. 7C). This enhancement was mainly contributed by removal of core-fucose¹¹ and addition of galactoses²³.

Fc-SCT enriched antibody and homogenous Fc-SCT antibody are similar in functions. Since the binding of Fc-SCT enriched antibody to FcγIIIA was elevated, we compared the binding with the homogenous Fc-SCT antibody. The result showed that Fc-SCT enriched antibody exhibited similar binding avidity to homogenous SCT antibody (Fig. 8A). Then we further evaluated if the Fc-SCT enriched antibody could evoke similar effect as homogenous Fc-SCT antibody in ADCC reporter assay³⁹. At the lowest antibody concentration used for induction, WT antibody did not induce any obvious cell activation, but the homogenous Fc-SCT and Fc-SCT enriched antibodies can trigger cell activation and rapidly reached a maximum at next concentration (Fig. 8B). Even at the higher concentration, the WT antibody can reach a maximum induction, but the activity was still much lower than that of homogenous Fc-SCT or Fc-SCT enriched antibody (Fig 8B). The overall response induced by homogenous Fc-SCT and Fc-SCT enriched antibodies were also very similar in receptor binding assay (Fig 8B). Thus, we concluded that the Fc-SCT enriched antibody derived from glycosylation pathway engineered cells could improve antibody effector functions in a similar manner as homogenous Fc-SCT antibody did.

In summary, we have simplified the process in different ways to generate functional Fc-SCT antibodies and evaluated the utility. First, we demonstrated that with endoglycosidase S2 or H expressed in the glycosyltransferase KO cells, the glycoengineered cells can produce antibodies with Fc-GlcNAc and the antibody made by this way can be used for *in vitro* enzymatic transglycosylation (Fig. 2-4). This strategy can be applied to engineer other *N*-glycosylated proteins to produce GlcNAc-decorated glycoproteins as well (Fig. 5). Second, we knocked out the core-fucosyltransferase and constitutively expressed the glycosyltransferases required for the enrichment of Fc-SCT glycan for cell-based production of glycoproteins. The Fc-SCT enriched antibodies generated by these cells (Fig. 6) exhibited similar FcR binding profiles to homogenous Fc-SCT antibody and was able to activate similar effector functions such as ADCC through binding to FcγIIIA (Fig. 9). Thus, the cell-based production of Fc-GlcNAc and Fc-SCT enriched antibodies may have the potential advantage of simplifying the production of therapeutic antibodies with improved effector functions, especially ADCC, ADCP and vaccinal effect. Work in progress to

evaluate the comparative activities of Fc-SCT enriched antibody glycoforms and homogeneous antibody in animals

ASSOCIATED CONTENT

Supporting Information.

This supporting information is available free of charge via the Internet at <http://pubs.acs.org>, including general methods and materials, IPM analysis of antibody glycan, observation of survival rate, receptor binding of antibody.

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Author Contributions

H.-W. H. and C.-H. W. designed research; H.-W. H. performed research and analyzed data; V. S. and T.-H. T assisted trans-glycosylation assay; H.-W. H and C.-H. W wrote the paper.

