



(51) International Patent Classification:

A61K 38/48 (2006.01) A61P 31/14 (2006.01)
A61K 39/00 (2006.01)

(21) International Application Number:

PCT/US2023/082361

(22) International Filing Date:

04 December 2023 (04.12.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/386,075 05 December 2022 (05.12.2022) US
63/483,911 08 February 2023 (08.02.2023) US

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM,

(54) Title: SOLUBLE ANGIOTENSIN-CONVERTING ENZYME 2 (ACE2) PROTEINS AND ACE2 FUSION PROTEINS

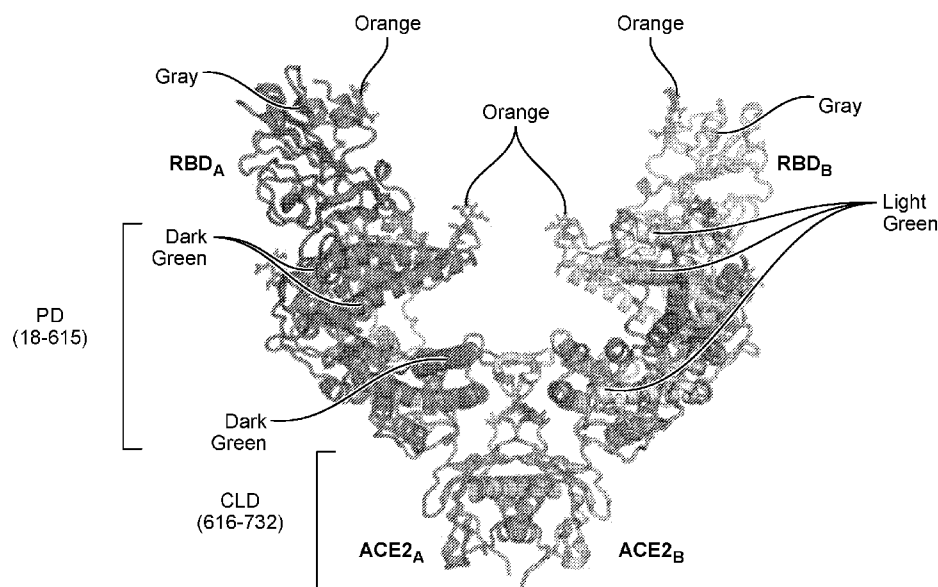


FIG. 5A

(57) Abstract: This disclosure relates generally to human angiotensin-converting enzyme 2 (ACE2) polypeptides and ACE2 fusion proteins that exhibit enhanced activity and/or pharmacokinetics, that can be used as therapeutic agents for the prophylaxis (pre- or post-exposure prophylaxis), or treatment of COVID-19, or a disease caused by any coronavirus that utilizes ACE2 as a cellular receptor.

DO, DZ, 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, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, 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, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

SOLUBLE ANGIOTENSIN-CONVERTING ENZYME 2 (ACE2) PROTEINS AND ACE2 FUSION PROTEINS

CROSS-REFERENCE

5 **[0001]** This application claims the benefit of U.S. Provisional Application No. 63/386,075, filed on December 5, 2022, and U.S. Provisional Application No. 63/483,911, filed on Feb 8, 2023, each of which are incorporated herein by reference in their entireties.

GOVERNMENT SUPPORT

10 **[0002]** This invention was made with government support under 6R43AI162329-02 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

15 **[0003]** This disclosure concerns modified angiotensin-converting enzyme 2 (ACE2) proteins and fusion proteins comprising the same having enhanced activity and pharmacokinetics as compared to wild-type ACE2.

BACKGROUND

20 **[0004]** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible respiratory virus that infects a broad range of cell types, but especially pneumocytes and mucosal epithelia of the respiratory tract. The virus' broad tropism is driven by host cell expression of its entry receptor, angiotensin converting enzyme 2 (ACE2) (*i.e.* the ACE2 receptor), as well as other accessory factors that facilitate the entry process. ACE2 is a dimeric protease at the plasma membrane that catalyzes the turnover of
25 vasoconstrictive and pro-inflammatory hormones. Trimeric Spikes (S) on the viral surface bind with moderate affinity to ACE2 via a receptor-binding domain (RBD), triggering conformational changes in S that facilitate fusion of the viral envelope and host cell membrane, releasing the viral genome into the infected host cell.

30 **[0005]** Antibodies targeting the RBD may block ACE2 interactions or block the necessary conformational changes associated with membrane fusion, thereby neutralizing the SARS-CoV-2 virus. Monoclonal anti-RBD antibodies have been developed as highly effective drugs and prophylactics for Coronavirus Disease 2019 (COVID-19), yet their efficacy against newly emerging SARS-CoV-2 variants of concern has waned. This was especially evident with sublineages of the omicron variant that were first reported in late 2021 in southern

Africa and within weeks had spread globally to become the dominant circulating variants, with many omicron variants now in co-circulation in what is colloquially called a “variant soup.” S proteins of omicron variants differ from original Wuhan isolates by approximately 30 mutations, with approximately 15 mutations in the RBD alone.

5 [0006] An alternative to monoclonal antibodies is to use the extracellular domains of the ACE2 receptor as a soluble decoy. Soluble decoy receptors have been explored as inhibitors of virus infection due to their perceived breadth against virus variants.

[0007] Provided herein are engineered, soluble ACE2 variant proteins and soluble ACE2 variant proteins fused to an immunoglobulin Fc polypeptide having one or more than one
10 amino acid substitution that elevates the protein’s *in vivo* catalytic activity and improves its pharmacokinetic (PK) properties as compared to wild-type ACE2.

SUMMARY OF THE DISCLOSURE

[0008] The present disclosure relates to human angiotensin-converting enzyme 2 (ACE2)
15 polypeptides and ACE2 fusion proteins that exhibit enhanced activity and/or pharmacokinetics, that can be used as therapeutic agents for the prophylaxis (pre- or post-exposure prophylaxis), or treatment of COVID-19, or a disease caused by any coronavirus that utilizes ACE2 as a cellular receptor.

[0009] In certain aspects, the present disclosure provides a protein including a modified
20 ACE2 polypeptide incorporating at least one substitution relative to wild-type human ACE2 of SEQ ID NO:1. In certain aspects, the at least one substitution introduces at least one glycosylation site not present in wild-type human ACE2.

[0010] In certain aspects, the present disclosure provides a protein including a modified ACE2 polypeptide incorporating a substitution or combination of substitutions relative to
25 wild-type human ACE2 of SEQ ID NO:1 selected from: (a) a substitution of valine at position 491 to isoleucine; a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine; (b) a substitution of isoleucine at position 663 to tryptophan, and a substitution of alanine at position 673 to tyrosine; (c) a substitution of alanine at position 673 to tyrosine, and a substitution of
30 isoleucine at position 694 to phenylalanine; (d) a substitution of valine at position 491 to isoleucine, and a substitution of isoleucine at position 679 to tyrosine; (e) a substitution of asparagine at position 682 to tryptophan, and a substitution of alanine at position 687 to phenylalanine; (f) a substitution of serine at position 167 to isoleucine, a substitution of alanine at position 246 to leucine, and a substitution of isoleucine at position 694 to

phenylalanine; (g) a substitution of valine at position 491 to isoleucine, a substitution of alanine at position 673 to tyrosine, a substitution of alanine at position 687 to phenylalanine, and a substitution of threonine at position 698 to glutamic acid; (h) a substitution of serine at position 167 to isoleucine, a substitution of valine at position 491 to isoleucine, a substitution of histidine at position 535 to phenylalanine; and a substitution of alanine at position 673 to valine; (i) a substitution of lysine at position 288 to cysteine, and a substitution of glutamic acid at position 433 to cysteine; (j) a substitution of leucine at position 624 to cysteine, and a substitution of alanine at position 632 to cysteine; (k) a substitution of glutamic acid at position 166 to cysteine, and a substitution of valine at position 691 to cysteine; (l) a substitution of methionine at position 662 to serine or threonine; (m) a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine, and a substitution of methionine at position 662 to serine or threonine; (n) a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine, a substitution of asparagine at position 580 to serine or threonine, and a substitution of methionine at position 662 to serine or threonine; (o) a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine; (p) a substitution of aspartic acid at position 615 to asparagine, a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine; (q) a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine; a substitution of methionine at position 662 to serine or threonine, and a substitution of glutamine at position 728 to asparagine; (r) a substitution of lysine at position 631 to asparagine, a substitution of tyrosine at position 633 to serine or threonine; a substitution of methionine at position 662 to serine or threonine, and a substitution of glutamine at position 728 to asparagine; and (s) a substitution of methionine at position 662 to serine or threonine, and a substitution of isoleucine at position 694 to phenylalanine. In certain aspects, the protein further incorporates a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, and a substitution of asparagine at position 330 to tyrosine. In certain aspects, the modified ACE2 polypeptide incorporates a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, a substitution of asparagine at position 330 to tyrosine, a substitution of valine at position 491 to isoleucine; a substitution of methionine at position 662 to threonine; and a substitution of asparagine at position 720 to serine.

[0011] In certain aspects, the modified ACE2 polypeptide has a sequence having at least 85%

sequence identity to SEQ ID NO:12. In certain aspects, the modified ACE2 polypeptide has a sequence having 100% sequence identity to SEQ ID NO:12.

[0012] In certain aspects, the modified ACE2 polypeptide comprises a signal peptide fused to the N-terminus of the modified ACE2 polypeptide. In certain aspects, the signal peptide has a sequence having at least 85% sequence identity to SEQ ID NO:11.

[0013] In certain aspects, the modified ACE2 polypeptide is fused to an immunoglobulin Fc domain polypeptide, or functional fragment thereof. In certain aspects, the immunoglobulin Fc domain polypeptide is a human IgG1, IgG2, IgG3, or IgG4 Fc domain polypeptide, or functional fragment thereof. In certain aspects, the immunoglobulin Fc domain, or functional fragment thereof, includes residues 221 to 447 of human IgG1 Fc, where positions are numbered according to EU numbering. In certain aspects, the human IgG1 Fc domain, or functional fragment thereof, incorporates one or more than one substitution relative to wild-type human IgG1 Fc domain of SEQ ID NO:4 selected from: (a) a substitution of methionine at position 252 to tyrosine; (b) a substitution of serine at position 254 to threonine; and (c) a substitution of threonine at position 256 to glutamic acid, where positions are numbered according to the EU numbering. In certain aspects, the immunoglobulin Fc domain polypeptide, or functional fragment thereof, is fused to the C-terminus of the modified ACE2 polypeptide. In certain aspects, the immunoglobulin Fc domain polypeptide, or functional fragment thereof, is fused to the C-terminus of the modified ACE2 polypeptide via a linker.

In certain aspects, the linker is a single serine residue.

[0014] In certain aspects, the present disclosure provides a protein including: (a) a modified ACE2 polypeptide incorporating a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, a substitution of asparagine at position 330 to tyrosine, a substitution of valine at position 491 to isoleucine, a substitution of methionine at position 662 to threonine, and a substitution of asparagine at position 720 to serine; and (b) an immunoglobulin Fc domain incorporating a substitution of methionine at position 252 to tyrosine, a substitution of serine at position 254 to threonine, and a substitution of threonine at position 256 to glutamic acid, where the positions are numbered according to the EU numbering. In certain aspects, the protein has a sequence having at least 85% or 100% sequence identity to SEQ ID NO:10.

[0015] In certain aspects, a protein of the present disclosure has increased levels of glycosylation as compared to wild-type ACE2.

[0016] In certain aspects, a protein of the present disclosure has two to fifty-fold increased affinity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein

as compared to wild-type ACE2 as measured by biolayer interferometry (BLI).

[0017] In certain aspects, a protein of the present disclosure has 1.2 to 2-fold increased catalytic activity as compared to wild-type ACE2 as measured in an *in vitro* fluorometric ACE2 activity assay.

5 [0018] In certain aspects, at least 20% by moles of the glycosylations of a protein of the present disclosure are sialylated.

[0019] In certain aspects, a protein of the present disclosure has a two to ten-fold decreased dissociation rate with FcRn at endosomal pH as compared to a protein comprising wild-type ACE2 fused to a wild-type human IgG1 Fc domain. In certain aspects, a protein of the
10 present disclosure has an equilibrium dissociation constant for FcRn at pH 6.0 of 10 nM to 50 nM as measured by BLI. In certain aspects, a protein of the present disclosure has an off rate for FcRn at pH 6.0 of $5.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$ as measured by BLI. In certain aspects, a protein of the present disclosure has a higher binding affinity to FcRn at pH 6.0 than at pH 7.0 as measured by BLI.

15 [0020] In certain aspects, the plasma half-life of a protein of the present disclosure is 40 to 240 hours when administered to a subject.

[0021] In certain aspects, a protein of the present disclosure has a plasma activity area under the curve over 48 hours ($\text{AUC}_{0-48\text{h}}$) of 50 to 150 ($\mu\text{M product/minute}$) $\times$ h when intravenously administered to a subject at a dose of 2 mg/kg. In certain aspects, a protein of the present
20 disclosure has an area under the curve over 120 hours ($\text{AUC}_{0-120\text{h}}$) of 100 to 9000 $\mu\text{g/ml} \times$ h when intravenously administered to a subject at a dose of 10 mg/kg.

[0022] In certain aspects, a protein of the present disclosure maintains a plasma concentration above 18 pM at 100 to 150 hours post administration to a subject.

[0023] In certain aspects, a protein of the present disclosure is nonimmunogenic when
25 administered to a subject.

[0024] In certain aspects, a protein of the present disclosure forms a stable homodimer.

[0025] In certain aspects, the present disclosure also provides a pharmaceutical formulation including a protein as described herein and a pharmaceutically acceptable carrier. The present disclosure additionally provides a nucleic acid vector encoding a protein as described
30 herein.

[0026] In certain aspects, the present disclosure provides a method of producing a protein as described herein where a vector encoding the protein is expressed in a cell line and at least 10% by moles of the expressed protein is glycosylated at each N-glycosylation motif. In some embodiments, glycosylation is measured by mass spectrometry. In certain

embodiments, the expressed protein is glycosylated with N-acetylhexosamine. In certain aspects, at least 20% of the glycosylations by moles are sialylated. In certain aspects, the cell line for expressing a protein of the present disclosure is Expi293F cells. In certain aspects, the cell line for expressing a protein of the present disclosure is a stable CHO line. In some
5 embodiments, the percentage is a mole percentage.

[0027] In certain aspects, the present disclosure provides a method of inhibiting SARS-CoV-2 replication including administering to a subject a therapeutically or prophylactically effective amount of a protein or pharmaceutical formulation as disclosed herein. In certain aspects, the protein or pharmaceutical formulation is administered to the subject
10 intravenously, subcutaneously, intratracheally, or by inhalation. In certain aspects, the SARS-CoV-2 variant is Wuhan, alpha, beta, gamma, delta, or omicron.

[0028] The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

15 [0029] Other embodiments and details of the disclosure are presented herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1A is a line graph showing catalytic activity in serum of soluble angiotensin-converting enzyme 2 v2.4-IgG1 fusion protein (sACE2₂.v2.4-IgG1) expressed and purified
20 from human Expi293F cell culture (broken line) or non-human ExpiCHO-S (solid line) cell culture and injected via tail vein at 10 mg/kg into human FcRn transgenic mice.

[0031] FIG. 1B is a line graph showing serum sACE2₂.v2.4-IgG1 protein concentrations in human FcRn transgenic mice injected via tail vein with 10 mg/kg sACE2₂.v2.4-IgG1. Data are mean ± SEM, n=3 mice per time point.

25 [0032] FIG. 2A and FIG. 2B are annotated MALDI-TOF-MS spectra (950-2500 m/z and 2500-5000 m/z, respectively) of N-glycans released from sACE2₂.v2.4-IgG1 expressed from ExpiCHO-S cells following 20-hour incubation with PNGase F at 37 °C, and permethylation. Peak intensities are representative of relative N-glycan abundance. N-glycan structures were assigned based on precursor masses and the common mammalian biosynthetic pathway.
30 GlcNAc, squares; Man, open circles; Gal, filled circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.

[0033] FIG. 3A and FIG. 3B are annotated MALDI-TOF-MS spectra (950-2500 m/z and 2500-5000 m/z, respectively) of N-glycans released from sACE2₂.v2.4-IgG1 expressed from Expi293F cells following 20-hour incubation with PNGase F at 37 °C, and permethylation.

Peak intensities are representative of relative N-glycan abundance. N-glycan structures were assigned based on precursor masses and the common mammalian biosynthetic pathway. GlcNAc, squares; Man, open circles; Gal, filled circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.

5 [0034] FIG. 4A and FIG. 4B are pie graphs showing the relative abundance of N-glycan types released from sACE2_{2.v2.4}-IgG1 expressed from ExpiCHO-S cells and Expi293F cells, respectively, following 48-hour incubation with PNGase F at 37 °C, permethylation, and analysis by MALDI-TOF-MS.

[0035] FIG. 4C is a bar graph showing the relative abundance of sialylated (Neu5Ac) and fucosylated N-glycan structures released from sACE2_{2.v2.4}-IgG1 expressed from ExpiCHO-S cells and Expi293F cells, respectively, following 48-hour incubation with PNGase F at 37°C, permethylation, and analysis by MALDI-TOF-MS.

[0036] FIG. 4D and FIG. 4E are bar graphs showing the relative abundance of O-glycans released from sACE2_{2.v2.4}-IgG1 expressed from ExpiCHO-S cells and Expi293F cells, respectively, following 18-hour incubation with sodium hydroxide and sodium borohydride at 45 °C, permethylation, and analysis by MALDI-TOF-MS. O-glycan structures were assigned based on precursor masses and the common mammalian biosynthetic pathway. Glycan structures are indicated on the x-axis by their m/z ratio. GlcNAc, filled squares; GalNAc, open squares; Gal, circles; Neu5Ac, diamonds.

20 [0037] FIG. 5A is a ribbon diagram of the structure of dimeric ACE2 (PDB 6M17) bound to the receptor-binding domain (RBD) of spike protein (S) of SARS-CoV-2 virus. ACE2 Chain 'A', dark green; ACE2 Chain 'B', light green; RBD, gray; glycans, orange; PD, protease domain; CLD, collectrin-like dimerization domain.

[0038] FIG. 5B, FIG. 5C, and FIG. 5D are ribbon diagrams of the structure of a single ACE2 subunit having residues substituted to fill cavities (blue spheres), introduce disulfide bonds (yellow spheres), or add N-glycosylation motifs (purple spheres), respectively.

[0039] FIG. 6A is a line graph showing the catalytic activity of purified sACE2_{2.v2.4}-IgG1 and derivatives (0.5 nM) containing cavity filling mutations or mutations introducing disulfide bonds as measured by an *in vitro* fluorometric assay.

30 [0040] FIG. 6B is a line graph showing the catalytic activity of purified sACE2_{2.v2.4}-IgG1 derivatives (0.5 nM) containing mutations introducing consensus glycosylation motifs as measured by an *in vitro* fluorometric assay.

[0041] FIG. 6C is a line graph showing the catalytic activity of purified sACE2_{2.v2.4}-IgG1 derivatives (0.5 nM) having M662T and N720S mutations as measured by an *in vitro*

fluorometric assay. Parental sACE2_{2.v2.4}-IgG1 in the presence (broken) or absence (solid) of ACE2 inhibitor is shown in black.

[0042] FIG. 7A is a line graph showing the catalytic activity of sACE2_{2.v2.4}-IgG1 and derivatives having cavity-filling mutations or disulfides in retroorbital blood draw samples from human FcRn mice injected with 2 mg/kg of protein via tail vein injection.

[0043] FIG. 7B is a line graph showing the catalytic activity of sACE2_{2.v2.4}-IgG1 and derivatives having introduced consensus glycosylation motifs in retroorbital blood draw samples from human FcRn mice injected with 2 mg/kg of protein via tail vein injection.

[0044] FIG. 7C is a line graph showing the catalytic activity of sACE2_{2.v2.4}-IgG1 and derivatives having M662T and N720S mutations in retroorbital blood draw samples from human FcRn mice injected with 2 mg/kg of protein via tail vein injection.

[0045] FIG. 7D is an image of a non-reducing polyacrylamide gel electrophoresis and anti-human IgG1 immunoblot of serum (1 µl each) sampled from a human FcRn mouse administered via tail vein injection with 2 mg/kg sACE2_{2.S14}-IgG1.

[0046] FIG. 8A and FIG. 8B are annotated MALDI-TOF-MS spectra (950-2500 m/z and 2500-5000 m/z, respectively) of N-glycans released from sACE2_{2.S19}-IgG1 expressed from Expi293F cells following 20-hour incubation with PNGase F at 37°C, and permethylation. Peak intensities are representative of relative N-glycan abundance. N-glycan structures were assigned based on precursor masses and the common mammalian biosynthetic pathway.

GlcNAc, squares; Man, open circles; Gal, filled circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.

[0047] FIG. 9A is a pie graph showing the relative abundance of N-glycan types released from sACE2_{2.S19}-IgG1 expressed from Expi293F cells, following 48-hour incubation with PNGase F at 37°C, permethylation, and analysis by MALDI-TOF-MS.

[0048] FIG. 9B is a bar graph showing the relative abundance of sialylated (Neu5Ac) and fucosylated N-glycan structures released from sACE2_{2.S19}-IgG1 expressed from Expi293F cells following 48-hour incubation with PNGase F at 37°C, permethylation, and analysis by MALDI-TOF-MS.

[0049] FIG. 9C is a bar graph showing the relative abundance of O-glycans released from sACE2_{2.S19}-IgG1 expressed from Expi293F cells following 18-hour incubation with sodium hydroxide and sodium borohydride at 45 °C, permethylation, and analysis by MALDI-TOF-MS. O-glycan structures were assigned based on precursor masses and the common mammalian biosynthetic pathway. Glycan structures are indicated on the x-axis by their m/z ratio. GlcNAc, filled squares; GalNAc, open squares; Gal, circles; Neu5Ac, diamonds.

[0050] **FIG. 10A** is a bar graph showing the percent occupancy of N-glycosylation sites in sACE2₂.S19-IgG1 expressed in Expi293F (white) cells, sACE2₂.v2.4-IgG1 expressed in Expi293F (dense diagonal hatching) cells, and sACE2₂.v2.4-IgG1 expressed in ExpiCHO-S (sparse diagonal hatching) cells.

5 [0051] **FIG. 10B** is a bar graph showing the percentage of glycoforms at each N-glycosylation site having at least one sialic acid in sACE2₂.S19-IgG1 expressed in Expi293F (white) cells, sACE2₂.v2.4-IgG1 expressed in Expi293F (dense diagonal hatching) cells, and sACE2₂.v2.4-IgG1 expressed in ExpiCHO-S (sparse diagonal hatching) cells.

[0052] **FIGs. 11A-11D** are sensogram plots of sACE2₂.v2.4-IgG1 (**FIG. 11A**), sACE2₂.v2.4-IgG1 (YTE) (**FIG. 11B**), sACE2₂.S19-IgG1 (**FIG. 11C**), and sACE2₂.S19-IgG1 (YTE) (**FIG. 11D**) binding to FcRn immobilized on biolayer interferometry (BLI) biosensors equilibrated at pH 6.0.

[0053] **FIGs. 11E-11H** are sensogram plots of sACE2₂.v2.4-IgG1 (**FIG. 11E**), sACE2₂.v2.4-IgG1 (YTE) (**FIG. 11F**), sACE2₂.S19-IgG1 (**FIG. 11G**), and sACE2₂.S19-IgG1 (YTE) (**FIG. 11H**) binding to FcRn immobilized on biolayer interferometry (BLI) biosensors equilibrated at pH 7.4.

[0054] **FIG. 12A** is a line graph showing the serum concentrations of sACE2₂.v2.4-IgG1, sACE2₂.S19-IgG1, and sACE2₂.S19-IgG1 (YTE) over 48 hours in retroorbital blood samples collected from human FcRn mice following 10 mg/kg administration into the tail vein.

20 [0055] **FIG. 12B** is a line graph showing the catalytic activity of sACE2₂.v2.4-IgG1 (10 mg/kg), sACE2₂.S19-IgG1 (10 mg/kg), sACE2₂.S19-IgG1 (100 mg/kg), and sACE2₂.S19-IgG1 (YTE) (10 mg/kg) over 120 hours in retroorbital blood samples collected from human FcRn mice following subcutaneous administration into the flank.

[0056] **FIG. 12C** is a line graph showing the serum concentrations of sACE2₂.v2.4-IgG1 (10 mg/kg), sACE2₂.S19-IgG1 (10 mg/kg), sACE2₂.S19-IgG1 (100 mg/kg), and sACE2₂.S19-IgG1 (YTE) (10 mg/kg) over 120 hours in retroorbital blood samples collected from human FcRn mice following subcutaneous administration into the flank.

25 [0057] **FIG. 13A** is an image of a Coomassie-stained, non-reducing, SDS-polyacrylamide gel electrophoresis of 20 µg of sACE2₂.S19-IgG1 (YTE) treated with PNGase F or neuraminidase.

[0058] **FIG. 13B** is an image of a Coomassie-stained, isoelectric focusing gel of 10 µg of sACE2₂.S19-IgG1 (YTE) treated with PNGase F or neuraminidase (NA) in the presence (+) or absence (-) of a gel filtration purification step.

[0059] **FIG. 13C** is a line graph showing the serum concentrations of untreated sACE2₂.v2.4-

IgG1 (YTE), untreated sACE2₂.S19-IgG1 (YTE), PNGase F treated sACE2₂.S19-IgG1 (YTE), and NA treated sACE2₂.S19-IgG1 (YTE) across 120 hours in retroorbital blood samples collected from human FcRn mice following 10 mg/kg administration into the tail vein.