Notes

The authors declare no competing financial interest

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REFERENCES

1. Lu, L. L.; Suscovich, T. J.; Fortune, S. M.; Alter, G., Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol* **2018**, *18* (1), 46-61.
2. Ben Mkaddem, S.; Benhamou, M.; Monteiro, R. C., Understanding Fc Receptor Involvement in Inflammatory Diseases: From Mechanisms to New Therapeutic Tools. *Front Immunol* **2019**, *10*, 811.
3. Boumazos, S.; Gupta, A.; Ravetch, J. V., The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* **2020**, *20* (10), 633-643.
4. Boumazos, S.; Corti, D.; Virgin, H. W.; Ravetch, J. V., Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection. *Nature* **2020**, *588* (7838), 485-490.
5. Ohtsubo, K.; Marth, J. D., Glycosylation in cellular mechanisms of health and disease. *Cell* **2006**, *126* (5), 855-67.
6. Reily, C.; Stewart, T. J.; Renfrow, M. B.; Novak, J., Glycosylation in health and disease. *Nat Rev Nephrol* **2019**, *15* (6), 346-366.
7. Li, C.; Wang, L. X., Chemoenzymatic Methods for the Synthesis of Glycoproteins. *Chem Rev* **2018**, *118* (17), 8359-8413.
8. Uebelhart, R.; Bach, M. P.; Eschbach, C.; Wossning, T.; Reth, M.; Jumaa, H., N-linked glycosylation selectively regulates autonomous precursor BCR function. *Nat Immunol* **2010**, *11* (8), 759-65.
9. Wright, J. F.; Shulman, M. J.; Isenman, D. E.; Painter, R. H., C1 binding by mouse IgM. The effect of abnormal glycosylation at position 402 resulting from a serine to asparagine exchange at residue 406 of the mu-chain. *J Biol Chem* **1990**, *265* (18), 10506-13.
10. Muraoka, S.; Shulman, M. J., Structural requirements for IgM assembly and cytolytic activity. Effects of mutations in the oligosaccharide acceptor site at Asn402. *J Immunol* **1989**, *142* (2), 695-701.

11. Shields, R. L.; Lai, J.; Keck, R.; O'Connell, L. Y.; Hong, K.; Meng, Y. G.; Weikert, S. H.; Presta, L. G., Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. *J Biol Chem* **2002**, 277 (30), 26733-40.
12. Duncan, A. R.; Winter, G., The binding site for C1q on IgG. *Nature* **1988**, 332 (6166), 738-40.
13. Kaneko, Y.; Nimmerjahn, F.; Ravetch, J. V., Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* **2006**, 313 (5787), 670-3.
14. Goh, J. B.; Ng, S. K., Impact of host cell line choice on glycan profile. *Crit Rev Biotechnol* **2018**, 38 (6), 851-867.
15. Ashwell, G.; Harford, J., Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* **1982**, 51, 531-54.
16. Ripka, J.; Adamany, A.; Stanley, P., Two Chinese hamster ovary glycosylation mutants affected in the conversion of GDP-mannose to GDP-fucose. *Arch Biochem Biophys* **1986**, 249 (2), 533-45.
17. Chandrasegaran, S.; Carroll, D., Origins of Programmable Nucleases for Genome Engineering. *J Mol Biol* **2016**, 428 (5 Pt B), 963-89.
18. Yang, Z.; Wang, S.; Halim, A.; Schulz, M. A.; Frodin, M.; Rahman, S. H.; Vester-Christensen, M. B.; Behrens, C.; Kristensen, C.; Vakhrushev, S. Y.; Bennett, E. P.; Wandall, H. H.; Clausen, H., Engineered CHO cells for production of diverse, homogeneous glycoproteins. *Nat Biotechnol* **2015**, 33 (8), 842-4.
19. Schulz, M. A.; Tian, W.; Mao, Y.; Van Coillie, J.; Sun, L.; Larsen, J. S.; Chen, Y. H.; Kristensen, C.; Vakhrushev, S. Y.; Clausen, H.; Yang, Z., Glycoengineering design options for IgG1 in CHO cells using precise gene editing. *Glycobiology* **2018**, 28 (7), 542-549.
20. Geisler, C.; Mabashi-Asazuma, H.; Jarvis, D. L., An Overview and History of Glyco-Engineering in Insect Expression Systems. *Methods Mol Biol* **2015**, 1321, 131-52.
21. Liu, C. P.; Tsai, T. I.; Cheng, T.; Shivatare, V. S.; Wu, C. Y.; Wong, C. H., Glycoengineering of antibody (Herceptin) through yeast expression and in vitro enzymatic glycosylation. *Proc Natl Acad Sci U S A* **2018**, 115 (4), 720-725.
22. Shivatare, S. S.; Huang, L. Y.; Zeng, Y. F.; Liao, J. Y.; You, T. H.; Wang, S. Y.; Cheng, T.; Chiu, C. W.; Chao, P.; Chen, L. T.; Tsai, T. I.; Huang, C. C.; Wu, C. Y.; Lin, N. H.; Wong, C. H., Development of glycosynthases with broad glycan specificity for the efficient glyco-remodeling of antibodies. *Chem Commun (Camb)* **2018**, 54 (48), 6161-6164.
23. Shivatare, V. S.; Chuang, P. K.; Tseng, T. H.; Zeng, Y. F.; Huang, H. W.; Veeranjanyulu, G.; Wu, H. C.; Wong, C. H., Study on antibody Fc-glycosylation for optimal effector functions. *Chem Commun (Camb)* **2023**, 59 (37), 5555-5558.
24. Lin, C. W.; Tsai, M. H.; Li, S. T.; Tsai, T. I.; Chu, K. C.; Liu, Y. C.; Lai, M. Y.; Wu, C. Y.; Tseng, Y. C.; Shivatare, S. S.; Wang, C. H.; Chao, P.; Wang, S. Y.; Shih, H. W.; Zeng, Y. F.; You, T. H.; Liao, J. Y.; Tu, Y. C.; Lin, Y. S.; Chuang, H. Y.; Chen, C. L.; Tsai, C. S.; Huang, C. C.; Lin, N. H.; Ma, C.; Wong, C. H., A common glycan structure on immunoglobulin G for enhancement of effector functions. *Proceedings of the National Academy of Sciences of the United States of America* **2015**, 112 (34), 10611-6.
25. Reeves, P. J.; Callewaert, N.; Contreras, R.; Khorana, H. G., Structure and function in rhodopsin: high-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line. *Proc Natl Acad Sci U S A* **2002**, 99 (21), 13419-24.
26. Sjogren, J.; Struwe, W. B.; Cosgrave, E. F.; Rudd, P. M.; Stervander, M.; Allhorn, M.; Hollands, A.; Nizet, V.; Collin, M., EndoS2 is a unique and conserved enzyme of serotype M49 group A Streptococcus that hydrolyses N-linked glycans on IgG and alpha1-acid glycoprotein. *Biochem J* **2013**, 455 (1), 107-18.