- 5 **[0060] FIG. 14A** is a gel image of Capillary Electrophoresis-SDS analysis of sACE2₂.v2.4-IgG1 (YTE) and sACE2₂.S19-IgG1 (YTE) purified from CHOK1SV GS-KO stable pools (left lanes) versus transiently transfected Expi293F (center-right lanes). Samples were analyzed under reducing (R) and non-reducing (NR) conditions, with accurate separation between the markers for 4.5 kD and 240 kD. IgG1 control is shown in the far right lanes.
- 10 **[0061] FIGs. 14B-14E** show traces from capillary Isoelectric Focusing analysis of sACE2₂.v2.4-IgG1(YTE) purified from Expi293F (**FIG. 14B**), sACE2₂.S19-IgG1(YTE) purified from Expi293F (**FIG. 14C**), sACE2₂.v2.4-IgG1(YTE) purified from CHOK1SV GS-KO (**FIG. 14D**), and sACE2₂.S19-IgG1(YTE) purified from CHOK1SV GS-KO (**FIG. 14E**). Major peaks are indicated by arrowheads and estimates of pI and % of total are tabulated.
- 15 **[0062] FIGs. 15A-15D** shows response traces from BLI analysis of highly sialylated decoy receptors binding to SARS-CoV-2 Spike RBD. Association (t = 0 to 60 s) and dissociation (t > 60 s) kinetics were measured by BLI between soluble monomeric RBD and immobilized decoy receptors. RBDs from Gamma (**FIGs. 15A and 15C**) and Delta (**FIGs. 15B and 15D**) SARS-CoV-2 variants were tested at three concentrations in duplicate. sACE2₂.v2.4-IgG1(YTE) (**FIGs. 15A and 15B**) and sACE2₂.S19-IgG1(YTE) (**FIGs. 15C and 15D**) were
- 20 purified from CHOK1SV GS-KO stable pools. Fitted curves are shown as dashed black lines.
- [0063] FIG. 16A** is a line graph showing plasma concentrations of sACE2₂.v2.4-IgG1(YTE) (solid line) and sACE2₂.S19-IgG1(YTE) (broken line) purified from stable CHOK1SV GS-KO pools after IV administration to human FcRn mice at a single dose of 10 mg/kg. Concentrations in plasma were measured by ELISA for 8 days.
- 25 **[0064] FIG. 16B** is a line graph showing plasma concentrations of sACE2₂.S19-IgG1(YTE) purified from stable CHOK1SV GS-KO pools after IV (solid line) or SC (broken line) administration to human FcRn mice at a single dose of 10 mg/kg. Concentrations in plasma were measured by ELISA for 14 days.
- 30 **[0065] FIG. 17** is an immunogenicity heat map of sACE2₂-IgG1 (WT), sACE2₂.v2.4-IgG1, sACE2₂.S19-IgG1 (YTE), 3N39v4 decoy, and 3J320v3 decoy for peptides predicted to have affinity for at least four HLA-II allotypes (Nhits ≥ 4). sACE2₂-IgG1 derivatives are shaded

gray except for regions where mutations are introduced.

[0066] FIG. 18 is a line graph showing the binding of wild type sACE2₂-IgG1 (circles), sACE2_{2.v2.4}-IgG1(YTE) (squares), or sACE2_{2.S19}-IgG1(YTE) (triangles) to cells expressing the S proteins of omicron sublineages (clockwise from top-left) BF.7, XBB, BA.2.75.2, and BQ.1.1. Bound proteins were detected by flow cytometry.

DETAILED DESCRIPTION

[0067] The present application provides modified human angiotensin-converting enzyme 2 (ACE2) proteins and fusion proteins comprising modified human ACE2 proteins having enhanced activity and pharmacokinetics as compared to wild-type ACE2. Also provided are methods of producing modified human ACE2 proteins and fusion proteins, and methods of treating SARS-CoV-2 infection in a subject using the same.

[0068] To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[0069] The terms “a” and “an” as used herein mean “one or more” and include the plural unless the context is inappropriate.

[0070] As used herein, the terms “subject” and “patient” refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably include humans.

[0071] As used herein, the term “effective amount” refers to the amount of a protein or fusion protein sufficient to effect beneficial or desired results (*e.g.*, a desired prophylactic or therapeutic effect). An effective amount can be administered in one or more administration(s), application(s) or dosage(s) and is not intended to be limited to a particular formulation or administration route. As used herein, the term “treating” includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof. As used herein, the term “therapeutically effective amount” refers to a quantity of a specific substance (such as a modified human ACE2 polypeptide) sufficient to achieve a desired effect in a subject being treated. For instance, this can be the amount necessary to inhibit CoV replication or reduce CoV titer in a subject. In one embodiment, a therapeutically effective amount is the amount necessary to inhibit CoV replication by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% (as compared to the absence of treatment). In another embodiment, a

therapeutically effective amount is the amount necessary to reduce CoV titer in a subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% (as compared to the absence of treatment). The therapeutically effective amount can also be the amount necessary to reduce or eliminate one of more

5 symptoms of CoV infection, such as the amount necessary to reduce or eliminate fever, cough or shortness of breath. Similarly, in certain aspects, a prophylactically effect amount is the amount necessary to reduce the risk of becoming infected with a CoV or developing disease, such as COVID-19, by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% (as compared to the absence of treatment).

10 **[0072]** As used herein, the term “pharmaceutical formulation” refers to the combination of an active agent (*e.g.*, protein or fusion protein) with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

[0073] As used herein, the term “pharmaceutically acceptable carrier” refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water,

15 emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, *see e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975].

[0074] The terms “protein” or “polypeptide” are used interchangeably herein and refer to a

20 polymer of repeating structural units connected by a peptide bond. Typically, the repeating structural units of the peptide are amino acids including naturally occurring amino acids, non-naturally occurring amino acids, analogues of amino acids or any combination of these. In certain aspects, proteins or polypeptides may be post-translationally modified (*e.g.*, glycosylated, phosphorylated, lapidated, acetylated, or conjugation with a labeling

25 component).

[0075] The term “heterologous” as used herein refers to a nucleic acid or polypeptide originating from a different genetic source or species.

[0076] The term “sequence identity” means the proportion of amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, *e.g.*, 85%,

30 the percentage denotes the fraction of matches over the length of sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; for example, wherein gap lengths of 5 amino acids or less, optionally 3 amino acids or less, are usually used.

[0077] Percent sequence identity can be any integer from 60% to 100%. Exemplary

embodiments include at least: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, as compared to a reference sequence using the programs described herein; preferably BLAST using standard parameters, as described below. One of skill will recognize that these values can be appropriately adjusted to

5 determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

[0078] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if
10 necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0079] Algorithms that are suitable for determining percent sequence identity and sequence
15 similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1977) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in
20 the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative
25 alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its
30 maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=1, N=-2, and a comparison of

both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

[0080] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.01, more preferably less than about 10^{-5} , and most preferably less than about 10^{-20} .

[0081] The terms “treating” or “treatment” refer to any indicia of success in the treatment or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to a subject, such as a patient in need of treatment; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a subject's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters, including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. As used herein, the terms “treating” or “treatment” include management, slowing the progression, and abating the symptoms of a disease, pathology or condition.

[0082] As defined herein, the term “inhibition,” “inhibit,” “inhibiting,” and the like in means negatively affecting (*e.g.*, decreasing) the activity, function, or number of a molecule or virus (*e.g.*, SARS-CoV-2) relative to the activity, function, or number of the molecule or virus in the absence of the inhibitor.

[0083] As used herein, the term “about” means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, “about” means within a standard deviation using measurements generally acceptable in the art. The term “about,” as used herein when referring to a measurable value such as an amount of mass, weight, time, volume, concentration, or percentage, is meant to encompass variations of in certain aspects $\pm 20\%$, in certain aspects $\pm 10\%$, in certain aspects $\pm 9\%$, in certain aspects $\pm 8\%$, in certain aspects $\pm 7\%$, in certain aspects $\pm 6\%$, in certain aspects $\pm 5\%$, in certain aspects $\pm 4\%$, in certain aspects $\pm 3\%$, in certain aspects $\pm 2\%$, in certain aspects $\pm 1\%$, in certain aspects $\pm 0.5\%$, and in certain

aspects $\pm 0.1\%$ from the specified amount, as such variations are appropriate to perform the disclosed methods and/or employ the disclosed compositions. Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently disclosed subject matter.

[0084] As used herein, the term “functional fragment thereof” refers to a portion of a protein or polypeptide that maintains the ability to perform a biological function of the whole protein or polypeptide. For example, a functional fragment of a polypeptide or protein of the present application maintains its ability to bind its cognate binding partner or ligand.

[0085] As used herein, the terms “mutation” and “substitution” are used interchangeably and refer to the alteration of an amino acid, in the context of a reference amino acid sequence, to another amino acid. An alteration of an amino acid in a reference amino acid sequence can occur at the N-terminal or C-terminal position or anywhere between those terminal positions. Substitutions may be interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0086] A substitution can be, but need not necessarily be, a conservative substitution. Conservative substitutions typically include substitutions within the following groups:

- (i) alanine and glycine;
- (ii) isoleucine, leucine, and valine;
- (iii) aspartic acid and glutamic acid;
- (iv) asparagine, glutamine, serine, and threonine;
- (v) arginine and lysine; and
- (vi) phenylalanine and tyrosine.

[0087] As used herein, the term “vector” refers to a nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art. In certain aspects, the vector is a virus vector, such as a lentivirus vector.

I. Modified ACE2 Polypeptides

[0088] The spike (S) glycoprotein of SARS-CoV-2 binds angiotensin-converting enzyme 2 (ACE2) on host cells. S is a trimeric class I viral fusion protein that is proteolytically processed into S1 and S2 subunits that remain noncovalently associated in a prefusion state. Upon engagement of ACE2 by a receptor binding domain (RBD) in S1, conformational rearrangements occur that cause S1 shedding, cleavage of S2 by host proteases, and exposure

of a fusion peptide adjacent to the S2' proteolysis site. Favorable folding of S to a post-fusion conformation is coupled to host cell/virus membrane fusion and cytosolic release of viral RNA. Atomic contacts with the RBD are restricted to the protease domain of ACE2, and soluble ACE2 (sACE2) in which the neck and transmembrane domains are removed, is sufficient for binding S and neutralizing infection. In principle, the virus has limited potential to escape sACE2-mediated neutralization without simultaneously decreasing affinity for native ACE2 receptors, thereby attenuating virulence. Furthermore, fusion of sACE2 to the Fc region of human immunoglobulin can provide an avidity boost while recruiting immune effector functions and increasing serum stability. Recombinant sACE2 has proven safe in healthy human subjects and patients with lung disease.

[0089] Modified ACE2 polypeptides of the present disclosure function to bind S and neutralize SARS-CoV2 infection. In certain aspects, modified ACE2 polypeptides of the present disclosure bind to a protein having a sequence at least 85% identical (*e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identical) to SEQ ID NO:32.

SARS-CoV2 S Spike (S) Glycoprotein

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQD
LFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFG
TTLDSKTQSLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRV
YSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINL
VRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT PGDSSSGWTAGAAAYY
VGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFR
VQPTE SIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLNSA
SFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKL
PDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGST
PCNGVEGFNCYFPLQSYGFQPTNGVGYQP YRVVVL SFELLHAPATVCGPKKS
TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTL
EILDITPCSFGGVS VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRV
YSTGSNVFQTRAGCLIGAEHVNNSECDIPIGAGICASYQTQTNSPRRARSVA
SQSIIAYTMSLGAENSVAYSNN SIAIPTNFTISVTTEILPVSMTKTSVDCTMYIC
GDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKD
FGGFNFSQILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLIC
AQKFNGLTVLPPLLTDemiaQYTSALLAGTITSGWTFGAGAAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSL SSTAALGKLQDVVNQNA

QALNTLVKQLSSNFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQ
 QLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVF
 LHVTVVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFTVQRNFYEPQII
 TTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD
 5 ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIA
 GLIAIVMVTIMLCCMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT
 (SEQ ID NO:32)

[0090] In certain aspects, modified ACE2 polypeptides of the present disclosure bind to a protein having a sequence at least 85% identical (*e.g.*, 85%, 90%, 91%, 92%, 93%, 94%,
 10 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identical) to SEQ ID NO:33.

SARS-CoV2 S Spike (S) Glycoprotein Receptor Binding Domain

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLVNS
 ASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYK
 LPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGS
 15 TPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKK
 STNLVKNKCVNF
 (SEQ ID NO:33)

[0091] In certain aspects, modified ACE2 polypeptides of the present disclosure have a sequence at least 85% identical (*e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 20 98%, 99%, 99.5%, or 100% identical) to SEQ ID NO:1.

Human Angiotensin-Converting Enzyme 2 (ACE2) (without signal peptide)

QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDK
 WSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTIL
 NTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEV
 25 GKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQ
 LIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRF
 WTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRFKEAEKFFVSVGLPNMTQ
 GFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHE
 MGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPD
 30 FQEDNETEINFLKQALTIVGTLPTFTYMLEKWRWMVFKGEIPKDQWMKKW
 WEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEAL
 CQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTALENVVGAKNMN
 VRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISLKSALGDKA

YEWNDNEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRISFNF
 FVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG
 (SEQ ID NO:1)

[0092] In certain aspects, modified ACE2 polypeptides of the present disclosure have a
 5 sequence at least 85% identical (*e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
 98%, 99%, 99.5%, or 100% identical) to SEQ ID NO:2

Human Angiotensin-Converting Enzyme 2 (ACE2) (with signal peptide)

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNT
 NITEENVQNMNNAAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQN
 10 GSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLD
 YNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGD
 YEYVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPI
 GCLPAHLLGDMWGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFK
 EAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRIL
 15 MCTKVTMDDFLTAAHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMS
 LSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTYMLEKWRWM
 VFKEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFI
 RYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEP
 WTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
 20 QSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYAMRQYFLKVKNQMILFG
 EEDVRVANLKPRISFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDN
 SLEFLGIQPTLG

(SEQ ID NO:2)

[0093] In certain aspects, modified ACE2 polypeptides of the present disclosure include at
 25 least one substitution introducing at least one glycosylation site not present in wild-type
 human ACE2. For example, in certain aspects, modified ACE2 polypeptides of the present
 disclosure include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve,
 thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty substitution(s)
 introducing one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen,
 30 fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty glycosylation site(s) not
 present in SEQ ID NO:1.

[0094] As used herein, all position numbering with reference to modified ACE2 polypeptides
 is relative to SEQ ID NO:2, unless otherwise stated.

[0095] In certain aspects, modified ACE2 polypeptides of the present disclosure include

T27Y, L79T, and N330Y substitutions.

[0096] In certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, and N330Y substitutions and one or more substitutions selected from E166C, S167I, A246L, K288C, E433C, V491I, H535F, E536N, P538S/T, N580S/T, D615N, L624C, K631N, A632C, Y633S/T, M662S/T, I663W, A673V, A673Y, I679Y, N682W, A687F, V691C, I694F, T698E, N720S/T, and Q728N.

[0097] In certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, and N330Y substitutions and one or more substitutions selected from S167I, A246L, V491I, H535F, I663W, A673V, A673Y, I679Y, N682W, A687F, I694F, and T698E.

For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, I663W, and A673Y substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, A673Y, and I694F substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, V491I, and I679Y substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, N682W, and A687F substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, S167I, A246L, and I694F substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, V491I, A673Y, A687F, and T698E substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, S167I, V491I, H535F, and A673V substitutions.

[0098] In certain aspects, modified ACE2 polypeptides of the present disclosure have T27Y, L79T, and N330Y substitutions and one or more substitutions that introduce a disulfide bond not present in wild-type human ACE2. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, and N330Y substitutions and one or more substitutions selected from K288C, E433C, L624C, A632C, E166C, and V691C. In certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, K288C and E433C substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, L624C and A632C substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E166C and V691C substitutions.

[0099] In certain aspects, modified ACE2 polypeptides of the present disclosure have T27Y, L79T, and N330Y substitutions and one or more substitutions that introduce a glycosylation site not present in wild-type human ACE2. For example, in certain aspects, modified ACE2

polypeptides of the present disclosure include T27Y, L79T, and N330Y substitutions and one or more substitutions selected from E536N, P538S/T, N580S/T, D615N, K631N, Y633S/T, M662S/T, N720S/T, and Q728N. In certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, and M662S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, and M662T substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S/T, and M662S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S, and M662T substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S/T, N580S/T and M662S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S, N580T, and M662T substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, M662S/T, and N720S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, M662T, and N720S substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, D615N, M662S/T, and N720S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, D615N, M662T, and N720S substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S/T, M662S/T, and Q728N substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, E536N, P538S, M662T, and Q728N substitutions. In other aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, K631N, Y633S/T, M662S/T, and Q728N substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, K631N, Y633T, M662T, and Q728N substitutions.

[00100] In certain aspects, modified ACE2 polypeptides of the present disclosure have T27Y, L79T, N330Y, M662S/T, and I694F substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y, K631N, M662T, and I694F substitutions.

[00101] In certain aspects, modified ACE2 polypeptides of the present disclosure have T27Y, L79T, N330Y, V491I, M662S/T, and N720S/T substitutions. For example, in certain aspects, modified ACE2 polypeptides of the present disclosure include T27Y, L79T, N330Y,

V491I, M662T, and N720S substitutions. In certain aspects, modified ACE2 polypeptides of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:12.

- 5 **[00102]** In certain aspects, modified ACE2 polypeptides of the present disclosure include a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to any one of the sequences listed in TABLE 1.

TABLE 1: Wild-type and Modified ACE2 Polypeptides

Identifier	Amino Acid Sequence
Wild-Type sACE2	QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:1)
sACE2 ₂ .v2.4	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG

Identifier	Amino Acid Sequence
	(SEQ ID NO:13)
sACE2 ₂ .S19	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQNTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDSSLEFLGIQPTLG
	(SEQ ID NO:12)
sACE2 ₂ .S01	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMWLFGEEDVRVYNLKPRI SFNFFVTAPKNVS DIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG
	(SEQ ID NO:14)
sACE2 ₂ .S02	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ

Identifier	Amino Acid Sequence
	FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDKQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVYNLKPRI SFNFVVTAPKNVSD FIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:15)
sACE ₂ .S03	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTL YQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDKQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SFNFVVTAPKNVS DIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:16)
sACE ₂ .S04	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTL YQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDKQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SFNFVVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:17)
sACE ₂ .S05	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWEIWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRLKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFQKPNIDVTDAMVDQAWDAQRIFKEAE

Identifier	Amino Acid Sequence
	KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD FIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:18)
sACE2 ₂ .S06	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVYNLKPRI SNFFVTFPKNVSDI IPREEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:19)
sACE2 ₂ .S07	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWEIWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKFEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVYNLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:20)
sACE2 ₂ .S08	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ

Identifier	Amino Acid Sequence
	QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECCLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQCPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWAYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNCTEINFL LKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWE MKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQ FQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEP WTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGW STDWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVA YAMRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNV SDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:21)
sACE2 ₂ .S09	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECCLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWAYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWE KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISCKSALGDKCYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:22)
sACE2 ₂ .S10	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECCLLE PGLNEIMANSLDYNERLWAWCSWRSEVGKQLRPLYEEYVVL KNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDV EHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWAYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWE KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA

Identifier	Amino Acid Sequence
	MRQYFLKVKNQMILFGEEDVRVANLKPRISFNFFVTAPKNCSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:23)
sACE2 ₂ .S11	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHGEPHLKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQITILFGEEDVRVANLKPRISFNFFVTAPKNVSDI IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:24)
sACE2 ₂ .S12	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHNGSLHKCDISNSTEAGQKLFNMLRLGKSEPW TLALENVVGAKNMNVRPLLNYFEPLFTWLKDNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQITILFGEEDVRVANLKPRISFNFFVTAPKNVSDI IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:25)
sACE2 ₂ .S13	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL

Identifier	Amino Acid Sequence
	KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHNGSLHKCDISNSTEAGQKLFNMLRLGKSEPW TLALENVVGAKNMTVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:26)
sACE2 ₂ .S14	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFW EYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDSSLEFLGIQPTLG (SEQ ID NO:27)
sACE2 ₂ .S15	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFW EYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYANQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDSSLEFLGIQPTLG (SEQ ID NO:28)
sACE2 ₂ .S16	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE

Identifier	Amino Acid Sequence
	HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHNGSLHKCDISNSTEAGQKLFNMLRLGKSEPW TLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGINPTLG (SEQ ID NO:29)
sACE ₂ .S17	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDNATEWNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGINPTLG (SEQ ID NO:30)
sACE ₂ .S18	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSSEKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQTILFGEEDVRVANLKPRI SFNFFVTAPKNVSD FIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLG (SEQ ID NO:31)

[00103] In certain aspects, modified ACE2 polypeptides of the present disclosure include a signal peptide fused to the N-terminus of the modified ACE2 polypeptide. In certain aspects, the signal peptide includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:11.

Human ACE2 Signal Peptide

MSSSSWLLLSLVAVTAA (SEQ ID NO:11)

II. Modified ACE2 Fusion Polypeptides

[00104] The present disclosure also provides modified ACE2 fusion proteins including a modified ACE2 polypeptide as previously described and a heterologous polypeptide. In certain aspects, the heterologous polypeptide is an Fc domain polypeptide, for example, a human Fc domain polypeptide. In certain aspects, the heterologous polypeptide is a human IgG1 Fc domain polypeptide, a human IgG2 Fc domain polypeptide, a human IgG3 Fc domain polypeptide, or a human IgG4 Fc domain polypeptide. In certain aspects, the heterologous polypeptide includes residues 221 to 447 of a human IgG1 Fc domain, wherein residue positions are numbered according to EU numbering.

[00105] In specific non-limiting examples, the modified ACE2 fusion protein incorporates an Fc domain polypeptide having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:4.

[00106] In certain aspects, modified ACE2 fusion proteins of the present disclosure include a human IgG1 Fc domain incorporating one or more substitution selected from: a substitution of methionine at position 252 to tyrosine; a substitution of serine at position 254 to threonine; and a substitution of threonine at position 256 to glutamic acid, wherein residue positions are numbered according to EU numbering. In certain aspects, modified ACE2 fusion proteins of the present disclosure include a human IgG1 Fc domain incorporating a substitution of methionine at position 252 to tyrosine; a substitution of serine at position 254 to threonine; and a substitution of threonine at position 256 to glutamic acid, wherein residue positions are numbered according to EU numbering. In certain aspects, the modified ACE2 fusion protein incorporates an Fc domain polypeptide having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:5.

[00107] In certain aspects, modified ACE2 fusion proteins of the present disclosure include a

human IgG1 Fc domain having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to any one of the sequences listed in TABLE 2.

TABLE 2: Wild-type and Modified Human IgG1 Fc Domain Polypeptide Sequences

Identifier	Amino Acid Sequence
Wild-Type Human IgG1 Fc [D221-K447] (GenBank Ref: KY432415.1 ¹) (G1m1 allotype)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVSVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:4)
“YTE” Human IgG1 Fc [D221-K447] (G1m1 allotype)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVSVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:5)
Wild-Type Human IgG1 Fc [D221-K447] (GenBank Ref: P01857.1) (G1m17 allotype)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVSVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:37)
“YTE” Human IgG1 Fc [D221-K447] (G1m17 allotype)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVSVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:38)

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[00108] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a human IgG1 Fc domain polypeptide fused to the C-terminus of the modified ACE2 polypeptide. In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a human IgG1 Fc domain polypeptide fused to the N-terminus of the modified ACE2 polypeptide.

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[00109] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes

¹ All references to accession numbers (*i.e.*, GenBank numbers) throughout the application refer to the versions that were current in the database as of the filing date of the application.

a human IgG1 Fc domain polypeptide fused to the modified ACE2 polypeptide via a linker. In certain aspects, the linker is one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, or twenty-five amino acid(s) in length. In certain aspects, the linker is a single amino acid in length. For example, in certain aspects, the linker is a single serine residue.

[00110] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a human IgG1 Fc domain polypeptide fused to the modified ACE2 polypeptide via a linker having a sequence selected from any one of the sequences listed in TABLE 3.

TABLE 3: Linker Sequences

Identifier	Amino Acid Sequence
“S”	S
“P”	P
“G”	G
“GS”	GS
“PP”	PP
“PPN”	PPN
“GGS”	GGS
“GSS”	GSS
“GGGS”	(GGGS) _n where n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 (SEQ ID NO: 39)
“GSGG”	(GSGG) _n where n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 (SEQ ID NO: 40)
“PPNQ”	PPNQ (SEQ ID NO: 41)
“PPNQP”	PPNQP (SEQ ID NO: 42)
“PPNQPP”	PPNQPP (SEQ ID NO: 43)
“PPNQPPV”	PPNQPPV (SEQ ID NO: 44)
“PPNQPPVS”	PPNQPPVS (SEQ ID NO: 45)

[00111] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes, from N-terminus to C-terminus, a modified ACE2 polypeptide having at least 85% sequence identity (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99%, 99.5%, or 100%) to any one of the sequences listed in TABLE 1, a linker sequence according to any one of the sequences listed in TABLE 3, and a human IgG1 Fc domain polypeptide having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to any one of the sequences listed in TABLE 2.

[00112] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:6. For example, a modified ACE2 fusion protein of the present disclosure has a sequence 100% identical to SEQ ID NO:6.

[00113] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:7. For example, a modified ACE2 fusion protein of the present disclosure has a sequence 100% identical to SEQ ID NO:7.

[00114] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:8. For example, a modified ACE2 fusion protein of the present disclosure has a sequence 100% identical to SEQ ID NO:8.

[00115] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:9. For example, a modified ACE2 fusion protein of the present disclosure has a sequence 100% identical to SEQ ID NO:9.

[00116] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to SEQ ID NO:10. For example, a modified ACE2 fusion protein of the present disclosure has a sequence 100% identical to SEQ ID NO:10.

[00117] In certain aspects, a modified ACE2 fusion protein of the present disclosure includes a sequence having at least 85% sequence identity (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100%) to any one of the sequences listed in TABLE 4.

TABLE 4: Exemplary Modified ACE2 Fusion Protein Sequences

Identifier	Amino Acid Sequence
Wild-Type sACE2 – hIgG1 Fc	QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECILLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO:6)
sACE2 ₂ .v2.4 – hIgG1 Fc	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECILLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWESMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPHLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPGK

Identifier	Amino Acid Sequence
	<u>(SEQ ID NO:7)</u>
sACE ₂ .v2.4 – “YTE” hIgG1 Fc	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHGEP LHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYA MRQYFLKVKNQMILFGEEDVRVANLKPRI SNFFVTAPKNVSD IIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKS LSLSPGK <u>(SEQ ID NO:8)</u>
sACE ₂ .S19 – hIgG1 Fc	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHGEP LHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNDNEMYLFRSSVAYA MRQYFLKVKNQ TILFGEEDVRVANLKPRI SNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDSSLEFLGIQPTLGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKS LSLSPGK

Identifier	Amino Acid Sequence
	(SEQ ID NO:9)
sACE2 ₂ .S19 – “YTE” hIgG1 Fc	QSTIEEQAKYFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQ NMNNAGDKWSAFLKEQSTTAQMYPLQEIQNLTVKLQLQALQ QNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLE PGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLK NEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM WGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDAQRIFKEAE KFFVSVGLPNMTQGFWWEYSMLTDPGNVQKAVCHPTAWDLGK GDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNG ANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFL KQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEM KREIVGVVEPIPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQ FQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWT LALENVVGAKNMNVRPLLNYFEPLFTWLKDNKNSFVGWST DWSPYADQSIKVRISLKSALGDKAYEWNENMYLFRSSVAYA MRQYFLKVKNQNTILFGEEDVRVANLKPRI SFNFFVTAPKNVSDI IPRTEVEKAIRMSRSRINDAFRLNDSSLEFLGIQPTLGSDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKS LSLSPGK (SEQ ID NO:10)

Bold = ACE2 substitution

Italicized = Linker

Underlined = hIgG1 Fc Sequence

Underlined = hIgG1 Fc Substitution

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III. Characteristics of Modified ACE2 Polypeptides and Modified ACE2 Fusion

Polypeptides

[00118] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion

polypeptides of the present disclosure have increased levels of glycosylation as compared to

10 wild-type ACE2. For example, in certain aspects modified ACE2 polypeptides and modified

ACE2 fusion polypeptides have 1 to 50% increased levels of glycosylation as compared to

wild-type ACE2 (*e.g.*, 1 to 50%, 5 to 50%, 10 to 50%, 15 to 50%, 20 to 50%, 25 to 50%, 30

to 50%, 35 to 50%, 40 to 50%, 45 to 50%, 1 to 45%, 5 to 45%, 10 to 45%, 15 to 45%, 20 to

45%, 25 to 45%, 30 to 45%, 35 to 45%, 40 to 45%, 1 to 40%, 5 to 40%, 10 to 40%, 15 to

15 40%, 20 to 40%, 25 to 40%, 30 to 40%, 35 to 40%, 1 to 35%, 5 to 35%, 10 to 35%, 15 to

35%, 20 to 35%, 25 to 35%, 30 to 35%, 1 to 30%, 5 to 30%, 10 to 30%, 15 to 30%, 20 to

30%, 25 to 30%, 1 to 25%, 5 to 25%, 10 to 25%, 15 to 25%, 20 to 25%, 1 to 20%, 5 to 20%,

10 to 20%, 15 to 20%, 1 to 15%, 5 to 15%, 10 to 15%, 1 to 10%, 5 to 10%, or 1 to 5%).