27. Huang, H. Y.; Liao, H. Y.; Chen, X.; Wang, S. W.; Cheng, C. W.; Shahed-Al-Mahmud, M.; Liu, Y. M.; Mohapatra, A.; Chen, T. H.; Lo, J. M.; Wu, Y. M.; Ma, H. H.; Chang, Y. H.; Tsai, H. Y.; Chou, Y. C.; Hsueh, Y. P.; Tsai, C. Y.; Huang, P. Y.; Chang, S. Y.; Chao, T. L.; Kao, H. C.; Tsai, Y. M.; Chen, Y. H.; Wu, C. Y.; Jan, J. T.; Cheng, T. R.; Lin, K. I.; Ma, C.; Wong, C. H., Vaccination with SARS-CoV-2 spike protein lacking glycan shields elicits enhanced protective responses in animal models. *Sci Transl Med* **2022**, 14 (639), eabm0899.
28. Chen, J. R.; Yu, Y. H.; Tseng, Y. C.; Chiang, W. L.; Chiang, M. F.; Ko, Y. A.; Chiu, Y. K.; Ma, H. H.; Wu, C. Y.; Jan, J. T.; Lin, K. I.; Ma, C.; Wong, C. H., Vaccination of monoglycosylated hemagglutinin induces cross-strain protection against influenza virus infections. *Proc Natl Acad Sci U S A* **2014**, 111 (7), 2476-81.
29. Nguyen, N. T. B.; Lin, J.; Tay, S. J.; Mariati, Yeo, J.; Nguyen-Khuong, T.; Yang, Y., Multiplexed engineering glycosyltransferase genes in CHO cells via targeted integration for producing antibodies with diverse complex-type N-glycans. *Sci Rep* **2021**, 11 (1), 12969.
30. Miyoshi, E.; Noda, K.; Yamaguchi, Y.; Inoue, S.; Ikeda, Y.; Wang, W.; Ko, J. H.; Uozumi, N.; Li, W.; Taniguchi, N., The alpha1-6-fucosyltransferase gene and its biological significance. *Biochimica et biophysica acta* **1999**, 1473 (1), 9-20.
31. Thomann, M.; Schlothauer, T.; Dashivets, T.; Malik, S.; Avenal, C.; Bulau, P.; Ruger, P.; Reusch, D., In vitro glycoengineering of IgG1 and its effect on Fc receptor binding and ADCC activity. *PLoS One* **2015**, 10 (8), e0134949.
32. Branstetter, E.; Duff, R. J.; Kuhns, S.; Padaki, R., Fc glycan sialylation of biotherapeutic monoclonal antibodies has limited impact on antibody-dependent cellular cytotoxicity. *FEBS Open Bio* **2021**, 11 (11), 2943-2949.
33. Qi, F.; Isaji, T.; Duan, C.; Yang, J.; Wang, Y.; Fukuda, T.; Gu, J., ST3GAL3, ST3GAL4, and ST3GAL6 differ in their regulation of biological functions via the specificities for the alpha2,3-sialylation of target proteins. *Faseb J* **2020**, 34 (1), 881-897.
34. Barb, A. W.; Brady, E. K.; Prestegard, J. H., Branch-specific sialylation of IgG-Fc glycans by ST6Gal-I. *Biochemistry* **2009**, 48 (41), 9705-7.
35. Geissner, A.; Baumann, L.; Morley, T. J.; Wong, A. K. O.; Sim, L.; Rich, J. R.; So, P. P. L.; Dullaghan, E. M.; Lessard, E.; Iqbal, U.; Moreno, M.; Wakarchuk, W. W.; Withers, S. G., 7-Fluorosialyl Glycosides Are Hydrolysis Resistant but Readily Assembled by Sialyltransferases Providing Easy Access to More Metabolically Stable Glycoproteins. *ACS Cent Sci* **2021**, 7 (2), 345-354.
36. Samuelsson, A.; Towers, T. L.; Ravetch, J. V., Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* **2001**, 291 (5503), 484-6.
37. Anthony, R. M.; Nimmerjahn, F.; Ashline, D. J.; Reinhold, V. N.; Paulson, J. C.; Ravetch, J. V., Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* **2008**, 320 (5874), 373-6.
38. Schwab, I.; Mihai, S.; Seeling, M.; Kasperkiewicz, M.; Ludwig, R. J.; Nimmerjahn, F., Broad requirement for terminal sialic acid residues and FcγRIIb for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. *Eur J Immunol* **2014**, 44 (5), 1444-53.
39. Parekh, B. S.; Berger, E.; Sibley, S.; Cahya, S.; Xiao, L.; LaCerte, M. A.; Vaillancourt, P.; Wooden, S.; Gately, D., Development and validation of an antibody-dependent cell-mediated cytotoxicity-reporter gene assay. *MAbs* **2012**, 4 (3), 310-8.

Supplementary Information

Methods and Material

Cell Culture

HEK 293T cells (ATCC) were cultured in the DMEM (Thermo Fisher) supplied with 10% FBS (Thermo Fisher) at 37°C with 5% CO₂ in the cell incubator. EXP1293F and EXP1293F GNT1 KO cells (Thermo Fisher) were cultured in the EXP1293 expression medium (Thermo Fisher) at 37°C with 8% CO₂ in the cell incubator.

Plasmid

The plasmids for CRISPR/Cas9 expression were constructed as kit manual (Thermo Fisher). Briefly, the validated sgRNA sequence targeting glycosyltransferase¹ was synthesized (Integrated DNA Technologies) and cloned into the vector for Cas9 and sgRNA expression. The synthetic codon-optimized gene insert (Integrated DNA Technologies), flanked by homologous arm of target gene at sgRNA target site, was cloned into empty vector as the donor plasmid.

Transfection

Transfection of 293T cells and EXPI293F cells were mediated by TransIT-293 (Mirus Bio) or expi293fectamine (Thermo Fisher) following the reagent manual.

Antibody production

The plasmids for antibody chMC81370 were transfected into cells followed by incubation. At the time of harvest, the culture medium was collected and then subjected to protein A sepharose beads (GE HealthCare) column to purify the antibody.

Protein gel analysis

The protein was heated at 95 °C for 5 mins in the LDS sample buffer supplied with 2-Mercaptoethanol and then subjected to gel electrophoresis with 12% SDS-PAGE. The protein on gel was stained by coomassie blue buffer (ApexBio).

Enzymatic transglycosylation assay

The antibody was incubated with SCT glycan-oxazoline and Endo S2 mutant in Tris buffer at 37 °C for the time indicated² before harvest for purification or gel electrophoresis.

Intact protein mass analysis

The samples were diluted with LCMS grade water at a concentration between 5-10uM and analyzed by 6230 TOF LC/MS with a Dual AJS ESI ion source (Agilent Technologies) with PLRP-S 1000Å 5µm column (Agilent Technologies). Solvent A was 0.1% Formic Acid in H₂O and Solvent B was 0.1% Formic Acid in ACN.

Glycosidase treatment

The proteins were incubated with alpha-2-3 neuraminidase, neuraminidase, or PNGase F (New England Biolabs) in the supplied buffer at 37 °C overnight followed by purification or gel electrophoresis.

ELISA binding assay

The recombinant soluble FcγRIIIA (R&D) was coated with 50 ng/well in bicarbonate/carbonate coating buffer (50 mM, pH. 10) at 4°C overnight before blocking the well by 5% BSA in TPBS (0.05% Tween 20 in PBS) at 4°C overnight. The antibody was added into wells with final concentration started at 100 µg/ml with 5x series dilution and incubated at room temperature for 1 hour followed by incubation with HRP-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch) for another 1 hour at room temperature. Finally, the TMB substrate (Bethyl Laboratories) was added to react with HRP at RT prior to stopping the reaction by H₂SO₄. The absorbance at 450 nm was detected by SpectraMax M5 spectrum reader (Molecular Device). The wells were washed by TPBS 3-5 times between each step.

ADCC reporter assay

ADCC reporter assay (Promega) was performed as kit manual. Briefly, the SSEA4 expressing target cells SK-OV3³ was seeded into 96-well plate followed by addition of antibody with final concentration started at 1 g/ml with 5x series dilution. Then the FcγRIIIA expressing effector cells (effector: target cell ratio, 6:1) was added and incubated with target cells in the incubator at 37 °C for 6 hrs. The plate was placed at room temperature for 15 min prior to adding the luciferase substrate. After 5 min incubation, the luminescence was measured by SpectraMax M5 spectrum reader. The induction fold was calculated by RLU (induced-background) /RLU (no antibody control-background).

Glycoform analysis

The FASP (filter-aided sample preparation) method⁴ was used for in-solution protease digestion. Briefly, 5 µg of protein samples were loaded onto filter units (Microcon YM-30) and mixed with 100 µl of 8 M urea in 0.1 M Tris-HCl pH 8.5 (UB) each for protein denaturation. The filter units were centrifuged with 14,000 x g for ~30 min until the solution was removed, followed by the addition of 100 µl of 25 mM DTT in UB each for protein reduction, and incubated at room temperature for 10 min. After centrifugation with 14,000 x g for ~30 min to remove the DTT solution, 100 µl of 50 mM iodoacetamide in UB was added to each sample for alkylation, and the filter units were incubated in the dark for 10 min, followed by centrifugation to remove iodoacetamide solution. To each filter unit, 100 µl of UB was added again. After another centrifugation to remove the solution, 100 µl of 50 mM ammonium bicarbonate in water (ABC) was added to change the buffer system to ABC for in-solution protease digestion. After centrifugation to remove the solution, 40 µl of ABC with 0.1 µg Trypsin/Lys-C Mix (Promega, sequencing grade) was added to each sample and incubated in a wet chamber at 37°C overnight. The digested peptides in samples were spun to collection tubes, and an additional rinse with 40 µl ABC for each sample was performed. The filter units were centrifuged at 14,000 x g for 10 min to collect the flow-through, which contains digested peptides. Samples were acidified with FA to a final concentration of 0.1%. The peptide samples were dried in a SpeedVac evaporator, and stored at -20°C until analysis with LC-MS/MS.