[00119] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion

polypeptides of the present disclosure have 2 to 50-fold increased affinity (*e.g.*, 2 to 50-fold, 4 to 50-fold, 6 to 50-fold, 8 to 50-fold, 10 to 50-fold, 15 to 50-fold, 20 to 50-fold, 25 to 50-fold, 30 to 50-fold, 35 to 50-fold, 40 to 50-fold, 45 to 50-fold, 2 to 40-fold, 4 to 40-fold, 6 to 40-fold, 8 to 40-fold, 10 to 40-fold, 15 to 40-fold, 20 to 40-fold, 25 to 40-fold, 30 to 40-fold, 35 to 40-fold, 2 to 35-fold, 4 to 35-fold, 6 to 35-fold, 8 to 35-fold, 10 to 35-fold, 15 to 35-fold, 20 to 35-fold, 25 to 35-fold, 30 to 35-fold, 2 to 30-fold, 4 to 30-fold, 6 to 30-fold, 8 to 30-fold, 10 to 30-fold, 15 to 30-fold, 20 to 30-fold, 25 to 30-fold, 2 to 25-fold, 4 to 25-fold, 6 to 25-fold, 8 to 25-fold, 10 to 25-fold, 15 to 25-fold, 20 to 25-fold, 2 to 20-fold, 4 to 20-fold, 6 to 20-fold, 8 to 20-fold, 10 to 20-fold, 15 to 20-fold, 2 to 15-fold, 4 to 15-fold, 6 to 15-fold, 8 to 15-fold, 10 to 15-fold, 2 to 10-fold, 4 to 10-fold, 6 to 10-fold, 8 to 10-fold, 2 to 5-fold, or 4 to 5-fold) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein as compared to wild-type ACE2. In some embodiments, the S protein is of a strain selected from Wuhan, alpha, beta, gamma, delta, and omicron. In some embodiments, the S protein is of BF.7, XBB, BA.2.75.2, or BQ.1.1 omicron sublineages. In certain aspects, affinity can be measured by any method known by persons of skill in the art. For example, in certain aspects, affinity is measured by biolayer interferometry (BLI).

[00120] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion

polypeptides of the present disclosure have 2 to 50-fold increased avidity (*e.g.*, 2 to 50-fold, 4 to 50-fold, 6 to 50-fold, 8 to 50-fold, 10 to 50-fold, 15 to 50-fold, 20 to 50-fold, 25 to 50-fold, 30 to 50-fold, 35 to 50-fold, 40 to 50-fold, 45 to 50-fold, 2 to 40-fold, 4 to 40-fold, 6 to 40-fold, 8 to 40-fold, 10 to 40-fold, 15 to 40-fold, 20 to 40-fold, 25 to 40-fold, 30 to 40-fold, 35 to 40-fold, 2 to 35-fold, 4 to 35-fold, 6 to 35-fold, 8 to 35-fold, 10 to 35-fold, 15 to 35-fold, 20 to 35-fold, 25 to 35-fold, 30 to 35-fold, 2 to 30-fold, 4 to 30-fold, 6 to 30-fold, 8 to 30-fold, 10 to 30-fold, 15 to 30-fold, 20 to 30-fold, 25 to 30-fold, 2 to 25-fold, 4 to 25-fold, 6 to 25-fold, 8 to 25-fold, 10 to 25-fold, 15 to 25-fold, 20 to 25-fold, 2 to 20-fold, 4 to 20-fold, 6 to 20-fold, 8 to 20-fold, 10 to 20-fold, 15 to 20-fold, 2 to 15-fold, 4 to 15-fold, 6 to 15-fold, 8 to 15-fold, 10 to 15-fold, 2 to 10-fold, 4 to 10-fold, 6 to 10-fold, 8 to 10-fold, 2 to 5-fold, or 4 to 5-fold) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein as compared to wild-type ACE2.

[00121] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion

polypeptides of the present disclosure comprise a binding affinity to SARS-CoV-2 of less than 1 nM, less than 1.2 nM, less than 2 nM, less than 5 nM, less than 10 nM, less than 11

nm, less than 13.5 nM, less than 15 nM, less than 20 nM, less than 25 nM, less than 30 nM, less than 35 nM, less than 40 nM, less than 45 nM, or less than 50 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 1 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 1.2 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 2 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 5 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 10 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 15 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 20 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 25 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 30 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 50 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding affinity of less than 75 nM.

[00122] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity to SARS- CoV-2 of less than 1 nM, less than 1.2 nM, less than 2 nM, less than 5 nM, less than 10 nM, less than 11 nm, less than 13.5 nM, less than 15 nM, less than 20 nM, less than 25 nM, less than 30 nM, less than 35 nM, less than 40 nM, less than 45 nM, or less than 50 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 1 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 1.2 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 2 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 5 nM. In some

instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 10 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 15 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 20 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 25 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 30 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 50 nM. In some instances, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure comprise a binding avidity of less than 75 nM.

[00123] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure have 1.2 to 2-fold increased catalytic activity as compared to wild-type ACE2 (*e.g.*, 1.2 to 2-fold, 1.3 to 2-fold, 1.4 to 2-fold, 1.5 to 2-fold, 1.6 to 2-fold, 1.7 to 2-fold, 1.8 to 2-fold, 1.9 to 2-fold, 1.2 to 1.8-fold, 1.3 to 1.8-fold, 1.4 to 1.8-fold, 1.5 to 1.8-fold, 1.6 to 1.8-fold, 1.7 to 1.8-fold, 1.2 to 1.6-fold, 1.3 to 1.6-fold, 1.4 to 1.6-fold, 1.5 to 1.6-fold, 1.2 to 1.4-fold, or 1.3 to 1.4-fold). In certain aspects, catalytic activity can be measured by any method known by persons of skill in the art. For example, in certain aspects, catalytic activity is measured by an *in vitro* fluorometric ACE2 activity assay.

[00124] In certain aspects, at least 10% (*e.g.* at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the glycosylation sites of a modified ACE2 polypeptide or modified ACE2 fusion polypeptide of the present disclosure are sialylated. For example, in some aspects at least 20% of the glycosylation sites of a modified ACE2 polypeptide or modified ACE2 fusion polypeptide of the present disclosure are sialylated. In some embodiments, the percentage is a mole percentage. In certain aspects, sialylation can be measured by any method known by persons of skill in the art. For example, in certain aspects, sialylation is measured by mass spectroscopy.

[00125] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a 2 to 10-fold (*e.g.*, 2 to 10-fold, 3 to 10-fold, 4 to 10-fold, 5 to 10-fold, 6 to 10-fold, 7 to 10-fold, 8 to 10-fold, 9 to 10-fold, 2 to 9-fold, 3 to 9-fold, 4 to 9-fold, 5 to 9-fold, 6 to 9-fold, 7

to 9-fold, 8 to 9-fold, 2 to 8-fold, 3 to 8-fold, 4 to 8-fold, 5 to 8-fold, 6 to 8-fold, 7 to 8-fold, 2 to 7-fold, 3 to 7-fold, 4 to 7-fold, 5 to 7-fold, 6 to 7-fold, 2 to 6-fold, 3 to 6-fold, 4 to 6-fold, 5 to 6-fold, 2 to 5-fold, 3 to 5-fold, 4 to 5-fold, 2 to 4-fold, 3 to 4-fold, or 2 to 3-fold) decreased dissociation rate with FcRn at endosomal pH as compared to a protein including a wild-type ACE2 polypeptide fused to a wild-type human IgG1 Fc domain polypeptide (*e.g.*, a modified ACE2 fusion protein having a sequence of SEQ ID NO:6). In certain aspects, dissociation rates can be measured by any method known by persons of skill in the art. For example, in certain aspects, dissociation rates are measured by BLI.

[00126] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have an equilibrium dissociation constant for FcRn at pH 6.0 of 10 nM to 50 nM (*e.g.*, 10 nM to 50 nM, 15 nM to 50 nM, 20 nM to 50 nM, 25 nM to 50 nM, 30 nM to 50 nM, 35 nM to 50 nM, 40 nM to 50 nM, 45 nM to 50 nM, 10 nM to 45 nM, 15 nM to 45 nM, 20 nM to 45 nM, 25 nM to 45 nM, 30 nM to 45 nM, 35 nM to 45 nM, 40 nM to 45 nM, 10 nM to 40 nM, 15 nM to 40 nM, 20 nM to 40 nM, 25 nM to 40 nM, 30 nM to 40 nM, 35 nM to 40 nM, 10 nM to 35 nM, 15 nM to 35 nM, 20 nM to 35 nM, 25 nM to 35 nM, 30 nM to 35 nM, 10 nM to 30 nM, 15 nM to 30 nM, 20 nM to 30 nM, 25 nM to 30 nM, 10 nM to 25 nM, 15 nM to 25 nM, 20 nM to 25 nM, 10 nM to 20 nM, 15 nM to 20 nM, or 10 nM to 15 nM). In certain aspects, equilibrium dissociation constants can be measured by any method known by persons of skill in the art. For example, in certain aspects, equilibrium dissociation constants are measured by BLI.

[00127] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have an off-rate for FcRn at pH 6.0 of $5.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$ (*e.g.*, $5.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $6.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $7.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $8.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $9.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $1.0 \times 10^{-3} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$, $5.0 \times 10^{-4} \text{s}^{-1}$ to $1.0 \times 10^{-3} \text{s}^{-1}$, $6.0 \times 10^{-4} \text{s}^{-1}$ to $1.0 \times 10^{-3} \text{s}^{-1}$, $7.0 \times 10^{-4} \text{s}^{-1}$ to $1.0 \times 10^{-3} \text{s}^{-1}$, $8.0 \times 10^{-4} \text{s}^{-1}$ to $1.0 \times 10^{-3} \text{s}^{-1}$, $9.0 \times 10^{-4} \text{s}^{-1}$ to $1.0 \times 10^{-3} \text{s}^{-1}$, $5.0 \times 10^{-4} \text{s}^{-1}$ to $9.0 \times 10^{-4} \text{s}^{-1}$, $6.0 \times 10^{-4} \text{s}^{-1}$ to $9.0 \times 10^{-4} \text{s}^{-1}$, $7.0 \times 10^{-4} \text{s}^{-1}$ to $9.0 \times 10^{-4} \text{s}^{-1}$, $8.0 \times 10^{-4} \text{s}^{-1}$ to $9.0 \times 10^{-4} \text{s}^{-1}$, $5.0 \times 10^{-4} \text{s}^{-1}$ to $8.0 \times 10^{-4} \text{s}^{-1}$, $6.0 \times 10^{-4} \text{s}^{-1}$ to $8.0 \times 10^{-4} \text{s}^{-1}$, $7.0 \times 10^{-4} \text{s}^{-1}$ to $8.0 \times 10^{-4} \text{s}^{-1}$, $5.0 \times 10^{-4} \text{s}^{-1}$ to $7.0 \times 10^{-4} \text{s}^{-1}$, $6.0 \times 10^{-4} \text{s}^{-1}$ to $7.0 \times 10^{-4} \text{s}^{-1}$, or $5.0 \times 10^{-4} \text{s}^{-1}$ to $6.0 \times 10^{-4} \text{s}^{-1}$). In certain aspects, off-rate for FcRn can be measured by any method known by persons of skill in the art. For example, in certain aspects, off-rate for FcRn is measured by BLI.

[00128] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a higher binding affinity to FcRn at pH 6.0 than at pH 7.0. In certain aspects, binding affinity can be measured by any method known by persons of skill in the art. For example, in certain

aspects, binding affinity is measured by BLI.

[00129] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life of 40 to 80 hours (*e.g.*, 40 to 80 hours, 42 to 80 hours, 44 to 80 hours, 46 to 80 hours, 48 to 80 hours, 50 to 80 hours, 52 to 80 hours, 54 to 80 hours, 54 to 80 hours, 56 to 80 hours, 58 to 80 hours, 60 to 80 hours, 62 to 80 hours, 64 to 80 hours, 66 to 80 hours, 68 to 80 hours, 70 to 80 hours, 72 to 80 hours, 74 to 80 hours, 76 to 80 hours, 78 to 80 hours, 40 to 70 hours, 42 to 70 hours, 44 to 70 hours, 46 to 70 hours, 48 to 70 hours, 50 to 70 hours, 52 to 70 hours, 54 to 70 hours, 54 to 70 hours, 56 to 70 hours, 58 to 70 hours, 60 to 70 hours, 62 to 70 hours, 64 to 70 hours, 66 to 70 hours, 68 to 70 hours, 40 to 60 hours, 42 to 60 hours, 44 to 60 hours, 46 to 60 hours, 48 to 60 hours, 50 to 60 hours, 52 to 60 hours, 54 to 60 hours, 54 to 60 hours, 56 to 60 hours, 58 to 60 hours, 40 to 58 hours, 42 to 58 hours, 44 to 58 hours, 46 to 58 hours, 48 to 58 hours, 50 to 58 hours, 52 to 58 hours, 54 to 58 hours, 54 to 58 hours, 56 to 58 hours, 40 to 56 hours, 42 to 56 hours, 44 to 56 hours, 46 to 56 hours, 48 to 56 hours, 50 to 56 hours, 52 to 56 hours, 54 to 56 hours, 54 to 56 hours, 40 to 54 hours, 42 to 54 hours, 44 to 54 hours, 46 to 54 hours, 48 to 54 hours, 50 to 54 hours, 52 to 54 hours, 40 to 52 hours, 42 to 52 hours, 44 to 52 hours, 46 to 52 hours, 48 to 52 hours, 50 to 52 hours, 40 to 50 hours, 42 to 50 hours, 44 to 50 hours, 46 to 50 hours, 48 to 50 hours, 40 to 48 hours, 42 to 48 hours, 44 to 48 hours, 46 to 48 hours, 40 to 46 hours, 42 to 46 hours, 44 to 46 hours, 40 to 44 hours, 42 to 44 hours, or 40 to 42 hours) when administered to a subject. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life of about 75 hours. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life in a range of about 70 to about 80 hours. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life of about 125 hours. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life in a range of about 110 to about 140 hours. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life of about 225 hours. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life in a range of about 210 to about 240 hours. In certain aspects, plasma half-life can be measured by any method known by persons of skill in the art. For example, in certain aspects, plasma half-life is measured by ELISA or an *in vitro* fluorometric ACE2 activity assay.

[00130] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma half-life of 40 to 240 hours (*e.g.*, 40 to 240 hours, 50 to 240 hours, 60 to 240 hours, 70 to 240 hours, 80 to 240 hours, 90 to 240 hours, 100 to 240 hours, 110 to 240 hours, 120 to

240 hours, 130 to 240 hours, 140 to 240 hours, 150 to 240 hours, 160 to 240 hours, 170 to 240 hours, 180 to 240 hours, 190 to 240 hours, 200 to 240 hours, 210 to 240 hours, 220 to 240 hours, 230 to 240 hours, 40 to 200 hours, 50 to 200 hours, 60 to 200 hours, 70 to 200 hours, 80 to 200 hours, 90 to 200 hours, 100 to 200 hours, 110 to 200 hours, 120 to 200 hours, 130 to 200 hours, 140 to 200 hours, 150 to 200 hours, 160 to 200 hours, 170 to 200 hours, 180 to 200 hours, 190 to 200 hours, 40 to 160 hours, 50 to 160 hours, 60 to 160 hours, 70 to 160 hours, 80 to 160 hours, 90 to 160 hours, 100 to 160 hours, 110 to 160 hours, 120 to 160 hours, 130 to 160 hours, 140 to 160 hours, 150 to 160 hours, 40 to 120 hours, 50 to 120 hours, 60 to 120 hours, 70 to 120 hours, 80 to 120 hours, 90 to 120 hours, 100 to 120 hours, 110 to 120 hours, 40 to 100 hours, 50 to 100 hours, 60 to 100 hours, 70 to 100 hours, 80 to 100 hours, 90 to 100 hours, 40 to 80 hours, 50 to 80 hours, 60 to 80 hours, 70 to 80 hours, 70 to 160 hours, 80 to 160 hours, 90 to 160 hours, 100 to 160 hours, 110 to 160 hours, 120 to 160 hours, 130 to 160 hours, 140 to 160 hours, 150 to 160 hours, 70 to 130 hours, 80 to 130 hours, 90 to 130 hours, 100 to 130 hours, 110 to 130 hours, 120 to 130 hours, 70 to 100 hours, 80 to 100 hours, or 90 to 100 hours) when administered to a subject.

[00131] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have a plasma activity area under the curve over 48 hours (AUC_{0-48h}) of 50 to 150 (μM product/minute) $\times$ h (e.g., 50 to 150 (μM product/minute) $\times$ h, 60 to 150 (μM product/minute) $\times$ h, 70 to 150 (μM product/minute) $\times$ h, 80 to 150 (μM product/minute) $\times$ h, 90 to 150 (μM product/minute) $\times$ h, 100 to 150 (μM product/minute) $\times$ h, 110 to 150 (μM product/minute) $\times$ h, 120 to 150 (μM product/minute) $\times$ h, 130 to 150 (μM product/minute) $\times$ h, 140 to 150 (μM product/minute) $\times$ h, 50 to 140 (μM product/minute) $\times$ h, 60 to 140 (μM product/minute) $\times$ h, 70 to 140 (μM product/minute) $\times$ h, 80 to 140 (μM product/minute) $\times$ h, 90 to 140 (μM product/minute) $\times$ h, 100 to 140 (μM product/minute) $\times$ h, 110 to 140 (μM product/minute) $\times$ h, 120 to 140 (μM product/minute) $\times$ h, 130 to 140 (μM product/minute) $\times$ h, 50 to 130 (μM product/minute) $\times$ h, 60 to 130 (μM product/minute) $\times$ h, 70 to 130 (μM product/minute) $\times$ h, 80 to 130 (μM product/minute) $\times$ h, 90 to 130 (μM product/minute) $\times$ h, 100 to 130 (μM product/minute) $\times$ h, 110 to 130 (μM product/minute) $\times$ h, 120 to 130 (μM product/minute) $\times$ h, 50 to 120 (μM product/minute) $\times$ h, 60 to 120 (μM product/minute) $\times$ h, 70 to 120 (μM product/minute) $\times$ h, 80 to 120 (μM product/minute) $\times$ h, 90 to 120 (μM product/minute) $\times$ h, 100 to 120 (μM product/minute) $\times$ h, 110 to 120 (μM product/minute) $\times$ h, 50 to 110 (μM product/minute) $\times$ h, 60 to 110 (μM product/minute) $\times$ h, 70 to 110 (μM product/minute) $\times$ h, 80 to 110 (μM product/minute) $\times$ h, 90 to 110 (μM product/minute) $\times$ h, 100 to 110 (μM product/minute) $\times$ h, 50 to 100 (μM product/minute) $\times$ h, 60 to 100 (μM product/minute) $\times$ h,

70 to 100 (μM product/minute) x h, 80 to 100 (μM product/minute) x h, 90 to 100 (μM product/minute) x h, 50 to 90 (μM product/minute) x h, 60 to 90 (μM product/minute) x h, 70 to 90 (μM product/minute) x h, 80 to 90 (μM product/minute) x h, 50 to 80 (μM product/minute) x h, 60 to 80 (μM product/minute) x h, 70 to 80 (μM product/minute) x h, 50 to 70 (μM product/minute) x h, 60 to 70 (μM product/minute) x h, or 50 to 60 (μM product/minute) x h) when intravenously administered to a subject at a dose of 2 mg/kg.

[00132] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have an area under the curve ($\text{AUC}_{0-120\text{h}}$) of 100 to 600 $\mu\text{g/ml}$ x h (e.g., 100 to 600 $\mu\text{g/ml}$ x h, 125 to 600 $\mu\text{g/ml}$ x h, 150 to 600 $\mu\text{g/ml}$ x h, 175 to 600 $\mu\text{g/ml}$ x h, 200 to 600 $\mu\text{g/ml}$ x h, 225 to 600 $\mu\text{g/ml}$ x h, 250 to 600 $\mu\text{g/ml}$ x h, 275 to 600 $\mu\text{g/ml}$ x h, 300 to 600 $\mu\text{g/ml}$ x h, 325 to 600 $\mu\text{g/ml}$ x h, 350 to 600 $\mu\text{g/ml}$ x h, 375 to 600 $\mu\text{g/ml}$ x h, 400 to 600 $\mu\text{g/ml}$ x h, 425 to 600 $\mu\text{g/ml}$ x h, 450 to 600 $\mu\text{g/ml}$ x h, 475 to 600 $\mu\text{g/ml}$ x h, 100 to 450 $\mu\text{g/ml}$ x h, 125 to 450 $\mu\text{g/ml}$ x h, 150 to 450 $\mu\text{g/ml}$ x h, 175 to 450 $\mu\text{g/ml}$ x h, 200 to 450 $\mu\text{g/ml}$ x h, 225 to 450 $\mu\text{g/ml}$ x h, 250 to 450 $\mu\text{g/ml}$ x h, 275 to 450 $\mu\text{g/ml}$ x h, 300 to 450 $\mu\text{g/ml}$ x h, 325 to 450 $\mu\text{g/ml}$ x h, 350 to 450 $\mu\text{g/ml}$ x h, 375 to 450 $\mu\text{g/ml}$ x h, 400 to 450 $\mu\text{g/ml}$ x h, 425 to 450 $\mu\text{g/ml}$ x h, 100 to 400 $\mu\text{g/ml}$ x h, 125 to 400 $\mu\text{g/ml}$ x h, 150 to 400 $\mu\text{g/ml}$ x h, 175 to 400 $\mu\text{g/ml}$ x h, 200 to 400 $\mu\text{g/ml}$ x h, 225 to 400 $\mu\text{g/ml}$ x h, 250 to 400 $\mu\text{g/ml}$ x h, 275 to 400 $\mu\text{g/ml}$ x h, 300 to 400 $\mu\text{g/ml}$ x h, 325 to 400 $\mu\text{g/ml}$ x h, 350 to 400 $\mu\text{g/ml}$ x h, 375 to 400 $\mu\text{g/ml}$ x h, 100 to 350 $\mu\text{g/ml}$ x h, 125 to 350 $\mu\text{g/ml}$ x h, 150 to 350 $\mu\text{g/ml}$ x h, 175 to 350 $\mu\text{g/ml}$ x h, 200 to 350 $\mu\text{g/ml}$ x h, 225 to 350 $\mu\text{g/ml}$ x h, 250 to 350 $\mu\text{g/ml}$ x h, 275 to 350 $\mu\text{g/ml}$ x h, 300 to 350 $\mu\text{g/ml}$ x h, 325 to 350 $\mu\text{g/ml}$ x h, 100 to 300 $\mu\text{g/ml}$ x h, 125 to 300 $\mu\text{g/ml}$ x h, 150 to 300 $\mu\text{g/ml}$ x h, 175 to 300 $\mu\text{g/ml}$ x h, 200 to 300 $\mu\text{g/ml}$ x h, 225 to 300 $\mu\text{g/ml}$ x h, 250 to 300 $\mu\text{g/ml}$ x h, 275 to 300 $\mu\text{g/ml}$ x h, 100 to 250 $\mu\text{g/ml}$ x h, 125 to 250 $\mu\text{g/ml}$ x h, 150 to 250 $\mu\text{g/ml}$ x h, 175 to 250 $\mu\text{g/ml}$ x h, 200 to 250 $\mu\text{g/ml}$ x h, 225 to 250 $\mu\text{g/ml}$ x h, 100 to 200 $\mu\text{g/ml}$ x h, 125 to 200 $\mu\text{g/ml}$ x h, 150 to 200 $\mu\text{g/ml}$ x h, 175 to 200 $\mu\text{g/ml}$ x h, 100 to 150 $\mu\text{g/ml}$ x h, or 125 to 150 $\mu\text{g/ml}$ x h) when intravenously administered to a subject at a dose of 10 mg/kg.

[00133] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure have an area under the curve (AUC_{0-t} , where t is 120 to 336 hours) of 100 to 9000 $\mu\text{g/ml}$ x h (e.g., 100 to 9000 $\mu\text{g/ml}$ x h, 500 to 9000 $\mu\text{g/ml}$ x h, 1000 to 9000 $\mu\text{g/ml}$ x h, 1500 to 9000 $\mu\text{g/ml}$ x h, 2000 to 9000 $\mu\text{g/ml}$ x h, 2500 to 9000 $\mu\text{g/ml}$ x h, 3000 to 9000 $\mu\text{g/ml}$ x h, 3500 to 9000 $\mu\text{g/ml}$ x h, 4000 to 9000 $\mu\text{g/ml}$ x h, 4500 to 9000 $\mu\text{g/ml}$ x h, 5000 to 9000 $\mu\text{g/ml}$ x h, 5500 to 9000 $\mu\text{g/ml}$ x h, 6000 to 9000 $\mu\text{g/ml}$ x h, 6500 to 9000 $\mu\text{g/ml}$ x h, 7000 to 9000 $\mu\text{g/ml}$ x h, 7500 to 9000 $\mu\text{g/ml}$ x h, 8000 to 9000 $\mu\text{g/ml}$ x h, 8500 to 9000 $\mu\text{g/ml}$ x h, 100 to 6500 $\mu\text{g/ml}$

x h, 500 to 6500 µg/ml x h, 1000 to 6500 µg/ml x h, 1500 to 6500 µg/ml x h, 2000 to 6500 µg/ml x h, 2500 to 6500 µg/ml x h, 3000 to 6500 µg/ml x h, 3500 to 6500 µg/ml x h, 4000 to 6500 µg/ml x h, 4500 to 6500 µg/ml x h, 5000 to 6500 µg/ml x h, 5500 to 6500 µg/ml x h, 6000 to 6500 µg/ml x h, 100 to 4000 µg/ml x h, 500 to 4000 µg/ml x h, 1000 to 4000 µg/ml x h, 1500 to 4000 µg/ml x h, 2000 to 4000 µg/ml x h, 2500 to 4000 µg/ml x h, 3000 to 4000 µg/ml x h, 3500 to 4000 µg/ml x h, 100 to 2000 µg/ml x h, 500 to 2000 µg/ml x h, 1000 to 2000 µg/ml x h, 1500 to 2000 µg/ml x h, 100 to 1000 µg/ml x h, 500 to 1000 µg/ml x h, 1000 to 6500 µg/ml x h, 1500 to 6500 µg/ml x h, 2000 to 6500 µg/ml x h, 2500 to 6500 µg/ml x h, 3000 to 6500 µg/ml x h, 3500 to 6500 µg/ml x h, 4000 to 6500 µg/ml x h, 4500 to 6500 µg/ml x h, 5000 to 6500 µg/ml x h, 5500 to 6500 µg/ml x h, 6000 to 6500 µg/ml x h, , or 100 to 500 µg/ml x h) when intravenously administered to a subject at a dose of 10 mg/kg.