Samples were analyzed using the LC-ESI-MS method on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an EASY-nLC 1200 system (Thermo, San Jose, CA, US) and an EASY-spray source (Thermo, San Jose, CA, US). A 5 μ l digestion solution was injected at a flow rate of 1 μ l/min onto an easy column (C18, 0.075 mm x 150 mm, ID 3 μ m; Thermo Scientific). Chromatographic separation utilized 0.1% formic acid in water as mobile phase A and 0.1% formic acid in 80% acetonitrile as mobile phase B, operated at a flow rate of 300 nl/min. The gradient employed was from 5% buffer B at 2 min to 60% buffer B at 55 min. For full-scan MS, the conditions included a mass range of m/z 375-1800 (AGC target 5E5) with a lock mass, resolution set at 60,000 at m/z 200, and a maximum injection time of 50 ms. MS/MS was conducted in top speed mode with 3 s cycles using both CID and HCD, with a dynamic exclusion duration of 60 s and a 10 ppm tolerance around the selected precursor and its isotopes. The electrospray voltage was maintained at 1.8 kV, and the capillary temperature was set to 275°C.

Byonic software version 4.2.10 (Protein Metric) was used for the identification of summary formulas of glycans associated with glycopeptides. The glycan database contained 132 entries, and the parameters used were: precursor mass tolerance of 10 ppm, fragment mass tolerance of 0.5 Da, maximum missed cleavages set to 5, and cysteine carbamidomethylation and methionine oxidation considered."

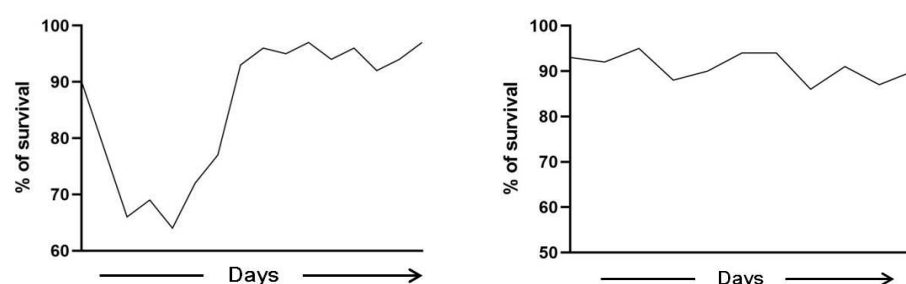


Figure S1. The survival curve of the GNT1-KO EXP1293F cells after CRISPR/Cas9 and Endo H (left) or Endo S2 (right) donor plasmid cotransfection.

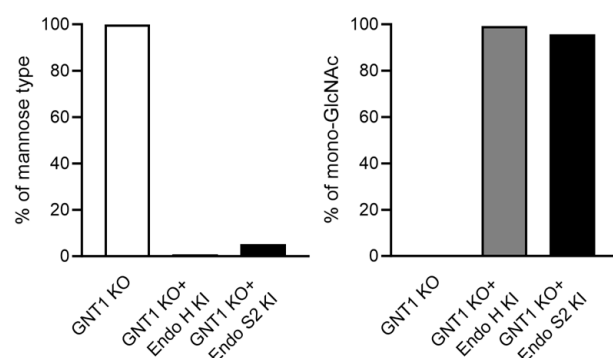


Figure S2. The glycoform analysis of chMC81370 antibody expressed by GNT1 KO and engineered GNT1 KO EXP1293 cells.