[00134] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure maintain a plasma concentration above 18 pM (*e.g.*, above 18 pM, above 20 pM, above 22 pM, above 24 pM, above 26 pM, above 28 pM, above 30 pM, above 32 pM, above 34 pM, above 36 pM, above 38 pM, above 40 pM, above 42 pM, above 44 pM, above 46 pM, above 48 pM, above 50 pM, above 75 pM, or above 100 pM) at 100 to 150 hours post-administration to a subject. In certain aspects, modified ACE2 fusion polypeptides of the present disclosure maintain a plasma concentration above 18 pM (*e.g.*, above 18 pM, above 100 pM, above 300 pM, above 1 nM, above 3 nM, above 10 nM, above 30 nM, or above 100 nM) at 100 to 150 hours post-administration to a subject.

[00135] In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure have reduced immunogenicity when administered to a subject as compared to a wild-type ACE2 protein administered to the same subject. In certain aspects, modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure are non-immunogenic when administered to a subject.

[00136] In certain aspects, modified ACE2 fusion polypeptides of the present disclosure form stable homodimers at physiological pH. For example, in certain aspects, dimerization of modified ACE2 fusion polypeptides is measured by gel filtration chromatography and/or size exclusion chromatography.

IV. Pharmaceutical Formulations

[00137] Modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure can be used in the manufacture of pharmaceutical formulations. In certain aspects, pharmaceutical formulations disclosed herein include modified ACE2 polypeptides and modified ACE2 fusion polypeptides of the present disclosure and a pharmaceutically

acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. By “pharmaceutically acceptable” it is meant a material that is not toxic or otherwise undesirable, i.e. the material may be administered to a subject without causing any undesirable biological effects.

5 **[00138]** In certain aspects, pharmaceutical formulations can comprise sterile aqueous and non-aqueous injection solutions, which are optionally isotonic with the blood of the subject to whom the pharmaceutical formulation is to be delivered. Pharmaceutical formulations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the composition isotonic with the blood of the intended subject to be administered. Aqueous and non-aqueous
10 sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In certain aspects pharmaceutical formulations comprise
15 pharmaceutically acceptable vehicles and can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[00139] In certain aspects, pharmaceutical formulations can be presented in unit/dose or
20 multi-dose containers, for example, in sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

[00140] In certain aspects, pharmaceutical formulations disclosed herein can be formulated for intravenous, intramuscular, subcutaneous, intraperitoneal, or inhalatory administration.

25 **V. Nucleic Acids and Vectors**

[00141] Effective concentrations of modified ACE2 polypeptides and modified ACE2 fusion polypeptides disclosed herein can be achieved via the transient or stable expression of a nucleic acid molecule encoding the polypeptides. For example, in certain aspects, a nucleic acid molecule encoding a modified ACE2 polypeptide or modified ACE2 fusion polypeptide
30 described herein can be incorporated into a vector and introduced into a cell. In certain aspects, a cell has one or more than one nucleic acid encoding a modified ACE2 polypeptide or modified ACE2 fusion polypeptide described herein.

[00142] Vectors can be introduced into a cell by a variety of methods, including transformation, transfection, direct uptake, projectile bombardment, and by encapsulation of

the vector in a liposome or nanoparticle. Examples of suitable methods of transfecting or transforming cells are calcium phosphate precipitation, electroporation, microinjection, infection, lipofection, and direct uptake. Such methods are described in more detail, for example, in Green *et al.*, *Molecular Cloning: A Laboratory Manual*, Fourth Edition (Cold Spring Harbor University Press, New York (2014)); and Ausubel *et al.*, *Current Protocols in Molecular Biology* (John Wiley & Sons, New York (2015)), the disclosures of each of which are incorporated herein by reference.

[00143] A variety of vectors for the delivery and expression of polynucleotides encoding exogenous polypeptides in a mammalian cell have been developed. Examples of expression vectors are disclosed in, *e.g.*, WO 1994/011026 and are incorporated herein by reference. Expression vectors for use in the compositions and methods described herein contain a polynucleotide sequence that encodes a modified ACE2 polypeptide or modified ACE2 fusion polypeptide as previously described as well as, *e.g.*, additional sequence elements used for the expression of the polypeptide and/or the integration of the polynucleotide sequence into the genome of a mammalian cell. Certain vectors that can be used include plasmids that contain regulatory sequences, such as promoter and enhancer regions, which direct gene transcription. Other useful vectors contain polynucleotide sequences that enhance the rate of translation or improve the stability or nuclear export of mRNA. These sequence elements include, *e.g.*, 5' and 3' UTR regions, an internal ribosomal entry site (IRES), and polyA in order to direct efficient transcription of the gene carried on the expression vector. The expression vectors suitable for use with the compositions and methods described herein may also contain a polynucleotide encoding a marker for selection of cells that contain such a vector. Examples of a suitable marker are a gene that encodes green fluorescent protein or a gene that encodes resistance to an antibiotic.

VI. Methods of Production

[00144] Also provided in the present disclosure are methods of producing modified ACE2 polypeptides and modified ACE2 fusion polypeptides described herein.

[00145] In certain aspects, a method is provided for producing a modified ACE2 polypeptide or modified ACE2 fusion polypeptide of the present disclosure by expressing a nucleic acid vector in a cell line. For example, in certain aspects, the expression cell line is ExpiCHO-S, Expi293F, or CHOK1SV GS-KO cells.

[00146] In certain aspects, expression methods of the present disclosure provide modified ACE2 polypeptides or modified ACE2 fusion polypeptides in which at least 10% (*e.g.*, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at

least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the polypeptides are glycosylated. In some embodiments, the percentage is a mole percentage. In some embodiments, glycosylation is measured by mass spectrometry.

5 [00147] In certain aspects, expression methods of the present disclosure provide modified ACE2 polypeptides or modified ACE2 fusion polypeptides in which at least 10% (*e.g.*, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the polypeptides are
10 glycosylated with N-acetylhexosamine. In some embodiments, the percentage is a mole percentage. In some embodiments, glycosylation is measured by mass spectrometry.

[00148] In certain aspects, expression methods of the present disclosure provide modified ACE2 polypeptides or modified ACE2 fusion polypeptides in which at least 20% (*e.g.*, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at
15 least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the glycosylations of the polypeptides are sialylated. In some embodiments, the percentage is a mole percentage. In some embodiments, sialylation is measured by mass spectrometry.

VII. Methods of Treatment

20 [00149] Also provided in the present disclosure are methods of treating SARS CoV-2 infection in a subject by administering a modified ACE2 polypeptide, a modified ACE2 fusion polypeptide, or a pharmaceutical formulation as described herein to the subject.

[00150] In certain aspects, the present disclosure provides a method of inhibiting SARS CoV-2 replication in a subject by administering to the subject a therapeutically or
25 prophylactically effective amount of a modified ACE2 polypeptide, a modified ACE2 fusion polypeptide, or a pharmaceutical formulation as described herein.

[00151] In certain aspects, the modified ACE2 polypeptide, the modified ACE2 fusion polypeptide, or the pharmaceutical formulation as described herein is administered to the subject intravenously, subcutaneously, intramuscularly, intratracheally, or by inhalation.

30 [00152] In certain aspects, the SARS CoV-2 strain is selected from Wuhan, alpha, beta, gamma, delta, or omicron variants.

EXAMPLES

[00153] The disclosure now being generally described, will be more readily understood by

reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present disclosure, and are not intended to limit the scope of the disclosure in any way.

EXAMPLE 1: Low sialylation of sACE2_{2.v2.4}-IgG1 is correlated with rapid clearance

in vivo

[00154] This example describes the *in vivo* clearance of modified ACE2 fusion proteins of the present disclosure.

[00155] Modified ACE2 fusion protein (sACE2_{2.v2.4}-IgG1) produced in either non-human ExpiCHO-S or human Expi293F cells, was intravenously (IV) administered to transgenic mice expressing human FcRn under the control of a human promoter (B6.Cg-Fcgrt^{tm1Dcr} Tg(FCGRT)32Dcr/DcrJ mice). As shown in FIGs. 1A and 1B, sACE2_{2.v2.4}-IgG1 produced in ExpiCHO-S cells was rapidly cleared. As shown in FIG. 1A, plasma ACE2 catalytic activity of sACE2_{2.v2.4}-IgG1 produced in ExpiCHO-S cells fell to levels close to background within 2 hours. In contrast, after IV administration of sACE2_{2.v2.4}-IgG1 protein produced in Expi293F cells, plasma ACE2 activity rapidly fell during the initial distribution phase for the first hour, but then remained elevated during a slower elimination phase (FIG 1A). At time points after 4 hours, catalytic activity in plasma of sACE2_{2.v2.4}-IgG1 produced in Expi293F cells was ~50-fold higher compared to sACE2_{2.v2.4}-IgG1 produced in ExpiCHO-S cells. As shown in FIG. 1B, these findings were confirmed by an ELISA optimized for detecting complete protein, in which the human IgG1 Fc moiety was captured on the assay plate surface and the ACE2 moiety detected with polyclonal antibody.

[00156] In brief, clear flat-bottom 96-well plates (Invitrogen) were coated overnight with donkey anti-human Fc γ at 0.3 μ g/ml in PBS. The plates were washed 4x with PBS containing 0.05% Tween 20 (PBS-T). Purified protein standards were prepared in mouse plasma. Samples and standards were diluted in PBS and incubated in wells for 1 h at room temperature. Plates were washed 4x with PBS-T and wells were incubated 1 h at room temperature with 0.3-1.0 μ g/ml polyclonal goat anti-human/mouse/rat/hamster ACE2 antibody in PBS. Plates were washed 4x with PBS-T and incubated 1 h at room temperature with 1:25,000 donkey anti-goat (H+L) antibody-HRP conjugate in PBS. Plates were washed 4x with PBS-T and developed with blotting solution containing soluble TMB (3,3',5,5'-tetramethylbenzidine) for 20 minutes. The reaction was ended with ELISA Stop Solution (0.16 M sulfuric acid) and absorbance at $\lambda = 450$ nm read on a microplate reader.

[00157] At 4 hours post-administration, serum levels of Expi293F-produced sACE2_{2.v2.4}-IgG1 were ~9 μ g/ml, whereas ExpiCHO-S-produced sACE2_{2.v2.4}-IgG1 levels had rapidly

fallen and were at ~0.2 µg/ml.

Glycomics and Glycopeptidomics

[00158] Analysis methods are previously described in Shajahan *et al.* (2021) *Glycobiology* 31:410-424 and incorporated by reference herein in its entirety.

- 5 **[00159]** In brief, for glycomics analysis, glycoproteins were diluted with 50 mM ammonium bicarbonate buffer, reduced with DTT, alkylated with iodoacetamide and desalted with 10 kDa molecular-weight cutoff centrifugal filtration devices. N-Glycans were released with PNGaseF (37 °C for 48 h) and permethylated. After the removal of N-glycans, the O-glycans were removed by beta elimination and samples were treated with 50 mM sodium hydroxide and sodium borohydride for 18 h at 45 °C. The released O-glycans were purified by Dowex H⁺ form ion exchange resin, lyophilized, borates were removed by a stream of nitrogen gas, and O-glycans were permethylated. Permethylated N- and O-glycans were analyzed by MALDI-TOF-MS. N- and O-glycan structures were assigned based on precursor masses (sodiated) and the common mammalian biosynthetic pathway.
- 10
- 15 **[00160]** Glycopeptide analysis and site mapping were performed using LC-MS/MS. Glycoprotein samples were reduced with DTT, alkylated with iodoacetamide, and digested with 0.5 µg/µl sequencing-grade trypsin at 37 °C for 16 h. Peptides were analyzed on an nanoscale liquid chromatography (nano-LC) column connected to a mass spectrometer. Nano-LC columns of 15 cm length with 75 µm internal diameter, filled with 3 µm C18
- 20 reverse phase material were used for chromatographic separation of the samples. The separation conditions were low to high acetonitrile in a solution containing 0.1% formic acid, and the separation time was 1 h. The precursor ion scan was acquired at 120,000 resolution in an ion trap mass analyzer and precursors at a time frame of 3 s were selected for subsequent fragmentation using HCD. Charge state screening was enabled, and precursors with unknown
- 25 charge state or a charge state of +1 were excluded. Dynamic exclusion was enabled (exclusion duration of 30 s). The fragment ions were analyzed on an ion trap mass analyzer for HCD at 30,000 resolution. The glycoproteomic data were processed with peptide and protein identification software and searched against the sequences and a catalogue of more than 180 N-glycans and 9 common O-glycans. The precursor mass tolerance and fragment
- 30 mass tolerances were set to 5 ppm and 10 ppm respectively. Additional modifications including deamidation of N and Q, carboxymethylation of C, and oxidation of precursor were also included in the search. Assignments were made using peptide and protein identification software (Delta Mod. Score ≥10, Log Prob>3) and manual interpretation. Raw files retrieved from nanoLC-MS were deconvoluted, and monoisotopic peak areas for each glycopeptide

found in the peptide and protein identification software were manually pooled. The relative percentages of each glycoform were determined by deconvolution of the LC-MS data at the full MS level, then determining the area under the curve for each full MH⁺. Relative abundance was then calculated. Suggested glycan structures were predicted. Glycoforms are based on the assumed biosynthetic pathway.

[00161] As shown in FIGs. 2A, 2B, 3A, and 3B, and summarized in FIGs. 4A, 4B, and 4C, glycomics analysis using mass spectrometry methods revealed that N-glycans of sACE2_{2.v2.4}-IgG1 produced in ExpiCHO-S cells (FIG. 2A and FIG. 2B) are 5.5-fold less sialylated than sACE2_{2.v2.4}-IgG1 produced in Expi293F cells (FIG. 3A and FIG. 3B).

Furthermore, O-glycans at Thr730 of ExpiCHO-S produced sACE2_{2.v2.4}-IgG1 are almost exclusively monosialylated core 1 structures (FIG. 4D), whereas O-glycans decorating sACE2_{2.v2.4}-IgG1 produced in Expi293F cells are more complex and 80% disialylated (FIG. 4E). High sialylation is thus correlated with extended activity of sACE2_{2.v2.4}-IgG1 *in vivo*.

EXAMPLE 2: Derivatives of sACE2_{2.v2.4}-IgG1 have higher catalytic activity *in vitro* and *in vivo*

[00162] This example describes the *in vitro* and *in vivo* catalytic activities of modified ACE2 fusion proteins derived from sACE2_{2.v2.4}-IgG1.

Rosetta-Based Stability Design

[00163] Stability was optimized using an *in silico* rational protein engineering approach based on the cryo-EM structure of human ACE2 (PDB 6M17). Stabilizing mutations in ACE2 were identified using the Rosetta software package (Leman *et al.* (2020) *Nat Methods* 17:665-680, incorporated by reference in its entirety). First, truncated models of ACE2 monomers (amino acids [a.a.] 21-615 and 21-732) underwent backbone and sidechain optimization via Rosetta Relax with score function ref2015. The protocol performed site saturation mutagenesis for both relaxed construct models, allowing neighboring amino acids to repack around a mutated residue (for example, as described in Frenz *et al.* (2020) *Front Bioeng Biotechnol* 8, incorporated by reference in its entirety). The mutants were ranked according to the difference in score between the wild type amino acid and the substitutions. Mutations with residues near the ACE2 active site, at the interface with SARS-CoV2 RBD, or conserved between human and mouse ACE2 were excluded, thus focusing design to residues that were expected to tolerate substitutions. Visual/manual inspection was performed for final selection of protein variants.

Disulfide Design

[00164] Disulfide bonds require specific geometric constraints for formation. The disulfidizer

mover within Rosetta substitutes all pairs of residues with Cys to evaluate proper geometries for disulfide formation (Bhardwaj *et al.* (2016) *Nature* 538: 329-335). Those that satisfied this geometric constraint were then sorted by disulfide score (dslf_fa13) and visually inspected.

- 5 **[00165]** As shown in FIG. 5A, sACE2_{2.v2.4}-IgG1 encompasses human ACE2 residues 18-732 and is a stable dimer in both the ACE2 and IgG1 Fc moieties. As shown in FIGs. 5B, 5C, and 5D, three additional design strategies were explored. First, human and mouse ACE2 sequences were compared to identify polymorphic residues. Mouse ACE2 fused to murine IgG1 Fc has a long serum half-life (elimination phase $t_{1/2\beta}$) of 174 hours following
- 10 intravenous (IV) administration (Liu *et al.* (2018) *Kidney Int* 94:114-125). The polymorphic residues were selected for computational saturation mutagenesis and modelling using ROSETTA software (Leman *et al.* (2020) *Nat Methods* 17: 665-680). Multiple mutations were identified that decrease the computed ΔG for folding. Second, residue pairs were identified that when mutated to cysteine have a high probability (based on ROSETTA
- 15 modeling) of oxidizing to form disulfides that constrain conformational dynamics. Seven designs with predicted stabilizing point substitutions (2 to 3 mutations per design) and three designs with engineered disulfides were expressed and purified (TABLE 5).

TABLE 5: SACE22.v2.4-IgG1 Derivatives Designed for Serum Stability

Construct Name	Class	Mutations
sACE2 ₂ .S01-IgG1	Stabilization (Packing)	I663W, A673Y
sACE2 ₂ .S02-IgG1	Stabilization (Packing)	A673Y, I694F
sACE2 ₂ .S03-IgG1	Stabilization (Packing)	V491I, I679Y
sACE2 ₂ .S04-IgG1	Stabilization (Packing)	N682W, A687F
sACE2 ₂ .S05-IgG1	Stabilization (Packing)	S167I, A246L, I694F
sACE2 ₂ .S06-IgG1	Stabilization (Packing)	V491I, A673Y, A687F, T698E
sACE2 ₂ .S07-IgG1	Stabilization (Packing)	S167I, V491I, H535F, A673V
sACE2 ₂ .S08-IgG1	Stabilization (Disulfide)	K288C, E433C
sACE2 ₂ .S09-IgG1	Stabilization (Disulfide)	L624C, A632C
sACE2 ₂ .S10-IgG1	Stabilization (Disulfide)	E166C, V691C

20 *N-Glycosylation Site Design*

[00166] N-glycosylation sites require a specific motif: Asn-X-Ser/Thr, where X is any amino acid other than proline. The previously described Rosetta-generated mutational data allowed

for the identification of energetically favorable substitutions that result in an N-glycosylation motif. Potential glycosylation sites were selected based on 1) Rosetta score, 2) minimal number of mutations compared to original sequence, 3) preference for Thr compared to Ser at the third position due to enhanced glycosylation efficiency, and 4) comparison to the mouse ACE2 sequence (UniProtKB - Q8R0I0), which suggested glycosylation sites unlikely to disrupt folding/function.

[00167] The third design strategy shielded sACE2₂.v2.4-IgG1 from proteases by introduction of novel N-glycosylation sites. Borrowing from the murine ACE2 sequence (which has two additional N-glycosylation motifs compared to human ACE2), as well as modeling the introduction of N-glycosylation motifs (Asn-X-Ser/Thr, where X is any amino acid other than proline) at other sites using ROSETTA, seven derivatives of sACE2₂.v2.4-IgG1 were designed with 1 to 3 new glycosylation motifs (TABLE 6).

TABLE 6: sACE2₂.v2.4-IgG1 Derivatives Designed with Additional Glycosylation Sites

Construct Name	Class	Mutations
sACE2 ₂ .S11-IgG1	Glycosylation	M662T
sACE2 ₂ .S12-IgG1	Glycosylation	E536N, P538S, M662T
sACE2 ₂ .S13-IgG1	Glycosylation	E536N, P538S, N580T, M662T
sACE2 ₂ .S14-IgG1	Glycosylation	M662T, N720S
sACE2 ₂ .S15-IgG1	Glycosylation	D615N, M662T, N720S
sACE2 ₂ .S16-IgG1	Glycosylation	E536N, P538S, M662T, Q728N
sACE2 ₂ .S17-IgG1	Glycosylation	K631N, Y633T, M662T, Q728N

[00168] Finally, two sACE2₂.v2.4-IgG1 proteins were designed that mixed features of the different design strategies (TABLE 7).

TABLE 7: sACE2₂.v2.4-IgG1 Derivatives Designed for Serum Stability and Additional Glycosylation Sites

Construct Name	Class	Mutations
sACE2 ₂ .S18-IgG1	Mixed	M662T, I694F
sACE2 ₂ .S19-IgG1	Mixed	V491I, M662T, N720S

Cell Lines and Transfection

[00169] Expi293F cells were cultured at 37 °C, 125 r.p.m., 8% CO₂, in Expi293 Expression Medium. Expi293F were transfected at a density of 2 x 10⁶ ml⁻¹ with 0.5-1.0 µg plasmid

DNA per mL of culture. Transfection enhancers 1 (5 μ l per ml of culture) and 2 (50 μ l per ml of culture) were added ~18 h post-transfection and medium was collected on day 5-7.

ExpiCHO-S cells were cultured at 37 °C, 125 r.p.m., 8% CO₂, in ExpiCHO Expression Medium. ExpiCHO-S were transfected at a density of 6×10^6 ml⁻¹ with 1.0 μ g plasmid DNA per ml of culture using Expifectamine CHO Reagent according to the manufacturer's directions. Expifectamine CHO Enhancer (6 μ l per ml of culture) and ExpiCHO Feed (240 μ l per ml of culture) were added ~20 h later and the temperature was lowered (33 °C). ExpiCHO Feed (240 μ l per ml of culture) was added again on day 5. CO₂ was decreased step-wise on days 9-12 to 5% final. The medium was harvested on days 12-14.

Plasmids

[00170] The expression plasmid for sACE2_{v2.4}-IgG1 is previously described (*Chan et al.* (2020) *Science* (1979)369: 1261-1265) and deposited. Briefly, the coding sequence is ligated into the NheI-XhoI sites of pcDNA3.1(+) and encompasses human ACE2 (GenBank NM_021804.1) amino acids (a.a.) M1-G732 fused via a 1x serine linker to human IgG1 Fc a.a. D221-K447 (nG1m1 isoallotype; GenBank KY432415.1). Targeted mutations were introduced by overlap extension PCR and confirmed by Sanger sequencing.

Protein Purification

[00171] Expression medium was centrifuged (800 g, 4 °C, 10 min) to remove cells and the pH adjusted to 7.5 with 1M Tris base. Insoluble particulates were removed by centrifugation (15,000 g, 4 °C, 20 min). The supernatant was incubated with Protein A affinity chromatography resin (2 ml resin per 100 ml medium) for 1-2 h at 4 °C. Resin was collected by passing through a chromatography column and washed with 10 column volumes (CV) of Dulbecco's phosphate-buffered saline (PBS). Fc-fusion proteins were eluted with 4 CV 60 mM sodium acetate pH 3.7 and rapidly neutralized by collecting in 2 CV 1M Tris pH 8.0. The pH was further raised to ~7.5 with an additional 1-2 CV 1M Tris base. Eluted proteins were concentrated with a centrifugal filtration device and injected on a dextran-agarose composite matrix gel filtration column equilibrated with PBS. Peak fractions were pooled, concentrated, and protein aliquots snap frozen in liquid nitrogen for storage at -80 °C. Concentrations were determined using calculated extinction coefficients and absorbance at $\lambda = 280$ nm.

Pharmacokinetics in Human FcRn Mice

[00172] Male B6.Cg-Fcgrt^{tm1Dcr} Tg(FCGRT)32Dcr/DcrJ mice (JAX stock # 014565; 6-8 weeks old; 3 mice per group) were housed in ventilated cages with HEPA filtered air and provided filtered tap water (pH 2.5-3.0) and standard lab chow ad libitum. Artificial

fluorescent lighting was on a 12 h light/dark cycle, temperature 20-26 °C and humidity 30-70%. Animals were administered protein solutions IV (tail vein) or SC (flank) diluted in PBS and sterile filtered for a final injection volume of 4 ml / kg. In life, blood was collected via retroorbital route into heparin tubes. At terminal time points, mice were euthanized via CO₂ asphyxiation and blood was collected via cardiocentesis into heparin. Processed plasma samples were stored at -80 °C. Experiments were conducted under institutional review and IACUC approval at JAX. Pharmacokinetic parameters were fitted with PKSolver 2.0 using the linear trapezoidal method.

ACE2 Catalytic Activity Assay

[00173] Hydrolysis of a quenched fluorescent peptide substrate was measured on a microplate reader using a Fluorometric ACE2 Activity Assay Kit according to the manufacturer's directions.

Biolayer Interferometry

[00174] For measuring kinetics of sACE2₂-IgG1 / RBD interactions, responses were recorded on a biolayer interferometry (BLI) biosensor and analyzed with a 1:1 binding model (global fit) using instrument software. sACE2₂-IgG1 proteins were immobilized at 100 nM for 60 s to AHC (anti-human IgG Fc) biosensors in assay buffer (10 mM HEPES pH 7.6, 150 mM NaCl, 3 mM EDTA, 0.05% polysorbate 20, 0.5% non-fat dry milk). Loaded sensors were equilibrated in assay buffer, transferred to delta RBD-8h solutions to measure association, and transferred back to buffer to measure dissociation. The purification of delta RBD is previously described (Zhang *et al.* (2022) *Nat Chem Biol* 18: 342-351).

[00175] For measuring kinetics of sACE2₂-IgG1 / FcRn interactions, responses were recorded on a biolayer interferometry (BLI) instrument and analyzed with a 1:1 binding model (global fit) using instrument software. FcRn with a C-terminal 6xHis tag (SEQ ID NO: 42) and complexed with beta-2-microglobulin was immobilized on anti-his biosensors to reach a response of 1 nm. The biosensors were equilibrated for 120 s to achieve a stable baseline in PBS containing 0.1% Tween 20 plus 0.02% bovine serum albumin at pH 6.0 or pH 7.4. Biosensors were then transferred to sACE2₂-IgG1 solutions in the same buffer for association kinetics and back to buffer for dissociation kinetics.

[00176] Of the 19 derivatives of sACE2₂.v2.4-IgG1, 15 were secreted at high levels in transiently transfected Expi293F culture and were purified. While the designs were all catalytically active, as shown in FIGs. 6A, 6B, and 6C, those with cavity-filling mutations tended to have reduced proteolytic activity. A subset of 3 designed proteins ("Stability/S" designs S14, S15, and S19), which shared mutations M662T and N720S to add N-glycans at

residues 660 and 718 in the collectrin-like domain (CLD), were found to have higher catalytic activity. This subset of sACE2₂.v2.4-IgG1 derivatives were also among the tighter binders to the receptor-binding domain (RBD) of the delta SARS-CoV-2 variant, as determined by biolayer interferometry (BLI) under conditions that measure monovalent affinity (TABLE 8).

TABLE 8: BLI Kinetics for Monovalent Binding to Delta Receptor Binding Domain ⁽¹⁾

Immobilized Protein	KD (nM)	k _{on} (M ⁻¹ s ⁻¹)	k _{off} (s ⁻¹)	χ ² (2)	R ² (2)
sACE2 ₂ .S01-IgG1	1.0	8.7 × 10 ⁵	8.4 × 10 ⁻⁴	0.016	0.998
sACE2 ₂ .S02-IgG1	1.1	8.2 × 10 ⁵	9.2 × 10 ⁻⁴	0.015	0.998
sACE2 ₂ .S03-IgG1	1.3	8.8 × 10 ⁵	1.2 × 10 ⁻³	0.021	0.996
sACE2 ₂ .S04-IgG1	1.4	8.3 × 10 ⁵	1.2 × 10 ⁻³	0.030	0.994
sACE2 ₂ .S06-IgG1	1.6	8.6 × 10 ⁵	1.4 × 10 ⁻³	0.032	0.994
sACE2 ₂ .S09-IgG1	1.4	8.6 × 10 ⁵	1.2 × 10 ⁻³	0.028	0.994
sACE2 ₂ .S11-IgG1	0.9	8.8 × 10 ⁵	8.2 × 10 ⁻⁴	0.010	0.998
sACE2 ₂ .S12-IgG1	1.1	9.3 × 10 ⁵	1.0 × 10 ⁻³	0.011	0.997
sACE2 ₂ .S13-IgG1	0.9	9.9 × 10 ⁵	8.7 × 10 ⁻⁴	0.009	0.997
sACE2 ₂ .S14-IgG1	0.9	9.2 × 10 ⁵	8.7 × 10 ⁻⁴	0.014	0.994
sACE2 ₂ .S15-IgG1	0.8	9.3 × 10 ⁵	7.3 × 10 ⁻⁴	0.012	0.997
sACE2 ₂ .S16-IgG1	0.9	9.3 × 10 ⁵	8.4 × 10 ⁻⁴	0.009	0.995
sACE2 ₂ .S17-IgG1	0.7	9.8 × 10 ⁵	6.9 × 10 ⁻⁴	0.010	0.997
sACE2 ₂ .S18-IgG1	0.8	9.0 × 10 ⁵	7.6 × 10 ⁻⁴	0.017	0.996
sACE2 ₂ .S19-IgG1	0.6	9.3 × 10 ⁵	5.8 × 10 ⁻⁴	0.016	0.996
(1) Dimeric sACE2 ₂ .v2.4-IgG1 proteins were immobilized to an anti-human IgG sensor. Sensors were transferred to monomeric RBD-8h at 90, 30, and 10 nM.					
(2) χ ² and R ² were determined from curves fitted to the entire data.					

[00177] The proteins were administered IV at 2 mg/kg to human FcRn mice and catalytic activity in plasma was measured (FIGs. 7A, 7B, and 7C). The half-life (calculated as a combination of distribution phase t_{1/2α} and elimination phase t_{1/2β}) of sACE2₂.v2.4-IgG1 was 15.3 hours and the area under the curve (AUC_{0-48h}) was 68.9 (μM product/minute) × h. Derivatives with cavity-filling mutations tended to have faster clearance, while derivatives with added glycosylation motifs were generally similar to the parental sACE2₂.v2.4-IgG1 protein. However, the subset of derivatives S14, S15, and S19 had higher activity in plasma, consistent with their higher activity *in vitro*. Full length protein was detected by immunoblot analysis of serum from a single mouse administered sACE2₂.S14-IgG1 as a representative example (FIG. 7D). The most active protein in mice, sACE2₂.S19-IgG1, had t_{1/2} = 21.0 hours and AUC_{0-48h} = 136.8 (μM product/minute) × h.

[00178] Glycomics analysis indicated that sACE2₂.S19-IgG1, expressed and purified from Expi293F cell culture, was highly sialylated and similar to parental sACE2₂.v2.4-IgG1

protein (FIG. 8A, 8B, 9A, 9B, and 9C). Glycopeptidomics analysis of sACE2₂.S19-IgG1 produced in Expi293F cell culture was compared to the parental sACE2₂.v2.4-IgG1 protein produced in Expi293F cells and ExpiCHO-S cells. This analysis provided quantitative determination of the glycoforms present at individual N-glycosylated sites (TABLES 9-18).

5 **TABLE 9: Relative Abundance of Glycoforms on Peptide YNTNITEENVQNMNAGD (SEQ ID NO: 43), Position N53**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(3)Hex(3) [2525.2440]		2.3%	2.6%	1.0%
HexNAc(2)Hex(5) [3214.4818]		2.1%	3.0%	0.2%
HexNAc(3)Hex(3)Fuc(1) [3360.5397]		0.7%	1.0%	0.4%
HexNAc(4)Hex(3) [3376.5346]		6.0%	5.2%	4.1%
HexNAc(4)Hex(3)Fuc(1) [3538.5875]		5.4%	5.7%	3.4%
HexNAc(4)Hex(4) [3563.6191]		1.7%	1.2%	0.8%
HexNAc(5)Hex(3) [3579.6140]		6.1%	5.5%	10.3%
HexNAc(4)Hex(4)Fuc(1) [3700.6403]		1.0%	1.0%	0.9%
HexNAc(5)Hex(3)Fuc(1) [3766.6985]		7.5%	8.8%	13.1%
HexNAc(5)Hex(4) [3782.6934]		2.5%	2.5%	0.8%
HexNAc(6)Hex(3) [3862.6931]		1.2%	0.8%	14.3%
HexNAc(4)Hex(4)NeuAc(1) [3870.7094]		0.9%	1.1%	ND
HexNAc(4)Hex(5)Fuc(1) [3928.7513]		ND	0.1%	0.6%
HexNAc(5)Hex(4)Fuc(1) [3944.7462]		3.5%	4.6%	1.0%
HexNAc(5)Hex(5) [3969.7778]		2.0%	1.1%	0.2%
HexNAc(6)Hex(3)Fuc(1) [3985.7727]		1.3%	1.3%	20.8%
HexNAc(6)Hex(4) [4016.7673]		0.2%	0.5%	2.2%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [4024.7459]		0.8%	0.9%	ND
HexNAc(4)Hex(5)NeuAc(1) [4026.7993]		1.8%	2.3%	ND
HexNAc(5)Hex(4)NeuAc(1) [4026.7993]		1.2%	1.1%	ND

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
[4032.7622]				
HexNAc(5)Hex(5)Fuc(1) [4073.7888]		2.1%	2.3%	0.6%
HexNAc(5)Hex(6) [4090.8041]		1.3%	0.7%	0.7%
HexNAc(6)Hex(4)Fuc(1) [4106.7990]		0.5%	0.5%	2.7%
HexNAc(6)Hex(5) [4131.8307]		0.3%	0.2%	0.5%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [4147.8256]		0.5%	0.7%	ND
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [4172.8572]		2.0%	2.8%	ND
HexNAc(5)Hex(5)NeuAc(1) [4219.8467]		1.3%	1.1%	ND
HexNAc(5)Hex(6)Fuc(1) [4235.8416]		0.4%	0.8%	2.5%
HexNAc(6)Hex(5)Fuc(1) [4276.8682]		0.3%	0.4%	1.5%
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [4293.8835]		2.1%	3.8%	ND
HexNAc(5)Hex(6)NeuAc(1) [4309.8784]		1.9%	3.3%	ND
HexNAc(6)Hex(6)Fuc(1) [4334.9100]		0.2%	0.2%	2.3%
HexNAc(6)Hex(7) [4422.9261]		1.4%	0.1%	5.4%
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [4438.9210]		2.5%	4.9%	ND
HexNAc(6)Hex(7)Fuc(1) [4455.9363]		0.3%	0.1%	7.4%
HexNAc(5)Hex(6)NeuAc(2) [4526.9370]		0.9%	1.5%	ND
HexNAc(6)Hex(7)NeuAc(1) [4584.9789]		2.8%	1.4%	0.4%
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [4600.9738]		1.8%	2.7%	ND
HexNAc(6)Hex(7)Fuc(1)NeuAc(1) [4659.0157]		1.5%	3.4%	1.2%
HexNAc(6)Hex(6)Fuc(1)NeuAc(2) [4672.9949]		1.5%	1.1%	ND
HexNAc(6)Hex(7)NeuAc(2) [4730.0164]		10.9%	6.5%	ND
HexNAc(6)Hex(7)Fuc(1)NeuAc(2) [4747.0317]		11.1%	8.3%	ND

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(6)Hex(7)NeuAc(3) [4788.0583]		1.5%	0.5%	ND
HexNAc(6)Hex(7)Fuc(1)NeuAc(3) [4821.0685]		2.2%	1.4%	ND

(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed.
(2) ND, Not Detected.
(3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.

TABLE 10: Relative Abundance of Glycoforms on Peptide**EQSTTAQMYPLQEIQNLTVK (SEQ ID NO: 44), Position N90**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(3)Hex(3) [3417.5612]		3.8%	4.9%	4.2%
HexNAc(2)Hex(5) [3538.5875]		3.0%	4.4%	1.8%
HexNAc(3)Hex(3)Fuc(1) [3563.6191]		1.5%	2.1%	4.5%
HexNAc(3)Hex(4) [3579.614]		0.2%	0.6%	0.5%
HexNAc(4)Hex(3) [3620.6405]		3.5%	4.2%	8.6%
HexNAc(4)Hex(3)Fuc(1) [3766.6985]		6.3%	6.7%	19.8%
HexNAc(4)Hex(4) [3782.6934]		3.7%	3.4%	3.8%
HexNAc(5)Hex(3) [3823.7199]		0.3%	0.5%	4.6%
HexNAc(4)Hex(4)Fuc(1) [3928.7513]		5.6%	5.1%	7.5%
HexNAc(4)Hex(5) [3944.7462]		1.8%	1.4%	3.3%
HexNAc(5)Hex(3)Fuc(1) [3969.7778]		1.3%	1.8%	16.1%
HexNAc(5)Hex(4) [3985.7727]		0.5%	0.6%	0.6%
HexNAc(4)Hex(4)NeuAc(1) [4073.7888]		5.4%	5.6%	0.0%
HexNAc(4)Hex(5)Fuc(1) [4090.8041]		1.8%	1.4%	5.9%
HexNAc(5)Hex(4)Fuc(1) [4131.8307]		1.2%	1.4%	1.8%
HexNAc(6)Hex(3)Fuc(1) [4172.8572]		0.1%	0.1%	7.0%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [4219.8467]		12.7%	11.6%	0.2%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(4)Hex(5)NeuAc(1) [4235.8416]		7.5%	6.8%	0.2%
HexNAc(5)Hex(5)Fuc(1) [4293.8835]		0.8%	0.8%	1.5%
HexNAc(5)Hex(6) [4309.8784]		0.1%	0.2%	0.6%
HexNAc(6)Hex(4)Fuc(1) [4334.91]		0.0%	0.1%	0.9%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [4381.8995]		13.7%	12.0%	0.6%
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [4422.9261]		1.8%	2.1%	0.1%
HexNAc(5)Hex(6)Fuc(1) [4455.9363]		0.1%	0.2%	2.0%
HexNAc(4)Hex(5)NeuAc(2) [4526.937]		2.0%	1.9%	ND
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [4584.9789]		1.7%	1.9%	0.0%
HexNAc(5)Hex(6)NeuAc(1) [4600.9738]		0.5%	0.3%	0.0%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [4672.9949]		6.1%	5.1%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [4747.0317]		1.7%	2.0%	0.3%
HexNAc(6)Hex(7)Fuc(1) [4821.0685]		0.0%	ND	0.5%
HexNAc(5)Hex(5)Fuc(1)NeuAc(2) [4876.0743]		1.2%	1.1%	ND
HexNAc(5)Hex(6)NeuAc(2) [4892.0692]		0.8%	0.7%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [5038.1271]		3.0%	2.7%	ND
HexNAc(6)Hex(6)Fuc(1)NeuAc(2) [5241.2065]		0.5%	0.4%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(3) [5329.2226]		1.3%	1.0%	ND
HexNAc(6)Hex(7)Fuc(1)NeuAc(2) [5403.2593]		0.9%	1.0%	0.0%
HexNAc(6)Hex(7)Fuc(1)NeuAc(3) [5694.3547]		0.6%	0.5%	ND
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.				

**TABLE 11: Relative Abundance of Glycoforms on Peptide
LQLQALQQNGSSVLSEDK (SEQ ID NO: 45), Position N103**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(3)Hex(3) [3053.4155]		0.5%	0.7%	0.9%
HexNAc(2)Hex(5) [3174.4418]		3.4%	3.2%	1.6%
HexNAc(3)Hex(3)Fuc(1) [3199.4734]		3.6%	3.0%	7.5%
HexNAc(4)Hex(3) [3256.4949]		0.9%	1.0%	2.6%
HexNAc(3)Hex(4)Fuc(1) [3361.5262]		1.5%	0.6%	0.6%
HexNAc(4)Hex(3)Fuc(1) [3402.5528]		24.0%	26.0%	41.5%
HexNAc(4)Hex(4)Fuc(1) [3564.6056]		16.6%	17.8%	15.0%
HexNAc(5)Hex(3)Fuc(1) [3605.6322]		3.1%	2.6%	10.0%
HexNAc(4)Hex(5)Fuc(1) [3726.6584]		6.2%	10.7%	16.5%
HexNAc(5)Hex(4)Fuc(1) [3767.685]		2.4%	2.2%	0.4%
HexNAc(6)Hex(3)Fuc(1) [3808.7115]		0.1%	0.0%	0.6%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [3855.701]		4.9%	5.5%	0.0%
HexNAc(5)Hex(5)Fuc(1) [3929.7378]		1.1%	0.8%	0.4%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [4017.7538]		9.4%	0.0%	ND
HexNAc(5)Hex(6)Fuc(1) [4091.7906]		0.6%	0.3%	0.7%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [4308.8493]		16.7%	22.0%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [4382.886]		0.7%	0.2%	0.0%
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [4673.9815]		1.0%	0.6%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(3) [4965.0769]		0.3%	0.6%	ND
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.				

TABLE 12: Relative Abundance of Glycoforms on Peptide**FFVSVGLPNMTQGFWEYSMLTDPGNVQK (SEQ ID NO: 46), Position N322**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(2)Hex(5) [4408.9398]		2.2%	3.2%	ND
HexNAc(3)Hex(3)Fuc(1) [4433.9714]		1.2%	4.6%	ND
HexNAc(4)Hex(3)Fuc(1) [4637.0508]		1.9%	7.2%	ND
HexNAc(5)Hex(3)Fuc(1) [4840.1301]		41.5%	33.6%	59.0%
HexNAc(2)Hex(8) [4895.0982]		2.3%	0.5%	ND
HexNAc(6)Hex(3) [4897.1516]		0.7%	2.4%	41.0%
HexNAc(5)Hex(4)Fuc(1) [5002.183]		22.9%	22.9%	ND
HexNAc(5)Hex(5)Fuc(1) [5164.2358]		21.5%	19.1%	ND
HexNAc(6)Hex(4)Fuc(1) [5205.2623]		2.8%	1.2%	ND
HexNAc(6)Hex(5)Fuc(1) [5367.3152]		3.0%	4.1%	ND
HexNAc(6)Hex(6)NeuAc(1) [5674.4055]		ND	1.2%	ND
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.				

5 TABLE 13: Relative Abundance of Glycoforms on Peptide**SIGLLSPDFQEDNETEINFLK (SEQ ID NO: 47), Position N432**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
Not Glycosylated [2522.2661]		10.9%	9.9%	0.9%
HexNAc(1) [2725.3454]		3.4%	6.4%	0.2%
HexNAc(2)Hex(3)Fuc(1) [3560.6412]		0.0%	0.0%	1.0%
HexNAc(3)Hex(3) [3617.6627]		0.3%	0.5%	0.2%
HexNAc(2)Hex(5) [3738.6889]		3.6%	2.8%	2.1%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(3)Hex(3)Fuc(1) [3763.7206]		1.6%	2.2%	6.9%
HexNAc(3)Hex(4)Fuc(1) [3925.7734]		0.1%	0.2%	0.8%
HexNAc(4)Hex(3)Fuc(1) [3966.7999]		10.4%	10.1%	23.2%
HexNAc(4)Hex(4)Fuc(1) [4128.8528]		6.4%	6.8%	5.4%
HexNAc(5)Hex(3)Fuc(1) [4169.8793]		7.3%	7.5%	30.3%
HexNAc(4)Hex(5)Fuc(1) [4290.9056]		5.8%	6.6%	5.9%
HexNAc(5)Hex(4)Fuc(1) [4331.9321]		9.4%	10.1%	4.0%
HexNAc(6)Hex(3)Fuc(1) [4372.9587]		0.6%	0.6%	3.7%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [4419.9482]		1.3%	1.0%	0.0%
HexNAc(5)Hex(5)Fuc(1) [4493.985]		7.6%	8.9%	5.3%
HexNAc(6)Hex(4)Fuc(1) [4535.0115]		1.0%	0.8%	0.2%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [4582.001]		5.7%	4.4%	0.0%
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [4623.0276]		1.2%	0.9%	0.0%
HexNAc(5)Hex(6)Fuc(1) [4656.0378]		5.0%	7.0%	8.3%
HexNAc(6)Hex(5)Fuc(1) [4697.0643]		0.9%	0.8%	0.0%
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [4785.0804]		3.7%	2.9%	0.0%
HexNAc(6)Hex(6)Fuc(1) [4859.1172]		0.8%	0.6%	0.8%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [4873.0964]		1.8%	1.0%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [4947.1332]		4.9%	4.2%	0.1%
HexNAc(6)Hex(7)Fuc(1) [5021.17]		0.6%	0.6%	0.5%
HexNAc(5)Hex(5)Fuc(1)NeuAc(2) [5076.1758]		0.5%	0.2%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [5238.2286]		3.0%	1.6%	0.0%
HexNAc(6)Hex(7)Fuc(1)NeuAc(1) [5312.2654]		0.5%	0.4%	0.0%
HexNAc(6)Hex(7)Fuc(1)NeuAc(3) [5894.4562]		0.6%	0.2%	0.0%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.				

TABLE 14: Relative Abundance of Glycoforms on Peptide CDISNSTEAGQK (SEQ ID NO: 48), Position N546

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(1) [1512.6482]		0.7%	1.7%	0.1%
HexNAc(2)Hex(3)Fuc(1) [2347.9440]		0.2%	0.3%	1.3%
HexNAc(3)Hex(3) [2404.9655]		0.6%	0.6%	1.7%
HexNAc(2)Hex(5) [2525.9917]		7.6%	7.8%	13.4%
HexNAc(3)Hex(3)Fuc(1) [2551.0234]		8.1%	8.3%	14.3%
HexNAc(3)Hex(4) [2567.0183]		0.4%	0.5%	0.0%
HexNAc(2)Hex(6) [2688.0446]		0.6%	0.7%	0.1%
HexNAc(3)Hex(4)Fuc(1) [2713.0762]		6.1%	6.5%	3.5%
HexNAc(4)Hex(3)Fuc(1) [2754.1027]		6.0%	5.4%	10.2%
HexNAc(2)Hex(7) [2850.0974]		0.6%	0.7%	0.0%
HexNAc(3)Hex(4)NeuAc(1) [2858.1137]		1.3%	1.3%	0.1%
HexNAc(3)Hex(5)Fuc(1) [2875.1290]		1.9%	2.5%	0.1%
HexNAc(4)Hex(3)NeuAc(1) [2899.1402]		2.7%	2.3%	3.2%
HexNAc(4)Hex(4)Fuc(1) [2916.1556]		4.8%	4.3%	4.5%
HexNAc(5)Hex(3)Fuc(1) [2957.1821]		6.2%	5.8%	20.4%
HexNAc(3)Hex(4)Fuc(1)NeuAc(1) [3004.1716]		7.2%	7.4%	ND
HexNAc(2)Hex(8) [3012.1502]		0.5%	0.6%	0.0%
HexNAc(4)Hex(4)NeuAc(1) [3061.1931]		1.7%	1.6%	2.6%
HexNAc(4)Hex(5)Fuc(1) [3078.2084]		2.7%	2.7%	3.1%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(5)Hex(4)Fuc(1) [3119.2349]		4.3%	3.8%	2.7%
HexNAc(6)Hex(3)Fuc(1) [3160.2615]		0.7%	0.6%	7.2%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [3207.2510]		4.3%	3.8%	0.1%
HexNAc(5)Hex(5)Fuc(1) [3281.2878]		2.6%	2.3%	2.3%
HexNAc(6)Hex(4)Fuc(1) [3322.3143]		0.5%	0.4%	0.9%
HexNAc(3)Hex(6)Fuc(1)NeuAc(1) [3328.2773]		0.2%	0.5%	ND
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [3369.3038]		4.9%	5.1%	0.9%
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [3410.3304]		2.5%	2.6%	0.1%
HexNAc(5)Hex(6)Fuc(1) [3443.3406]		1.7%	1.6%	3.1%
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [3572.3832]		2.9%	2.9%	0.3%
HexNAc(6)Hex(6)Fuc(1) [3646.4200]		0.2%	0.1%	0.5%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [3660.3992]		1.8%	1.8%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [3734.4360]		3.3%	3.2%	1.0%
HexNAc(6)Hex(7)Fuc(1) [3808.4728]		0.2%	0.1%	0.6%
HexNAc(5)Hex(5)Fuc(1)NeuAc(2) [3863.4786]		0.6%	0.7%	ND
HexNAc(6)Hex(6)Fuc(1)NeuAc(1) [3937.5154]		0.6%	0.4%	0.0%
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [4025.5314]		3.1%	3.5%	0.0%
HexNAc(6)Hex(7)Fuc(1)NeuAc(1) [4099.5682]		0.7%	0.6%	0.4%
HexNAc(6)Hex(6)Fuc(1)NeuAc(2) [4228.6108]		0.7%	0.7%	ND
HexNAc(6)Hex(7)Fuc(1)NeuAc(2) [4390.6636]		1.4%	1.5%	ND
(1) Only glycopeptides with relative abundance ≥ 0.5% are listed. (2) ND, Not Detected.				

TABLE 15: Relative Abundance of Glycoforms on Peptide NQTILFGEEDVR (SEQ ID NO: 49), Position N660

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)
Not Glycosylated [1420.7067]		3.8%
HexNAc(1) [1623.7861]		3.5%
HexNAc(2)Hex(5) [2637.1295]		5.0%
HexNAc(3)Hex(3)Fuc(1) [2662.1612]		7.5%
HexNAc(3)Hex(4)Fuc(1) [2824.2140]		1.5%
HexNAc(4)Hex(3)Fuc(1) [2865.2406]		13.3%
HexNAc(2)Hex(7) [2961.2352]		0.5%
HexNAc(4)Hex(4)Fuc(1) [3027.2934]		11.7%
HexNAc(5)Hex(3)Fuc(1) [3068.3199]		2.9%
HexNAc(3)Hex(4)Fuc(1)NeuAc(1) [3115.3094]		0.5%
HexNAc(4)Hex(5)Fuc(1) [3189.3462]		3.2%
HexNAc(5)Hex(4)Fuc(1) [3230.3728]		2.5%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [3318.3888]		23.8%
HexNAc(5)Hex(5)Fuc(1) [3392.4256]		0.8%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [3480.4416]		13.8%
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [3521.4682]		1.0%
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [3683.5210]		1.0%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [3771.5370]		0.8%
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [3845.5738]		1.2%
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.		

TABLE 16: Relative Abundance of Glycoforms on Peptide NVSDIIPR (SEQ ID NO: 50), Position N690

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
Not Glycosylated [913.5102]		1.3%	1.2%	0.3%
HexNAc(2)Hex(3) [1805.8274]		1.1%	0.5%	0.4%
HexNAc(3)Hex(3) [2008.9067]		7.8%	4.5%	6.4%
HexNAc(2)Hex(5) [2129.933]		5.1%	4.1%	3.1%
HexNAc(3)Hex(3)Fuc(1) [2154.9647]		3.4%	5.1%	8.2%
HexNAc(3)Hex(4) [2170.9596]		0.4%	0.5%	0.1%
HexNAc(4)Hex(3) [2211.9861]		5.8%	6.2%	25.3%
HexNAc(2)Hex(6) [2291.9858]		0.7%	0.3%	0.3%
HexNAc(3)Hex(4)Fuc(1) [2317.0175]		1.3%	0.9%	0.0%
HexNAc(3)Hex(5) [2333.0124]		1.1%	0.0%	0.2%
HexNAc(4)Hex(3)Fuc(1) [2358.044]		3.6%	12.9%	32.3%
HexNAc(4)Hex(4) [2374.0389]		4.1%	2.9%	2.7%
HexNAc(5)Hex(3) [2415.0655]		2.1%	0.1%	1.4%
HexNAc(2)Hex(7) [2454.0387]		0.6%	0.2%	0.1%
HexNAc(3)Hex(4)NeuAc(1) [2462.055]		3.1%	0.2%	ND
HexNAc(4)Hex(4)Fuc(1) [2520.0969]		2.8%	8.8%	4.1%
HexNAc(4)Hex(5) [2536.0918]		3.8%	1.4%	0.0%
HexNAc(5)Hex(3)Fuc(1) [2561.1234]		2.3%	1.4%	10.4%
HexNAc(5)Hex(4) [2577.1183]		1.9%	0.0%	0.1%
HexNAc(3)Hex(4)Fuc(1)NeuAc(1) [2608.1129]		1.0%	0.2%	ND
HexNAc(2)Hex(8) [2616.0915]		0.6%	0.1%	0.1%
HexNAc(3)Hex(5)NeuAc(1) [2624.1078]		0.7%	0.1%	ND
HexNAc(4)Hex(4)NeuAc(1) [2665.1344]		2.4%	2.0%	0.1%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(4)Hex(5)Fuc(1) [2682.1497]		3.3%	6.1%	0.1%
HexNAc(5)Hex(4)Fuc(1) [2723.1762]		2.3%	0.7%	0.9%
HexNAc(5)Hex(5) [2739.1711]		1.6%	0.0%	0.0%
HexNAc(6)Hex(3)Fuc(1) [2764.2028]		ND	0.0%	1.9%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [2811.1923]		1.8%	6.6%	0.2%
HexNAc(4)Hex(5)NeuAc(1) [2827.1872]		8.3%	5.5%	0.0%
HexNAc(5)Hex(4)NeuAc(1) [2868.2137]		0.5%	0.0%	0.0%
HexNAc(5)Hex(5)Fuc(1) [2885.229]		2.0%	0.8%	0.3%
HexNAc(5)Hex(6) [2901.224]		2.0%	0.0%	ND
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [2973.2451]		4.6%	15.2%	0.0%
HexNAc(5)Hex(4)Fuc(1)NeuAc(1) [3014.2716]		0.6%	0.3%	0.1%
HexNAc(5)Hex(5)NeuAc(1) [3030.2666]		0.9%	0.1%	0.0%
HexNAc(5)Hex(6)Fuc(1) [3047.2819]		1.9%	0.8%	0.1%
HexNAc(4)Hex(5)NeuAc(2) [3118.2826]		0.3%	0.6%	ND
HexNAc(5)Hex(5)Fuc(1)NeuAc(1) [3176.3245]		1.5%	1.0%	0.0%
HexNAc(5)Hex(6)NeuAc(1) [3192.3194]		2.3%	0.1%	0.0%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [3264.3405]		0.8%	3.7%	0.1%
HexNAc(5)Hex(6)Fuc(1)NeuAc(1) [3338.3773]		3.3%	1.9%	0.0%
HexNAc(5)Hex(6)NeuAc(2) [3483.4148]		0.9%	0.1%	ND
HexNAc(5)Hex(6)Fuc(1)NeuAc(2) [3629.4727]		1.5%	1.3%	ND

(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed.