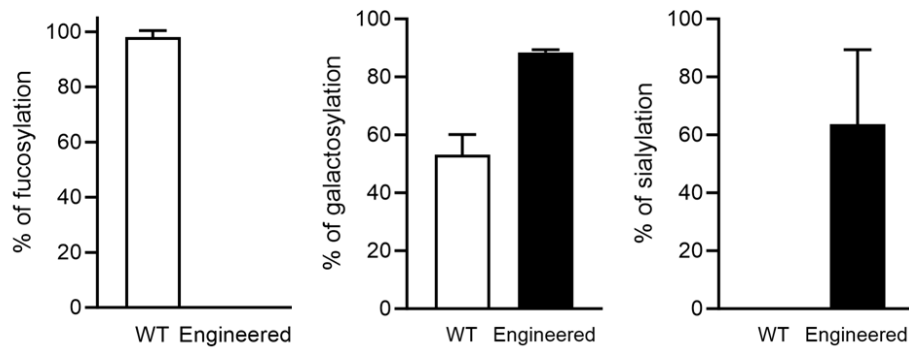


Figure S3. The glycoform analysis of chMC81370 antibody expressed by WT or engineered 293T cells.

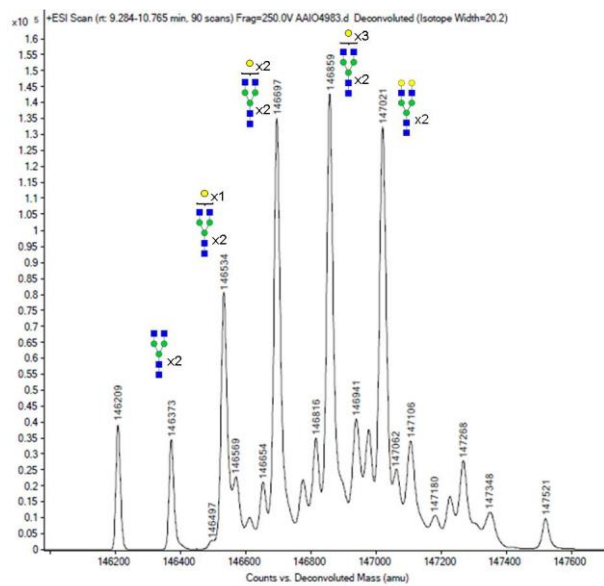


Figure S4. The IPM analysis of chMC81370 antibody expressed by FUT 8KO/B4GalT1 KI 293T cells

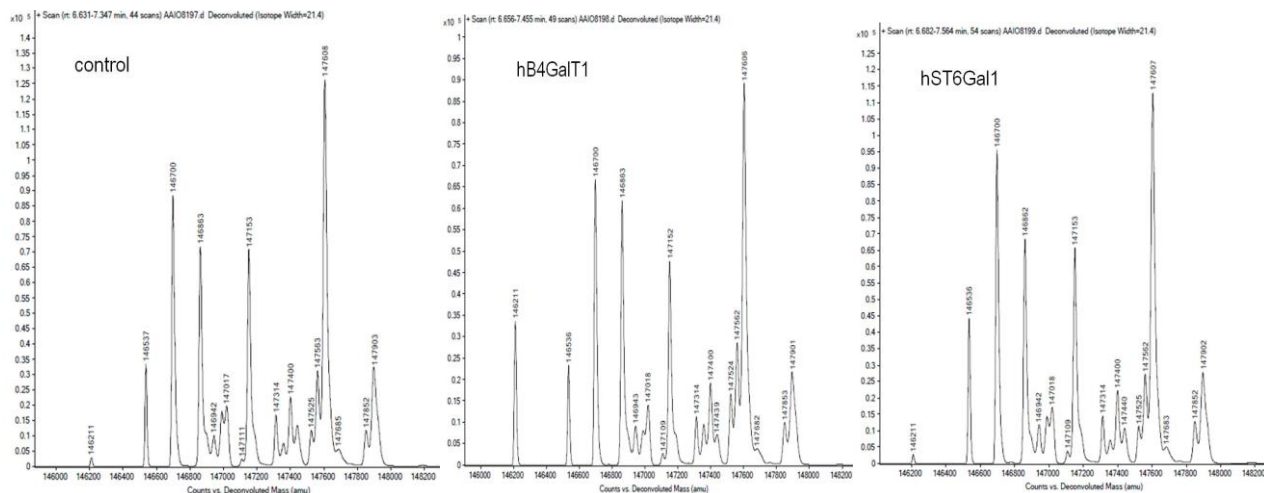


Figure S5. The IPM analysis of chMC81370 antibody expressed by untreated, hB4Gal1 transiently overexpressed, and hST6GalT1 transiently overexpressed, engineered 293T cells.