(2) ND, Not Detected.

(3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.

**TABLE 17: Relative Abundance of Glycoforms on Peptide
LNDSSEFLGIQPTLGSDK (SEQ ID NO: 51), Position N718**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)
Not Glycosylated [2034.039]		77.5%
HexNAc(1) [2237.118]		2.0%
HexNAc(2)Hex(5) [3250.462]		2.0%
HexNAc(4)Hex(3)Fuc(1) [3478.573]		2.6%
HexNAc(4)Hex(4)Fuc(1) [3640.626]		2.5%
HexNAc(5)Hex(3)Fuc(1) [3681.652]		1.2%
HexNAc(4)Hex(5)Fuc(1) [3802.679]		1.8%
HexNAc(5)Hex(4)Fuc(1) [3843.705]		1.4%
HexNAc(4)Hex(4)Fuc(1)NeuAc(1) [3931.721]		1.3%
HexNAc(5)Hex(5)Fuc(1) [4005.758]		0.5%
HexNAc(4)Hex(5)Fuc(1)NeuAc(1) [4093.774]		4.5%
HexNAc(4)Hex(5)Fuc(1)NeuAc(2) [4384.869]		1.0%
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.		

**TABLE 18: Relative Abundance of Glycoforms on Peptide EEQYNSTYR, Position
N810 (SEQ ID NO: 52), (Position N297 of IgG1-Fc using EU numbering)**

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
Not Glycosylated [1189.5120]		0.5%	0.5%	0.3%
HexNAc(1) [1392.5914]		0.5%	0.9%	0.3%
HexNAc(2)Hex(3)Fuc(1) [2227.8871]		0.6%	0.5%	1.2%
HexNAc(2)Hex(4) [2243.8820]		0.6%	0.7%	0.9%
HexNAc(3)Hex(3) [2284.9086]		0.3%	0.2%	0.9%
HexNAc(2)Hex(5) [2405.9349]		5.8%	6.3%	9.8%

Glycans / Mass	Proposed Structure	sACE2 ₂ .S19-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (Expi293F)	sACE2 ₂ .v2.4-IgG1 (ExpiCHO-S)
HexNAc(3)Hex(3)Fuc(1) [2430.9665]		12.5%	12.8%	16.1%
HexNAc(2)Hex(6) [2567.9877]		0.6%	0.5%	0.3%
HexNAc(3)Hex(4)Fuc(1) [2593.0193]		1.9%	1.7%	0.3%
HexNAc(4)Hex(3)Fuc(1) [2634.0459]		61.8%	60.9%	64.4%
HexNAc(2)Hex(7) [2730.0405]		0.7%	0.7%	0.1%
HexNAc(3)Hex(5)Fuc(1) [2755.0722]		0.8%	0.9%	0.0%
HexNAc(4)Hex(4)Fuc(1) [2796.0987]		10.8%	11.0%	3.8%
HexNAc(5)Hex(3)Fuc(1) [2837.1253]		0.3%	0.3%	0.7%
HexNAc(4)Hex(5)Fuc(1) [2958.1515]		0.8%	0.8%	0.1%
(1) Only glycopeptides with relative abundance $\geq 0.5\%$ are listed. (2) ND, Not Detected. (3) GlcNAc, squares; Man, dark gray circles; Gal, light gray circles; Fuc, triangles; Neu5Ac (sialic acid), diamonds.				

[00179] All the native N-glycosylation sites had close to 100% glycan occupancy except for N432, which was 90% glycosylated in proteins produced in Expi293F cells and 99% glycosylated in sACE2₂.v2.4-IgG1 produced in ExpiCHO-S (FIG. 10A). Of the two consensus N-glycosylation motifs added to sACE2₂.S19-IgG1, position 660 was 96% glycosylated but position 718 was only 23% glycosylated (FIG. 10A). The glycoforms present at each site are highly heterogeneous (TABLES 9-18). For the proteins produced in Expi293F cells, ~20-60% of the glycoforms contained at least one sialic acid, with the exceptions of N322 and N810 (equivalent to N297 of IgG1 Fc using EU numbering) where sialylation was absent (FIG. 10B). Sialylated glycans had very low relative abundance (< 2%) at all positions in ExpiCHO-S-produced sACE2₂.v2.4-IgG1, although it was moderately higher (8.7%) at position N546.

[00180] The O-glycosylation site at T730 was poorly glycosylated in the proteins produced in Expi293F cells (2.3-5.0% glycosylated) and approximately half the glycoforms were sialylated (TABLE 19). By comparison, sACE2₂.v2.4-IgG1 expressed in ExpiCHO-S cells was more highly O-glycosylated (11.1%) and almost exclusively with a single N-acetylhexosamine (TABLE 19). This differs from a previous report in which T730 was found to be 97% glycosylated (Shajahan *et al.* (2021) *Glycobiology* 31:410-424) and may

reflect differences in expression systems, construct length, and fusion to IgG1 (here, sACE2_{2.v2.4}-IgG1 and sACE2_{2.S19}-IgG1 are fused at residue G732 to IgG1 Fc, thus changing the environment around T730).

TABLE 19: Relative Abundance of Glycoforms on Peptide LGIQPTLGSDK (SEQ ID NO: 53), Position T730 (O-glycosylation)

Glycans / Mass	sACE2 _{2.S19} -IgG1 (Expi293F)	sACE2 _{2.v2.4} -IgG1 (Expi293F)	sACE2 _{2.v2.4} -IgG1 (ExpiCHO-S)
Not Glycosylated [1128.6259]	97.7%	95.0%	88.9%
HexNAc(2)Hex(2)NeuAc(1) [2149.9857]	0.2%	0.2%	ND
HexNAc(1) [1331.7053]	0.6%	1.1%	10.9%
HexNAc(1)Hex(1) [1493.7581]	0.3%	1.3%	0.2%
HexNAc(1)Hex(1)NeuAc(2) [2075.9489]	1.1%	2.5%	ND
(1) ND, Not Detected.			

EXAMPLE 3: Mutations in the Fc moiety of Modified ACE2 Fusion Proteins Improve Pharmacokinetics

[00181] This example describes the pharmacokinetics of modified ACE2 fusion proteins having mutations in the IgG1 Fc domain polypeptide.

[00182] Substitutions M252Y, S254T, and T256E (EU numbering; referred to as “YTE” mutations) in human IgG1 decrease the dissociation rate between Fc and FcRn 10-fold at endosomal pH, leading to a 2- to 5-fold increase in the serum half-life of antibodies (Robbie *et al.* (2013) *Antimicrob Agents Chemother* 57:6147-6153; Acqua *et al.* (2002) *The Journal of Immunology* 169: 5171-5180; Dall’Acqua *et al.* (2006) *Journal of Biological Chemistry* 281:23514-23524).

[00183] As shown in FIGs. 11A-11H, YTE mutations enhanced the affinities of sACE2_{2.v2.4}-IgG1 and sACE2_{2.S19}-IgG1 for FcRn using biolayer interferometry (BLI). FcRn binding at pH 6.0 was tighter than at pH 7.4, reflecting the biological role of FcRn to capture internalized IgG in the acidic endosome and recycle it to the cell surface for release. Dissociation constants of modified ACE2 fusion protein in the absence of YTE mutations were estimated to be > 100 nM (accurate fitting of kinetic rate constants was not possible for these samples due to incomplete dissociation of the analyte). By comparison, sACE2_{2.v2.4}-IgG1(YTE) and sACE2_{2.S19}-IgG1(YTE) had dissociation constants for FcRn at pH 6.0 of 21 nM and 39 nM, respectively, with slow off rates of $5.9 \times 10^{-4} \text{ s}^{-1}$ and $1.2 \times 10^{-3} \text{ s}^{-1}$.

[00184] 10 mg/kg of sACE2₂.v2.4-IgG1 with or without YTE mutations, or 10 mg/kg sACE2₂.S19-IgG1 with and without YTE mutations were administered to human FcRn transgenic mice intravenously and plasma protein concentrations were measured by ELISA (FIG. 12A). sACE2₂.S19-IgG1(YTE) had the highest exposure ($AUC_{0-48h} = 490 \mu\text{g/ml} \times \text{h}$), followed by sACE2₂.S19-IgG1 ($310 \mu\text{g/ml} \times \text{h}$) and sACE2₂.v2.4-IgG1 ($290 \mu\text{g/ml} \times \text{h}$). When administered subcutaneously (SC); the serum half-lives of the modified ACE2 fusion proteins were extended due to sustained absorption from the injection site. Based on catalytic activity in plasma (FIG. 12B), adding the YTE mutations to sACE2₂.S19-IgG1 increased the half-life ($t_{1/2}$) by 11 hours and exposure to ACE2 activity by 3-fold. Using ELISA to measure serum concentrations (FIG. 12C), the $t_{1/2}$ of sACE2₂.S19-IgG1(YTE) was 51.1 hours and the AUC_{0-120h} was $320 \mu\text{g/ml} \times \text{h}$. Exposures were less for sACE2₂.S19-IgG1 ($160 \mu\text{g/ml} \times \text{h}$) and sACE2₂.v2.4-IgG1 ($150 \mu\text{g/ml} \times \text{h}$). Protein concentrations remained above the EC90 for virus neutralization (Chan *et al.* (2020) *Science* (1979)369: 1261-1265; Zhang *et al.* (2022) *Nat Chem Biol* 18: 342-351; Zhang *et al.* (2022) *Embo Biol Med*) at 120 hours post-administration (FIG. 12C).

Deglycosylation

[00185] Purified sACE2₂.S19-IgG1(YTE) was deglycosylated in PBS under native conditions for 20 h at room temperature. For PNGase F treatment, substrate protein (5 mg/ml) was incubated with PNGase F (New England Biolabs; 125 units/ μl) in 1x Glycobuffer (New England Biolabs) prepared in PBS. For neuraminidase treatment, substrate protein (6.8 mg/ml) was incubated with *Arthrobacter ureafaciens* neuraminidase (Roche; 1.7 units/ml) in PBS. Mixtures were separated on a gel filtration column to purify the treated sACE2₂.S19-IgG1(YTE) proteins.

[00186] PNGase F cleaves the amide linkage between asparagine and the innermost N-acetylglucosamine, releasing the entire glycan. After incubation of sACE2₂.S19-IgG1(YTE) produced in Expi293F cells with PNGase F under native conditions, the modified ACE2 fusion protein had increased electrophoretic mobility consistent with removal of large glycans (FIG. 13A). Multiple charged species of the protein were still observed on an isoelectric focusing (IEF) gel (FIG. 13B), suggesting heterogenous sialylated glycans remained and deglycosylation was incomplete. Treatment of sACE2₂.S19-IgG1(YTE) from Expi293F culture with *Arthrobacter ureafaciens* neuraminidase (an enzyme that removes all terminal sialic acids), did not change electrophoretic mobility on a standard denaturing gel but did reduce heterogeneity on an IEF gel, as expected for removal of charged sialic acids (FIG. 13A and 13B).

[00187] The pharmacokinetics of the deglycosylated proteins were compared to untreated sACE2₂.S19-IgG1(YTE) and sACE2₂.v2.4-IgG1(YTE) (FIG. 13C and TABLE 20). Human FcRn transgenic mice were given a single IV dose of 10 mg/kg and concentrations in plasma were measured over 5 days. At later days, the concentrations of the untreated decoy receptors were measured over 5 days. At later days, the concentrations of the untreated decoy receptors closely aligned with those observed following SC administration. The elimination phase $t_{1/2\beta}$ values were 73 and 60 h for sACE2₂.S19-IgG1(YTE) and sACE2₂.v2.4-IgG1(YTE), respectively, and exposures (AUC_{0-120h}) were 510 and 330 $\mu\text{g/mL}\times\text{h}$. Exposure was decreased following PNGase F treatment of sACE2₂.S19-IgG1(YTE), but the most dramatic effect was seen following treatment with neuraminidase. Desialylated sACE2₂.S19-IgG1(YTE) was rapidly cleared and its exposure was decreased by two orders of magnitude. Sialylation of the decoy receptor is thus necessary for optimal pharmacokinetics.

TABLE 20: Pharmacokinetic properties of optimized decoy receptors following IV administration ⁽¹⁾

sACE2 ₂ -IgG1 protein	Enzyme Treatment	$t_{1/2\beta}$ ⁽²⁾ (h)	AUC_{0-120h} ($\mu\text{g/mL}\times\text{h}$)	MRT _{0-inf obs} ⁽³⁾ (h)
sACE2 ₂ .v2.4-IgG1(YTE)	None	60.1	330	43.4
sACE2 ₂ .S19-IgG1(YTE)	None	73.0	510	60.8
sACE2 ₂ .S19-IgG1(YTE)	NA/sialidase	30.8	48	2.1
(1) Proteins were produced in Expi293F cells and were administered as a single IV dose (10 mg/kg) to human FcRn mice. Plasma concentrations were measured by ELISA for 120 h.				
(2) Beta half-life is determined from 24-120 h.				
(3) MRT, mean residence time.				

EXAMPLE 4: Decoy receptors from stable pooled expression in CHOK1SV GS-KO cells

[00188] This example describes representative manufacture from stable CHOK1SV GS-KO pools of modified ACE2 fusion proteins derived from sACE2₂.v2.4-IgG1 and their pharmacokinetics.

[00189] Biologic drugs are generally purified from CHO expression systems that have well described safety characteristics and for which there is extensive clinical experience. To assess the feasibility of stable CHO expression for the manufacture of sialylated decoy receptors, CHOK1SV GS-KO stable pools were generated for the production of sACE2₂.v2.4-IgG1(YTE) and sACE2₂.S19-IgG1 (YTE).

[00190] Genes encoding sACE2₂.v2.4-IgG1(YTE) and sACE2₂.S19-IgG1(YTE) were designed with a consensus Kozak sequence and codon optimized for *Cricetulus griseus*. Genes were synthesized and subcloned into the HindIII and EcoRI sites of pPV-A. The pPV-

A parts vectors were assembled into piggyBac destination vectors in an assembly reaction containing a 2:1 ratio of part vector to destination vector, T4 DNA ligase, and Esp3I. The reaction was cycled between 37 °C and 16 °C for the digestion and ligation phases of the assembly reaction, respectively.

- 5 **[00191]** CHOK1SV GS-KO cells were cultured in CD-CHO media supplemented with 6 mM L-glutamine. Cells were grown at 36.5 °C, 5% CO₂, 85% humidity, 140 rpm. Cells were transfected via electroporation. 1 x 10⁷ cells in 700 µL CD-CHO medium were combined with 100 µL (40 µg) linearized DNA and 5 µg piggyBac Transposase mRNA in a 0.4 cm gap electroporation cuvette and pulsed at 300 V, 900 µF. Cells were transferred to 30 mL pre-
- 10 warmed CD-CHO supplemented with 1% SP4 to generate a stable pool and incubated at 36.5 °C, 5% CO₂, 85% humidity, 140 rpm. Three stable pool transfectants were established per gene, one of which was a no-mRNA control. Once the density was >1 x 10⁶ cells/mL, stable recombinant CHOK1SV GS-KO cells were maintained in CD-CHO media supplemented with 50 µM L-methionine sulfoximine and 1% SP4.
- 15 **[00192]** Clarified and 0.22 µm filtered supernatants from stable pools were analyzed by BLI and yields of sACE2₂-IgG1 fusion proteins were similar between the pools. The top relative stable pool for each protein was progressed for protein production. The stable pools were adapted for one passage in CM76 media and production cultures in shake flasks were seeded with 0.2 x 10⁶ cells/ml in CM125 media. Bolus feeds were administered on days 4 and 8
- 20 consisting of a mixture of feeds. Production cultures were harvested on day 10 and sterile filtered. Fusion proteins were purified using affinity chromatography. The column was equilibrated with 50 mM sodium phosphate pH 7.0, 125 mM NaCl, and washed with 50 mM sodium phosphate pH 7.0, 1 M NaCl, followed by a second wash with equilibration buffer. Proteins were eluted with 10 mM NaHCOO pH 3.5 and eluents were neutralized with 10x
- 25 PBS, pH 7.4, and 1 M Tris-HCl pH 8.0. to pH ~7.3. Eluted protein fractions were concentrated and separated on a dextran-agarose matrix gel filtration column.
- [00193]** Capillary Electrophoresis-Sodium Dodecyl Sulfate (CE-SDS) analysis was performed on an automated electrophoresis bioanalyzer instrument. Protein solutions were mixed 1:3 with denaturing solution with and without β-mercaptoethanol for reduced and non-
- 30 reduced samples, respectively. Samples were heated to 95 °C for five minutes, diluted 1:14 with water, and loaded onto a primed microfluidic chip for electrophoresis.
- [00194]** Capillary isoelectric focusing electrophoresis (cIEF) was performed on a capillary electrophoresis system. Samples were prepared at 0.2 mg/ml in sample buffer (0.35% methyl cellulose, 4% pharmalyte 3-10, 10 mM arginine) containing 4.05 and 9.99 pI markers.

[00195] Sialic acids were released by acid hydrolysis (2 M acetic acid) of the test samples under heat and the α -keto functionality of the free sialic acids underwent a condensation reaction with 1,2-diamino-4,5-methylenedioxybenzene (DMB) to form fluorescent reaction products. Sialic acid standards were also labeled with DMB. Using RP-HPLC with

5 fluorescence detection, N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) were quantified.

[00196] Based on capillary electrophoresis, decoy receptors purified from stable CHOK1SV GS-KO culture had equivalent purity to those from transiently transfected Expi293F cells (FIG. 14A) but had sharper peaks with lower isoelectric points (pI) under isoelectric focusing

10 (FIGs. 14B-14E), suggesting reduced glycoform heterogeneity with high levels of negatively charged sugars. Sialic acids were released by acid hydrolysis and reacted to form a fluorescent product that was quantified by reverse phase high performance liquid chromatography (RP-HPLC). Decoy receptors from CHOK1SV GS-KO stable pools had

15 twice the molar content of N-acetylneuraminic acid (Neu5Ac) than proteins transiently expressed by Expi293F (TABLE 21). Levels of N-glycolylneuraminic acid (Neu5Gc), a sialic acid that is not produced by human cells and has immunogenic potential, were low (TABLE 21). Furthermore, decoy receptors from CHOK1SV GS-KO stable pools tightly

20 bound the RBDs of gamma and delta SARS-CoV-2 variants with monovalent binding affinities equivalent to proteins with less sialylation from Expi293F culture (FIG. 15 and TABLE 22). The data demonstrate that a stable CHO expression system is suitable for producing decoy receptors with high levels of sialylated N-glycans and potent affinity for virus spikes.

TABLE 21: Sialylation of decoy receptors purified from different sources ⁽¹⁾

sACE2 ₂ -IgG1 protein	Source	Neu5Ac ⁽²⁾	Neu5Gc ⁽³⁾
sACE2 ₂ .v2.4-IgG1(YTE)	Expi293F	8.3	Not Detected
sACE2 ₂ .S19-IgG1(YTE)	Expi293F	7.1	Not Detected
sACE2 ₂ .v2.4-IgG1(YTE)	CHOK1SV GS-KO	14.4	0.46
sACE2 ₂ .S19-IgG1(YTE)	CHOK1SV GS-KO	14.8	0.50
(1) Measured by RP-HPLC.			
(2) Mol Neu5Ac / mol protein monomer. Limit of detection is 2.2 mol/mol protein.			
(3) Mol Neu5Gc / mol protein monomer. Limit of detection is 0.12 mol/mol protein.			

TABLE 22: BLI Kinetics for Monovalent Binding of Decoy Receptors Purified from Stable CHOK1SV GS-KO Cells to SARS-CoV-2 RBD ⁽¹⁾

Immobilized Protein	RBD	KD (nM)	k _{on} (M ⁻¹ s ⁻¹)	k _{off} (s ⁻¹)	R ² (2)
sACE2 ₂ .v2.4-IgG1(YTE)	Delta	0.6	7.6 × 10 ⁵	4.9 × 10 ⁻⁴	0.991
sACE2 ₂ .v2.4-IgG1(YTE)	Gamma	0.8	8.2 × 10 ⁵	6.6 × 10 ⁻⁴	0.991

sACE2 ₂ .S19-IgG1(YTE)	Delta	0.7	7.9×10^{-5}	5.3×10^{-4}	0.992
sACE2 ₂ .S19-IgG1(YTE)	Gamma	0.8	8.6×10^{-5}	7.1×10^{-4}	0.991
(1) Dimeric sACE2 ₂ .v2.4-IgG1 derivative proteins were immobilized to an anti-human IgG sensor. Sensors were transferred to monomeric RBD-8h at 90, 30, and 10 nM. Samples were tested in duplicate.					
(2) R ² were determined from curves fitted to the entire data.					

[00197] sACE2₂.v2.4-IgG1(YTE) and sACE2₂.S19-IgG1(YTE) from CHOK1SV GS-KO stable pooled expression were administered IV to human FcRn mice at a single 10 mg/kg dose and plasma concentrations were measured over 8 days. The highly sialylated decoy receptors had superior PK, with plasma concentrations remaining >10-fold higher than the EC90 for virus neutralization for at least 8 days (FIG 16A and TABLE 22). Half-lives of the proteins were extended ($t_{1/2\beta}$ = 124 and 224 h for sACE2₂.v2.4-IgG1(YTE) and sACE2₂.S19-IgG1(YTE), respectively) and exposures were increased by an order of magnitude (AUC_{0-192h} = 4100 and 6400 $\mu\text{g/ml}\times\text{h}$, respectively) (TABLE 22).

TABLE 23: Pharmacokinetic properties of optimized decoy receptors produced by stable CHOK1SV GS-KO ⁽¹⁾

sACE2 ₂ -IgG1 protein	ROA, Study Length	$t_{1/2\beta}$ (h)	AUC_{0-120h} ($\mu\text{g/ml}\times\text{h}$)	C_{last} ($\mu\text{g/ml}$)
sACE2 ₂ .v2.4-IgG1(YTE)	IV, 8 days	124 ⁽²⁾	4100	10 ± 2
sACE2 ₂ .S19-IgG1(YTE)	IV, 8 days	224 ⁽²⁾	6400	23 ± 3
sACE2 ₂ .S19-IgG1(YTE)	IV, 14 days	122 ⁽³⁾	8500	9 ± 1
sACE2 ₂ .S19-IgG1(YTE)	SC, 14 days	196 ⁽⁴⁾	6200	8 ± 2
(1) Proteins were administered as a single dose (10 mg/kg) to human FcRn mice.				
(2) Beta half-life is determined from 24-192 h.				
(3) Beta half-life is determined from 24-336 h.				
(4) Beta half-life is determined from 120-336 h				

[00198] To better understand the PK of the decoy receptors over a longer period of time, the experiment was repeated out to 14 days with cohorts of human FcRn mice receiving a single dose (10 mg/kg) of sACE2₂.S19-IgG1(YTE) from stable CHOK1SV GS-KO expression either by IV or SC route (FIG. 16B). Plasma concentrations following IV administration very closely aligned with the prior experiment for the first 8 days (compare FIGs. 16A and 16B), with $t_{1/2\beta}$ = 122 h and AUC_{0-336h} = 8500 $\mu\text{g/ml}\times\text{h}$ (TABLE 23). When sACE2₂.S19-IgG1(YTE) was administered SC, the protein's concentration in plasma rose in 8 h to ~30 $\mu\text{g/ml}$ and remained steady until Day 5, when concentration fell ($t_{1/2\beta}$ = 196 h) with comparable kinetics to IV administration. Bioavailability following SC administration was high (>70%) and concentrations in plasma remained ~10-fold above the EC90 for SARS-

CoV-2 neutralization at Day 14. Considering the variability between experiments, the data demonstrate that the elimination half-life is approximately 1 week for the highly sialylated decoy receptors. Overall, decoy receptors produced from stable CHO expression have favorable pharmacokinetic properties and improved half-life.

EXAMPLE 5: Modified ACE2 Fusion Proteins Have Low Probability of Presenting Immunogenic Epitopes on HLA Class II

[00199] This example describes the predicted immunogenicity of modified ACE2 fusion proteins of the present disclosure.

[00200] Using an algorithm trained on large empirical data sets (King *et al.* (2014) *Proc Natl*

Acad Sci U S A 111: 8577-8582), the sequences of modified ACE2 fusion proteins were scanned for peptides predicted to be displayed on a set of 14 common HLA-II allotypes.

Peptides that fall within the top 10% of all possible sequences for calculated binding affinity, and are predicted to bind a minimum threshold of 4 HLA-II allotypes, have a high probability of being presented on HLA-II. Wild-type recombinant ACE2 has been evaluated in clinical

trials and is nonimmunogenic (Haschke *et al.* (2013) *Clin Pharmacokinet* 52: 783-792; Khan *et al.* (2017) *Crit Care* 21). Compared to wild-type sACE2₂-IgG1, sACE2_{2.v2.4}-IgG1 has 3 mutations: there are no high affinity antigenic peptides predicted to encompass the T27Y mutation; the L79T mutation may be found at the end positions of a small number of presented peptides; and the N330Y mutation is predicted to remove an HLA-II epitope (FIG.