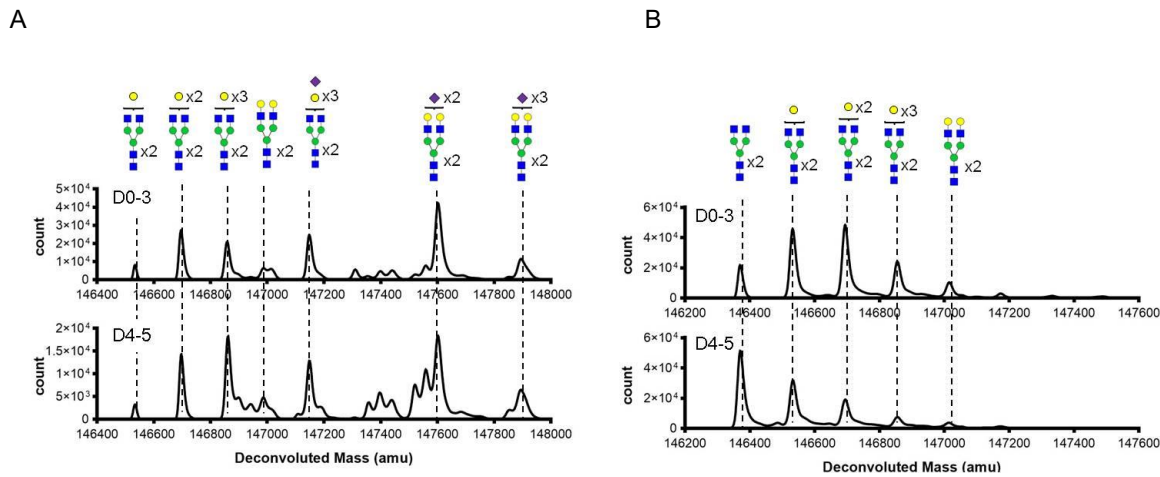


Figure S6. The IPM analysis of chMC81370 antibody expressed by multiple engineered (A) and FUT8 KO (B) cells at different collection time point.

Reference

1. Narimatsu, Y.; Joshi, H. J.; Yang, Z.; Gomes, C.; Chen, Y. H.; Lorenzetti, F. C.; Furukawa, S.; Schjoldager, K. T.; Hansen, L.; Clausen, H.; Bennett, E. P.; Wandall, H. H., A validated gRNA library for CRISPR/Cas9 targeting of the human glycosyltransferase genome. *Glycobiology* **2018**, *28* (5), 295-305.
2. Shivatare, V. S.; Chuang, P. K.; Tseng, T. H.; Zeng, Y. F.; Huang, H. W.; Veeranjanyulu, G.; Wu, H. C.; Wong, C. H., Study on antibody Fc-glycosylation for optimal effector functions. *Chem Commun (Camb)* **2023**, *59* (37), 5555-5558.
3. Lou, Y. W.; Wang, P. Y.; Yeh, S. C.; Chuang, P. K.; Li, S. T.; Wu, C. Y.; Khoo, K. H.; Hsiao, M.; Hsu, T. L.; Wong, C. H., Stage-specific embryonic antigen-4 as a potential therapeutic target in glioblastoma multiforme and other cancers. *Proc Natl Acad Sci U S A* **2014**, *111* (7), 2482-7.
4. Wisniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M., Universal sample preparation method for proteome analysis. *Nat Methods* **2009**, *6* (5), 359-62.