17). In sACE2_{2.S19}-IgG1, there are three substitutions in addition to those present in sACE2_{2.v2.4}-IgG1: V491I falls within a predicted epitope but is a highly conservative change of a single methyl group; while the mutations that add N-glycans at residues 660 and 718 were not analyzed as the computational algorithm is not trained on glycopeptides, although glycosylation may reduce display and T Cell receptor (TCR) recognition. Overall, the mutations introduced into ACE2 are not anticipated to be strongly immunogenic. The YTE mutations in the Fc region remove a predicted HLA-II epitope (FIG. 17) and are known to be safe in antibodies approved for clinical use.

[00201] The predicted immunogenicity of two other engineered ACE2 proteins, 3N39v4 and 3J320v3 were analyzed. These proteins each harbor 6 mutations in ACE2. As shown in FIG.

17, computational epitope scanning identified new HLA-II epitopes created by mutations at the N-termini of 3N39v4 and 3J320v3. Furthermore, these proteins remove the N-glycan at position N90, which occurs in a region predicted to generate many high affinity antigenic peptides.

EXAMPLE 6: The optimized decoys broadly bind to S proteins of omicron sublineages

[00202] This example describes the binding to Spike (S) proteins from SARS-CoV-2 omicron sublineages of modified ACE2 fusion proteins of the present disclosure.

[00203] S sequences were subcloned into vector pcDNA3.1(+). The final plasmids encode the mature S proteins (a.a. V16-T1273; numbering based on Wuhan variant S protein in GenBank YP_009724390.1) downstream of an influenza HA signal peptide and N-terminal myc epitope tag. Expi293F cells were transfected with 500 ng pcDNA3-myc-S plasmid per mL of culture using Expifectamine. Cells were harvested (600 × g, 1 min) 24 h post-transfection without the addition of transfection enhancers. Cells were washed with cold PBS supplemented with 0.2% bovine serum albumin (PBS-BSA) to reduce non-specific binding. Cells were resuspended in PBS-BSA and incubated with serial dilutions of sACE2₂-IgG1 proteins on ice for 30 minutes. Cells were washed with PBS-BSA and were resuspended in 1:150 polyclonal chicken anti-MYC-FITC and 1:300 APC anti-human IgG Fc for 30 minutes on ice. Cells were washed twice with PBS-BSA and analyzed on a flow cytometer. The main population of viable cells was gated by forward and side scatter. Mean APC fluorescence was recorded for the myc-positive population. Background fluorescence of transfected cells incubated without sACE2₂-IgG1 proteins was subtracted and data were normalized based on the total fluorescence signal for each experiment.

[00204] Numerous variants of the SARS-CoV-2 omicron lineage have evolved, with mutations arising in the S gene that influence ACE2 binding and immune evasion. The optimized decoy receptors remained highly active for tight binding to S proteins of omicron sublineages that are currently prevalent in the community. Using flow cytometry to measure avid binding of decoy receptors to trimeric S proteins expressed at the plasma membrane, sACE2₂.v2.4-IgG1(YTE) and sACE2₂.S19-IgG1(YTE) were shown to tightly bind the S proteins of BF.7, XBB, BA.2.75.2, and BQ.1.1 omicron sublineages at low nanomolar concentrations (FIG. 18). Binding of the optimized decoy receptors across the panel of S proteins was almost an order of magnitude tighter than that of wild type sACE2₂-IgG1 in this assay. Exceptional breadth of the decoy receptors is thus maintained following modifications for improved pharmacokinetics.

INCORPORATION BY REFERENCE

[00205] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00206] The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the disclosure described herein.

- 5 Various structural elements of the different embodiments and various disclosed method steps may be utilized in various combinations and permutations, and all such variants are to be considered forms of the disclosure. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

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CLAIMS

WHAT IS CLAIMED IS:

1. A protein comprising:
a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising at least one substitution relative to wild-type human ACE2 of SEQ ID NO:1, wherein the at least one substitution introduces at least one glycosylation site not present in wild-type human ACE2.
2. A protein comprising:
a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising a substitution or combination of substitutions relative to wild-type human ACE2 of SEQ ID NO:1 selected from the group consisting of:
 - a. a substitution of valine at position 491 to isoleucine; a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine;
 - b. a substitution of isoleucine at position 663 to tryptophan, and a substitution of alanine at position 673 to tyrosine;
 - c. a substitution of alanine at position 673 to tyrosine, and a substitution of isoleucine at position 694 to phenylalanine;
 - d. a substitution of valine at position 491 to isoleucine, and a substitution of isoleucine at position 679 to tyrosine;
 - e. a substitution of asparagine at position 682 to tryptophan, and a substitution of alanine at position 687 to phenylalanine;
 - f. a substitution of serine at position 167 to isoleucine, a substitution of alanine at position 246 to leucine, and a substitution of isoleucine at position 694 to phenylalanine;
 - g. a substitution of valine at position 491 to isoleucine, a substitution of alanine at position 673 to tyrosine, a substitution of alanine at position 687 to phenylalanine, and a substitution of threonine at position 698 to glutamic acid;
 - h. a substitution of serine at position 167 to isoleucine, a substitution of valine at position 491 to isoleucine, a substitution of histidine at position 535 to phenylalanine; and a substitution of alanine at position 673 to valine;
 - i. a substitution of lysine at position 288 to cysteine, and a substitution of glutamic acid at position 433 to cysteine;
 - j. a substitution of leucine at position 624 to cysteine, and a substitution of alanine at position 632 to cysteine;

- k. a substitution of glutamic acid at position 166 to cysteine, and a substitution of valine at position 691 to cysteine;
 - l. a substitution of methionine at position 662 to serine or threonine;
 - m. a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine, and a substitution of methionine at position 662 to serine or threonine;
 - n. a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine, a substitution of asparagine at position 580 to serine or threonine, and a substitution of methionine at position 662 to serine or threonine;
 - o. a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine;
 - p. a substitution of aspartic acid at position 615 to asparagine, a substitution of methionine at position 662 to serine or threonine; and a substitution of asparagine at position 720 to serine or threonine;
 - q. a substitution of glutamic acid at position 536 to asparagine, a substitution of proline at position 538 to serine or threonine; a substitution of methionine at position 662 to serine or threonine, and a substitution of glutamine at position 728 to asparagine;
 - r. a substitution of lysine at position 631 to asparagine, a substitution of tyrosine at position 633 to serine or threonine; a substitution of methionine at position 662 to serine or threonine, and a substitution of glutamine at position 728 to asparagine; and
 - s. a substitution of methionine at position 662 to serine or threonine, and a substitution of isoleucine at position 694 to phenylalanine.
3. The protein as in claim 1 or 2, wherein the modified ACE2 polypeptide further comprises a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, and a substitution of asparagine at position 330 to tyrosine, wherein the substitutions are relative to wild-type human ACE2 of SEQ ID NO:1.
4. The protein as in any one of claims 1 to 3, wherein the modified ACE2 polypeptide comprises a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, a substitution of asparagine at position 330 to tyrosine, a substitution of valine at position 491 to isoleucine; a substitution of methionine at position 662 to threonine; and a substitution of asparagine at position 720 to serine, wherein the substitutions are relative to wild-type human ACE2 of SEQ ID NO:1.

5. The protein as in claim 4, wherein the modified ACE2 polypeptide comprises a sequence having at least 85% sequence identity to SEQ ID NO:12.
6. The protein as in claim 5, wherein the modified ACE2 polypeptide comprises a sequence having 100% sequence identity to SEQ ID NO:12.
7. The protein as in any one of claims 1 to 6, wherein the protein further comprises a signal peptide fused to the N-terminus of the modified ACE2 polypeptide.
8. The protein as in claim 7, wherein the signal peptide comprises a sequence having at least 85% sequence identity to SEQ ID NO:11.
9. The protein as in any one of claims 1 to 8, wherein the modified ACE2 polypeptide is fused to an immunoglobulin Fc domain polypeptide, or functional fragment thereof.
10. The protein as in any one of claims 1 to 9, wherein the immunoglobulin Fc domain polypeptide is a human IgG1, IgG2, IgG3, or IgG4 Fc domain polypeptide, or functional fragment thereof.
11. The protein as in claim 10, wherein the immunoglobulin Fc domain polypeptide is a human IgG1 Fc domain polypeptide, or functional fragment thereof.
12. The protein as in claim 10 or 11, wherein the immunoglobulin Fc domain polypeptide, or functional fragment thereof, comprises residues 221 to 447 of human IgG1 Fc, wherein positions are numbered according to EU numbering.
13. The protein as in any one of claims 10 to 12, wherein the human IgG1 Fc domain polypeptide, or functional fragment thereof, comprises one or more than one substitution relative to wild-type human IgG1 Fc domain of SEQ ID NO:4 selected from the group consisting of:
 - a. a substitution of methionine at position 252 to tyrosine;
 - b. a substitution of serine at position 254 to threonine; and
 - c. a substitution of threonine at position 256 to glutamic acid,wherein positions are numbered according to the EU numbering.
14. The protein as in claim 13, wherein the human IgG1 Fc domain polypeptide comprises:
 - a. a substitution of methionine at position 252 to tyrosine;
 - b. a substitution of serine at position 254 to threonine; and
 - c. a substitution of threonine at position 256 to glutamic acid,wherein the positions are numbered according to the EU numbering.
15. The protein as in any one of claims 9 to 14, wherein the immunoglobulin Fc domain polypeptide, or functional fragment thereof, is fused to the C-terminus of the modified ACE2 polypeptide.

16. The protein as in claim 15, wherein the immunoglobulin Fc domain polypeptide, or functional fragment thereof, is fused to the C-terminus of the modified ACE2 polypeptide via a linker.
17. The protein as in claim 16, wherein the linker consists of a single serine residue.
18. The protein as in any one of claims 9 to 17, wherein:
 - a. the modified ACE2 polypeptide comprises a substitution of threonine at position 27 to tyrosine, a substitution of leucine at position 79 to threonine, a substitution of asparagine at position 330 to tyrosine, a substitution of valine at position 491 to isoleucine, a substitution of methionine at position 662 to threonine, and a substitution of asparagine at position 720 to serine; and
 - b. the immunoglobulin Fc domain comprises a substitution of methionine at position 252 to tyrosine, a substitution of serine at position 254 to threonine, and a substitution of threonine at position 256 to glutamic acid,wherein the positions are numbered according to the EU numbering.
19. The protein as in any one of claims 9 to 18, wherein the protein comprises a sequence having at least 85% sequence identity to SEQ ID NO:10.
20. The protein as in claim 19, wherein the protein comprises a sequence having 100% sequence identity to SEQ ID NO:10.
21. The protein as in any one of claims 1 to 20, wherein the protein has increased levels of glycosylation as compared to wild-type ACE2.
22. The protein as in any one of claims 1 to 21, wherein the protein has two to fifty-fold increased affinity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein as compared to wild-type ACE2 as measured by biolayer interferometry (BLI).
23. The protein as in any one of claims 1 to 22, wherein the protein has 1.2 to 2-fold increased catalytic activity as compared to wild-type ACE2 as measured in an *in vitro* fluorometric ACE2 activity assay.
24. The protein as in any one of claims 1 to 23, wherein at least 20% by moles of the glycosylations are sialylated.
25. The protein as in any one of claims 9 to 24, wherein the protein has a two to ten-fold decreased dissociation rate with FcRn at endosomal pH as compared to a protein comprising wild-type ACE2 fused to a wild-type human IgG1 Fc domain.
26. The protein as in any one of claims 9 to 25, wherein the protein has an equilibrium dissociation constant for FcRn at pH 6.0 of 10 nM to 50 nM as measured by BLI.
27. The protein as in any one of claims 9 to 26, wherein the protein has an off rate for FcRn at pH 6.0 of $5.0 \times 10^{-4} \text{s}^{-1}$ to $2.0 \times 10^{-3} \text{s}^{-1}$ as measured by BLI.

28. The protein as in any one of claims 9 to 27, wherein the protein has a higher binding affinity to FcRn at pH 6.0 than at pH 7.0 as measured by BLI.
29. The protein as in any one of claims 9 to 28, wherein the plasma half-life of the protein is 40 to 240 hours when administered to a subject.
30. The protein as in any one of claims 9 to 29, wherein the protein has a plasma activity area under the curve over 48 hours (AUC_{0-48h}) of 50 to 150 ($\mu\text{M product/minute}$) $\times$ h when intravenously administered to a subject at a dose of 2 mg/kg.
31. The protein as in any one of claims 9 to 30, wherein the protein has an area under the curve over 120 hours (AUC_{0-120h}) of 100 to 9000 $\mu\text{g/mL} \times \text{h}$ when intravenously administered to a subject at a dose of 10 mg/kg.
32. The protein as in any one of claims 9 to 31, wherein the protein maintains a plasma concentration above 18 pM at 100 to 150 hours post administration to a subject.
33. The protein as in any one of claims 1 to 32, wherein the protein is nonimmunogenic when administered to a subject.
34. The protein as in any one of claims 9 to 33, wherein the protein forms a stable homodimer.
35. A pharmaceutical formulation comprising a protein as in any one of claims 1 to 34 and a pharmaceutically acceptable carrier.
36. A nucleic acid vector encoding a protein as in any one of 1 to 34.
37. A method of producing a protein as in any one of 1 to 34, wherein a vector encoding the protein is expressed in a cell line and at least 10% by moles of the expressed protein is glycosylated.
38. The method as in claim 37, wherein the expressed protein is glycosylated with N-acetylhexosamine.
39. The method as in claim 37 or 38, wherein at least 20% by moles of the glycosylations are sialylated.
40. The method as in any one of claims 37 to 39, wherein the cell line is a CHO cell line.
41. A method of inhibiting SARS-CoV-2 replication comprising administering to a subject a therapeutically or prophylactically effective amount of a protein as in any one of claims 1 to 34 or a pharmaceutical formulation as in claim 35.
42. The method as in claim 41, comprising administering the protein or pharmaceutical formulation to the subject intravenously, subcutaneously, intratracheally, or by inhalation.
43. The method as in claim 41 or claim 42, wherein the SARS-CoV-2 is selected from Wuhan, alpha, beta, gamma, delta, or omicron variants.

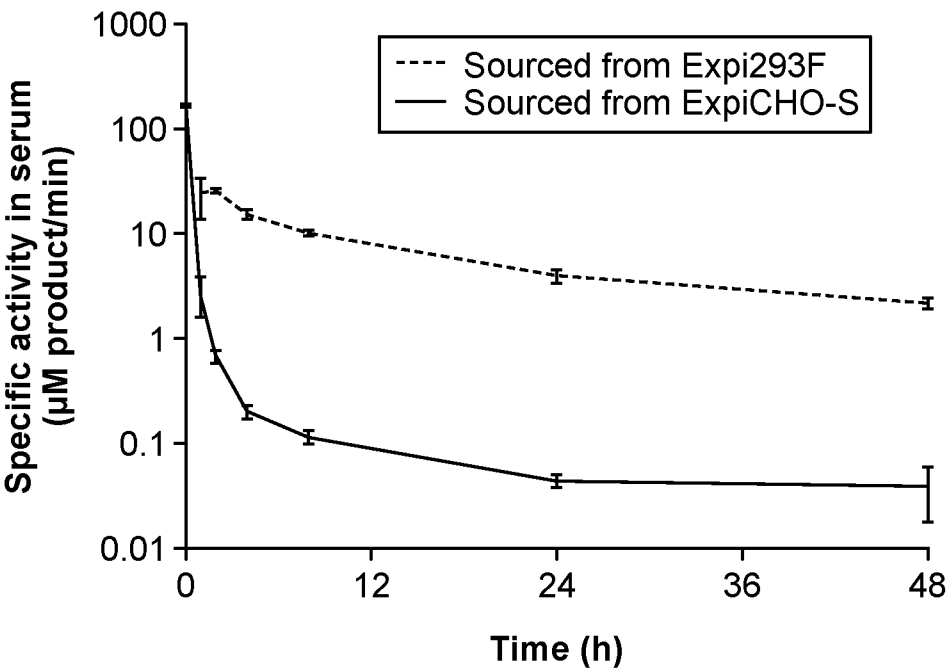


FIG. 1A

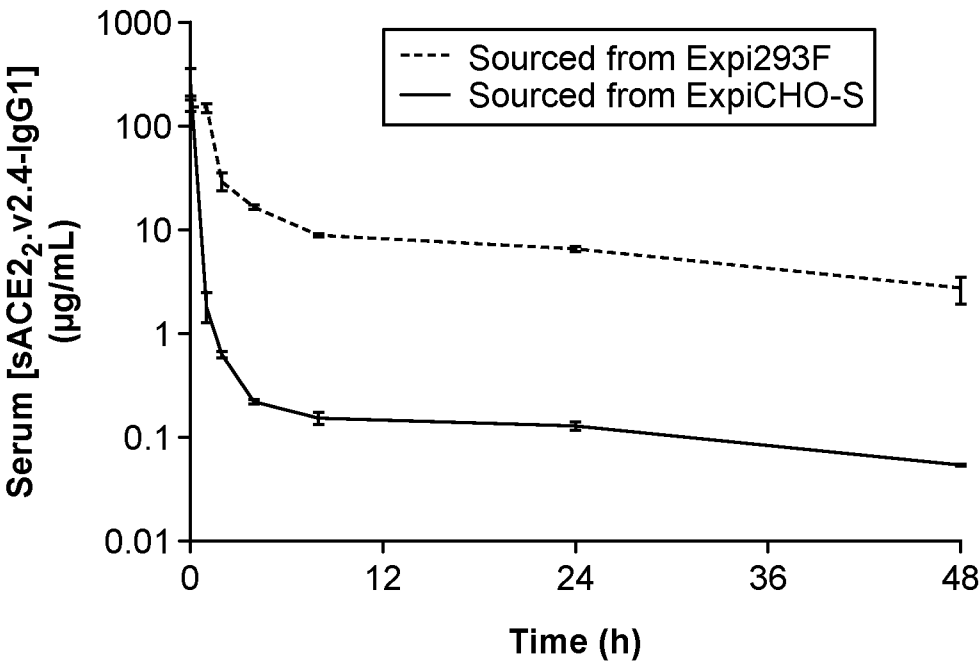


FIG. 1B

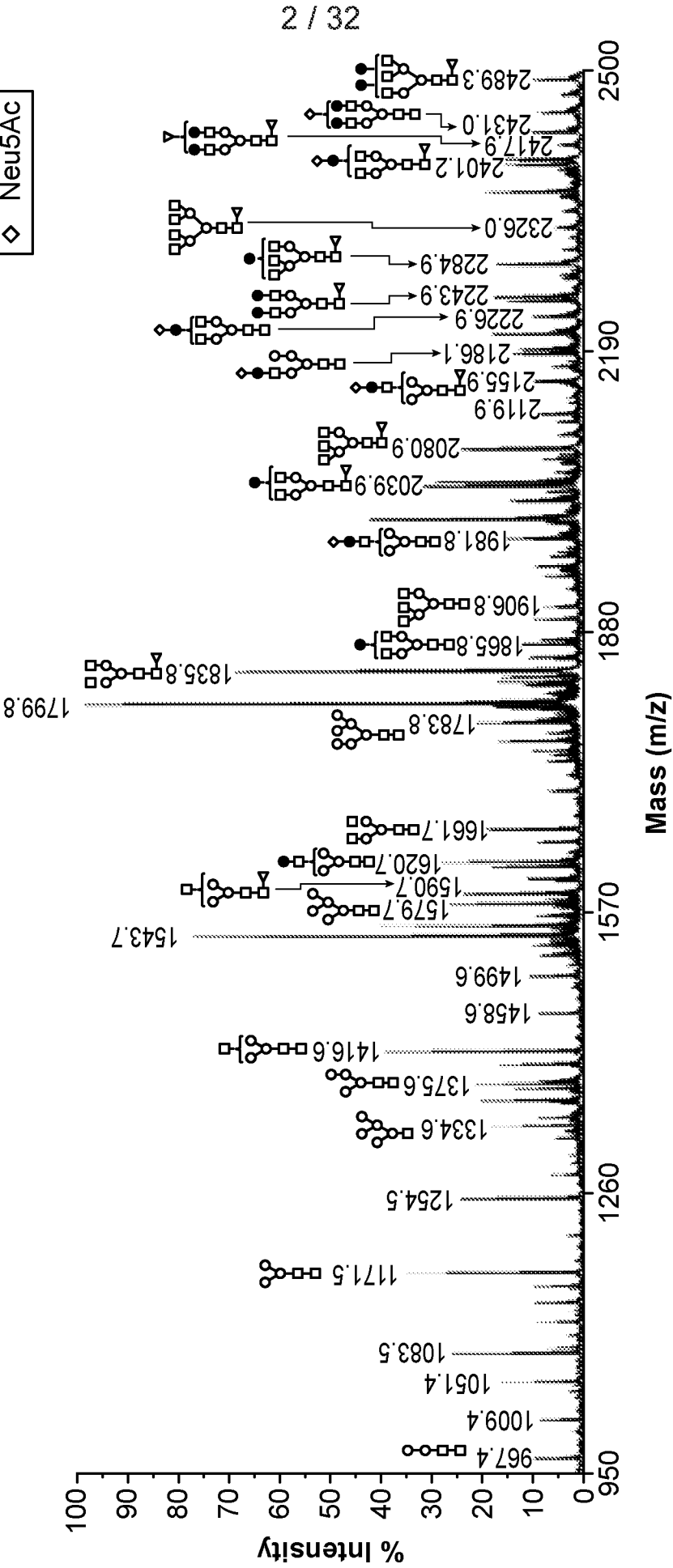


FIG. 2A

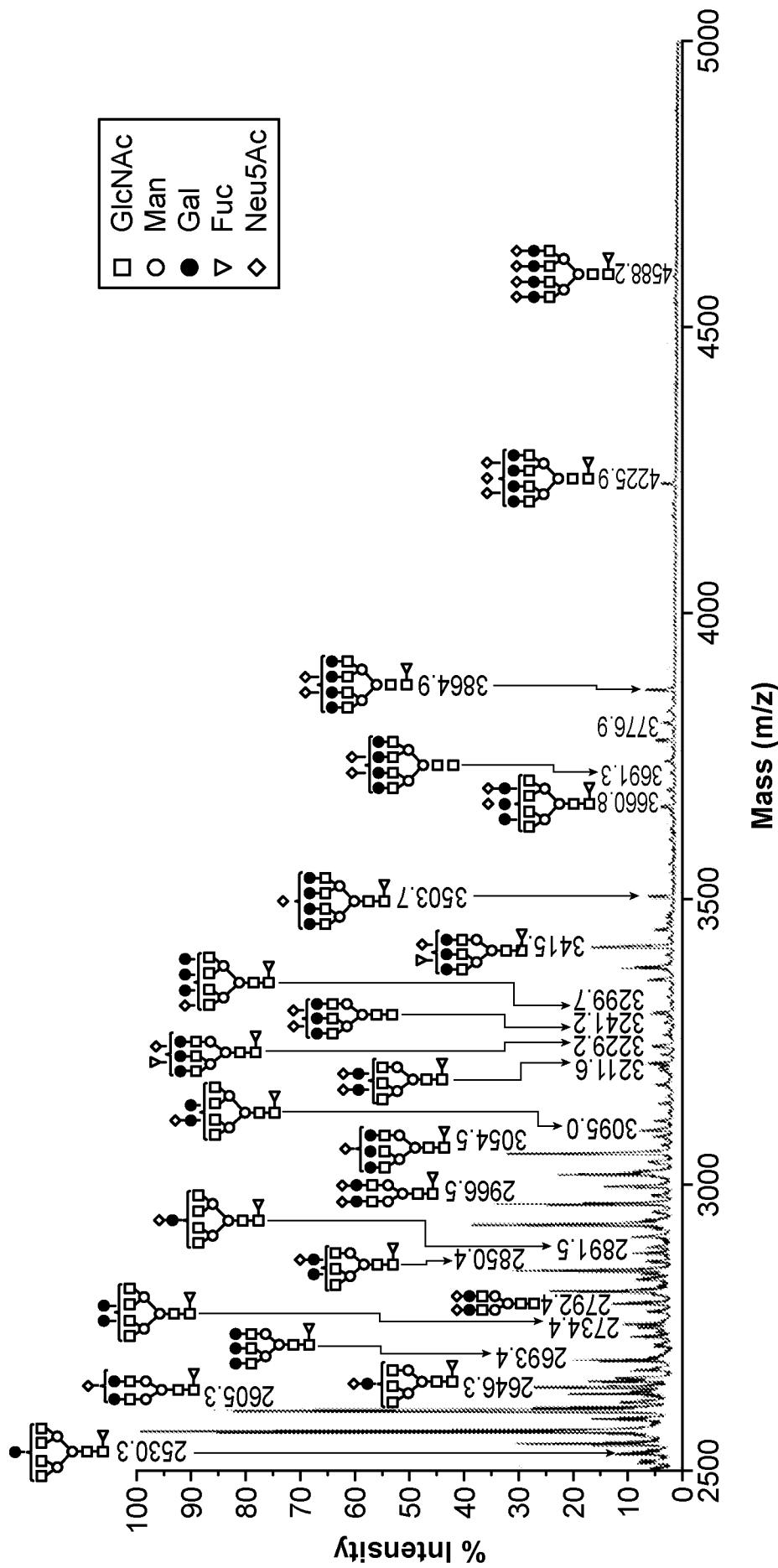


FIG. 2B

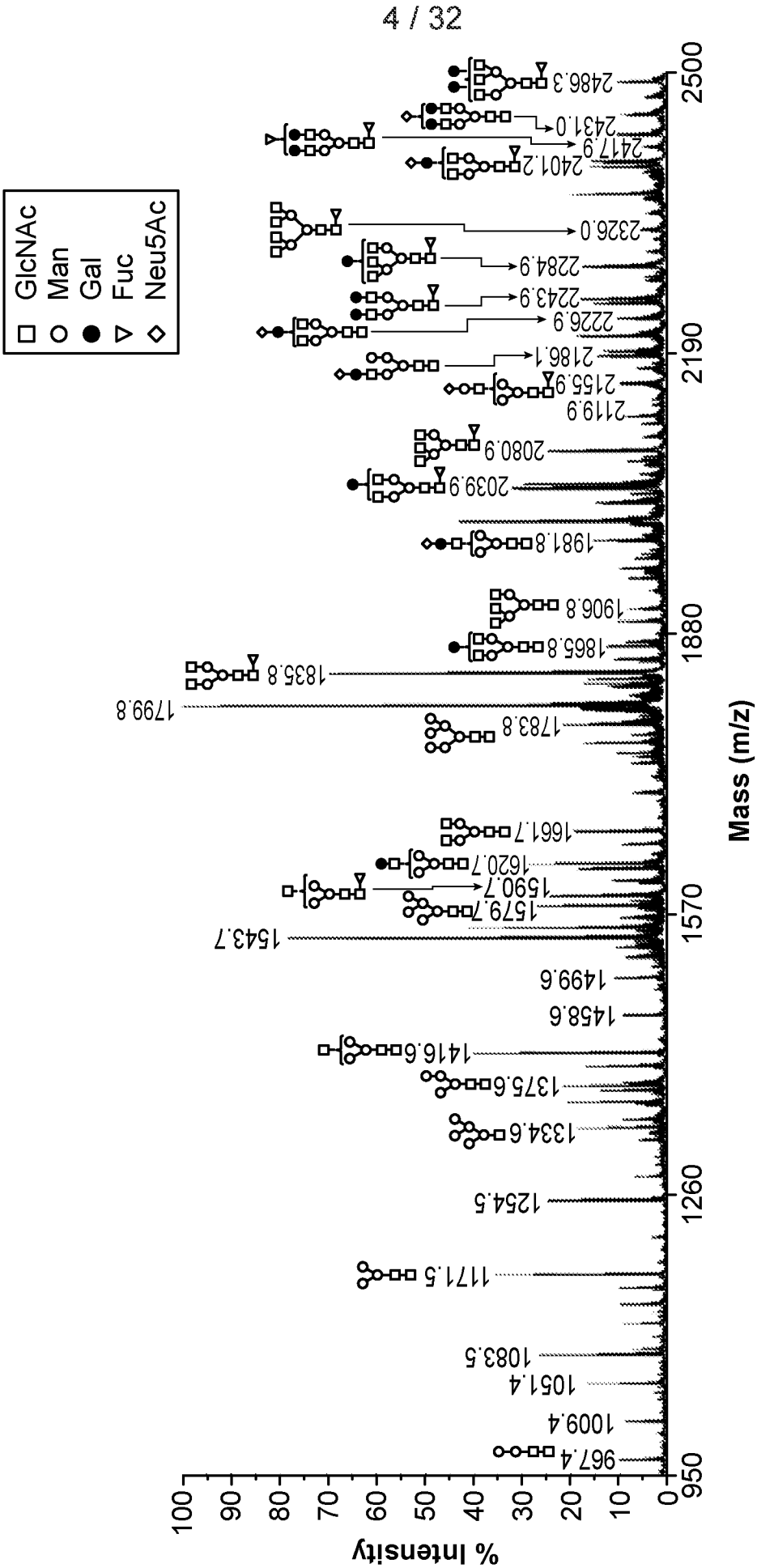


FIG. 3A

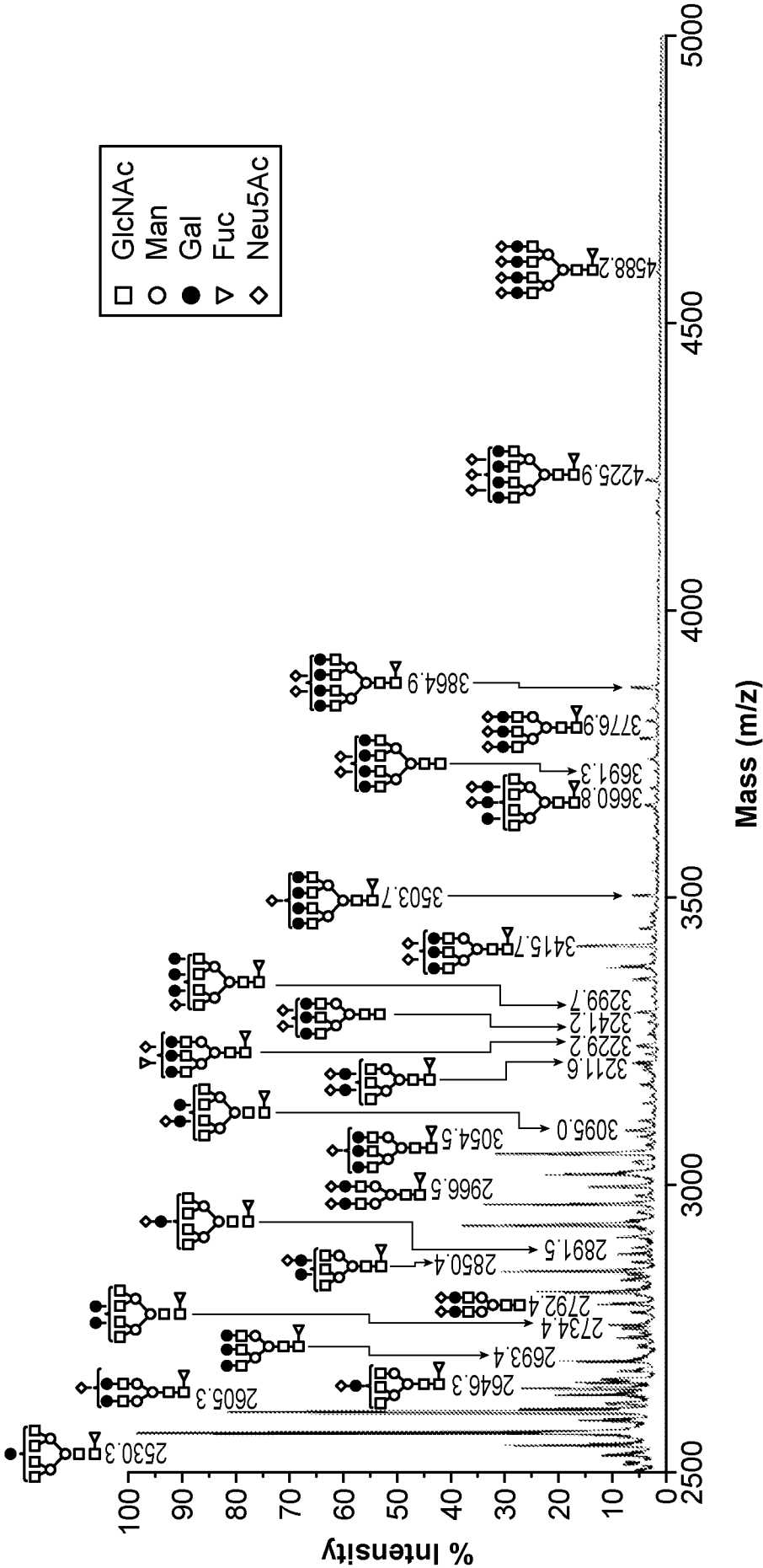


FIG. 3B

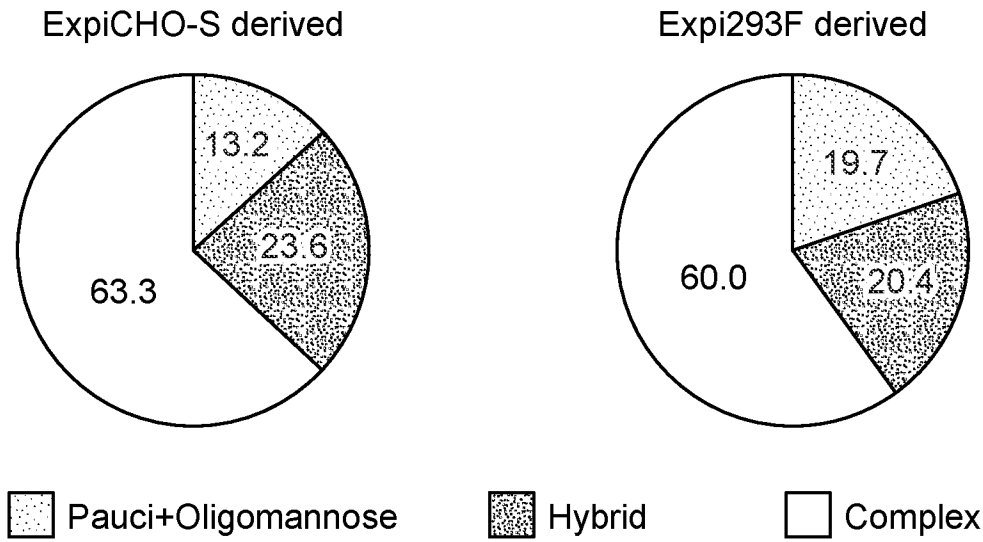


FIG. 4A

FIG. 4B

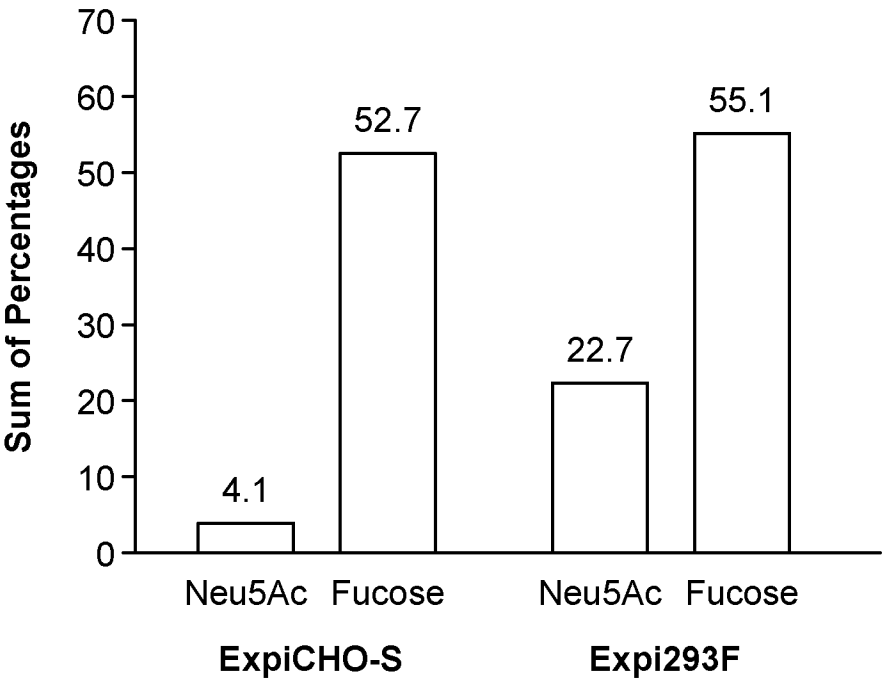


FIG. 4C

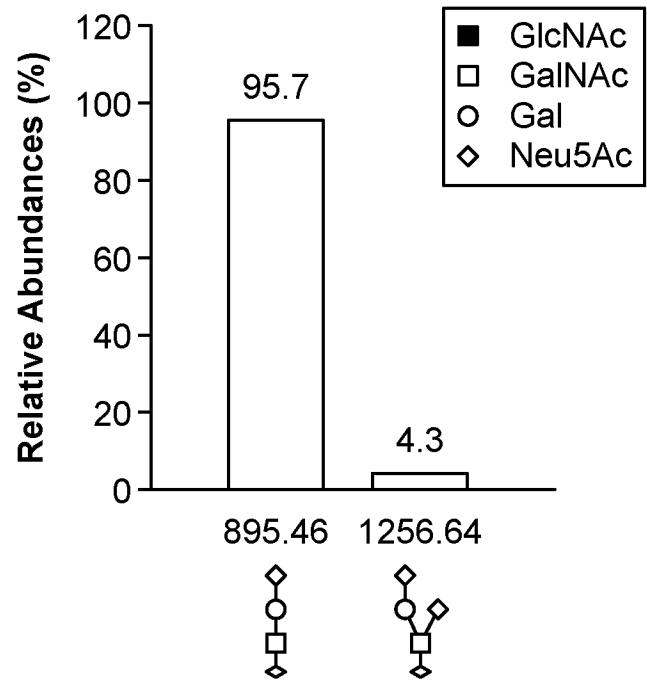


FIG. 4D

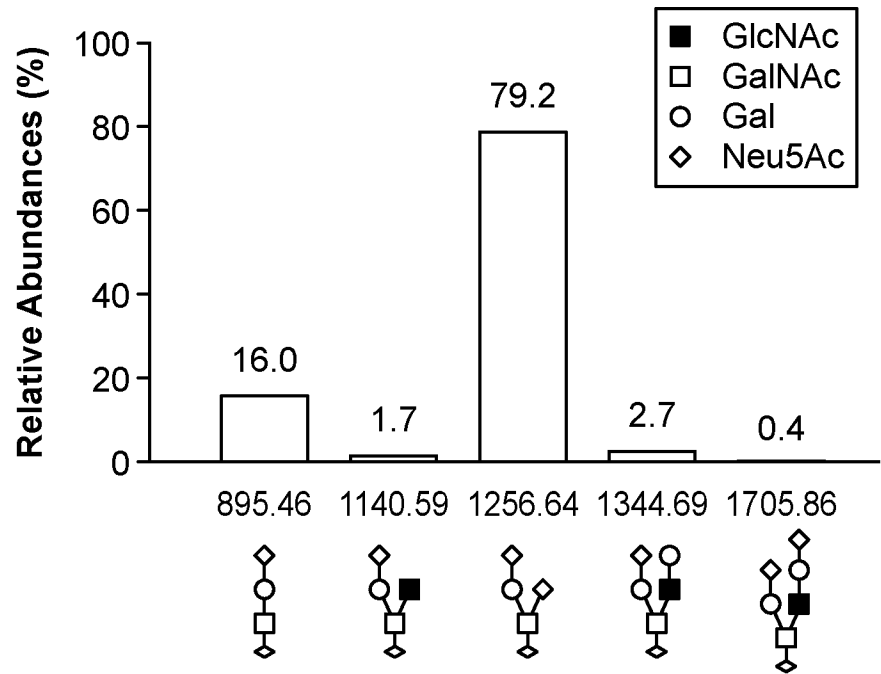


FIG. 4E

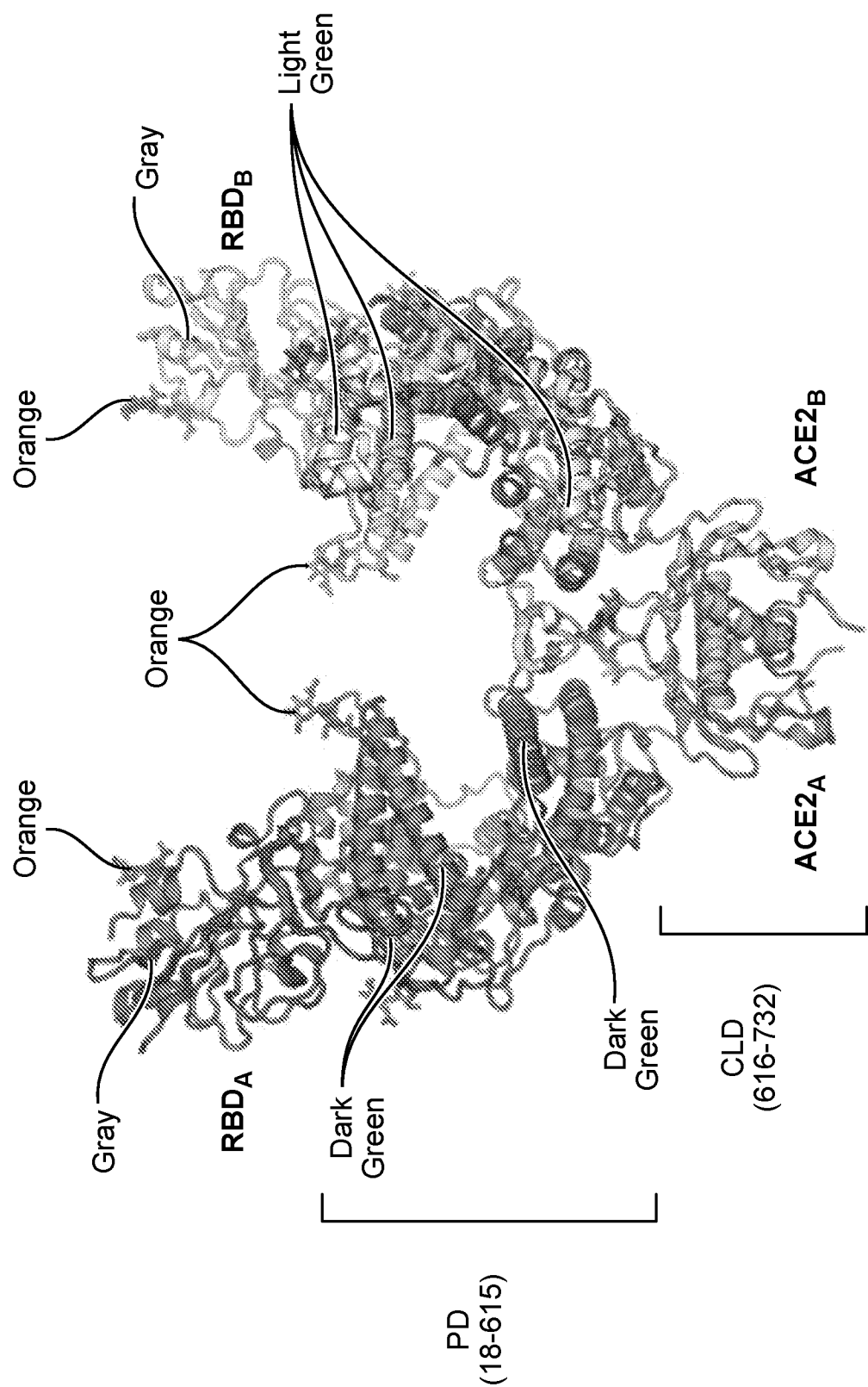


FIG. 5A

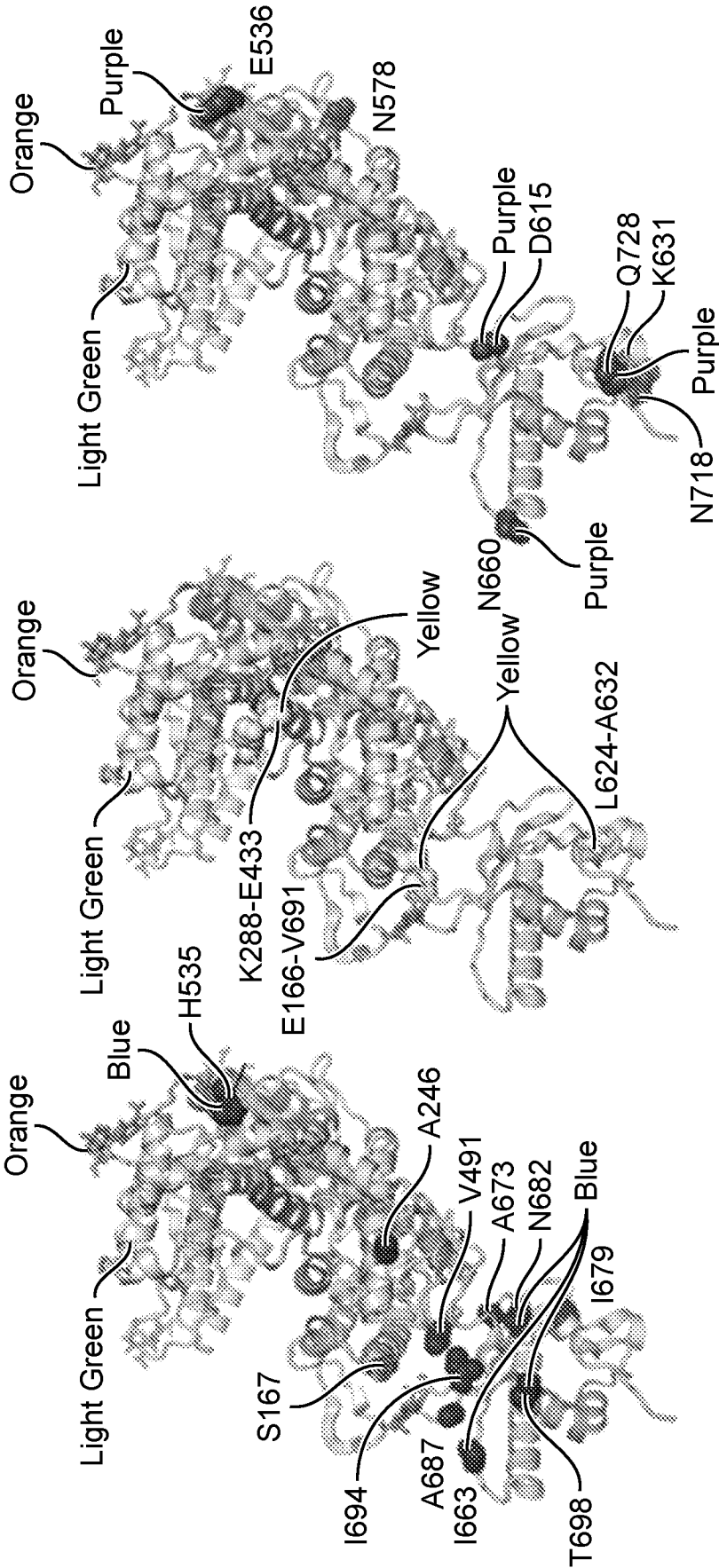


FIG. 5B

FIG. 5C

FIG. 5D

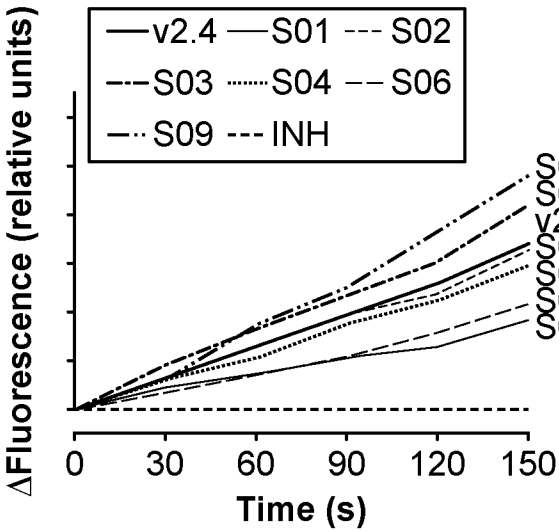


FIG. 6A

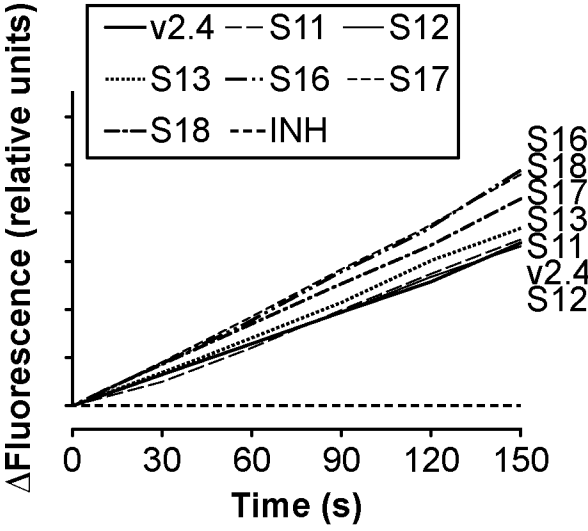


FIG. 6B

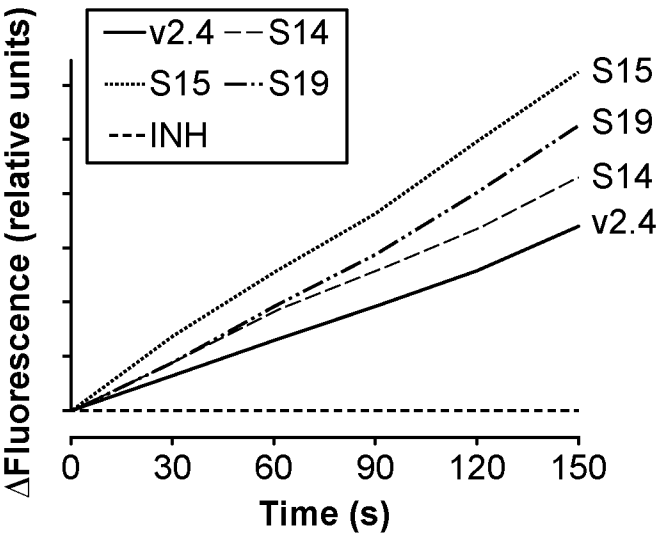


FIG. 6C

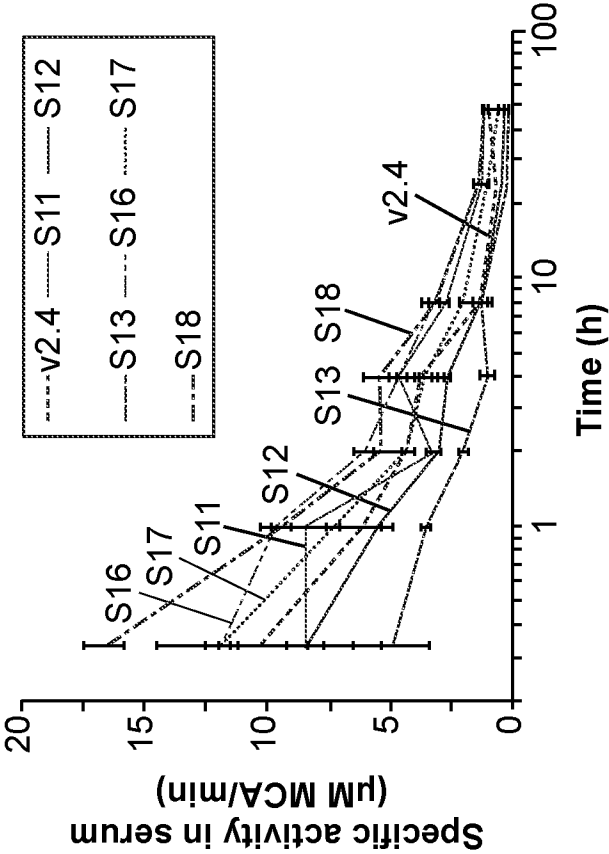


FIG. 7B

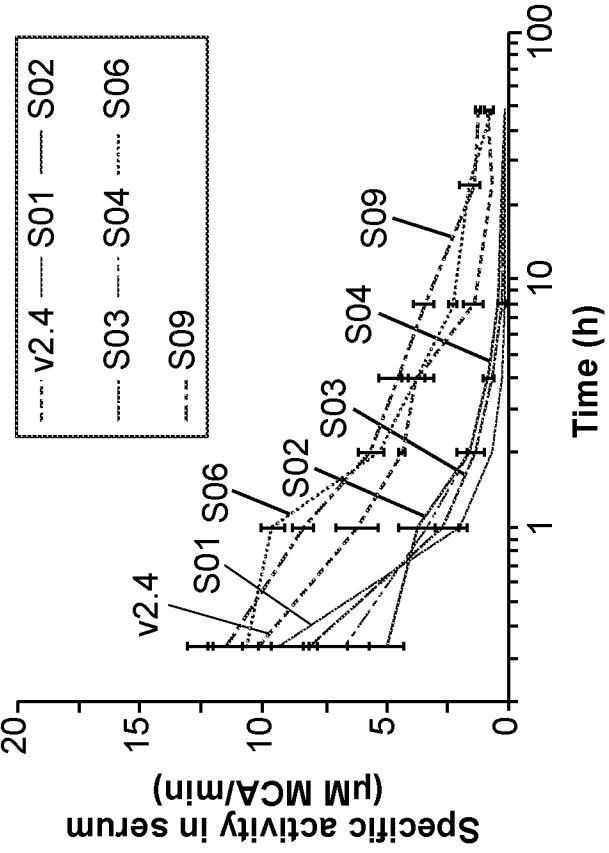
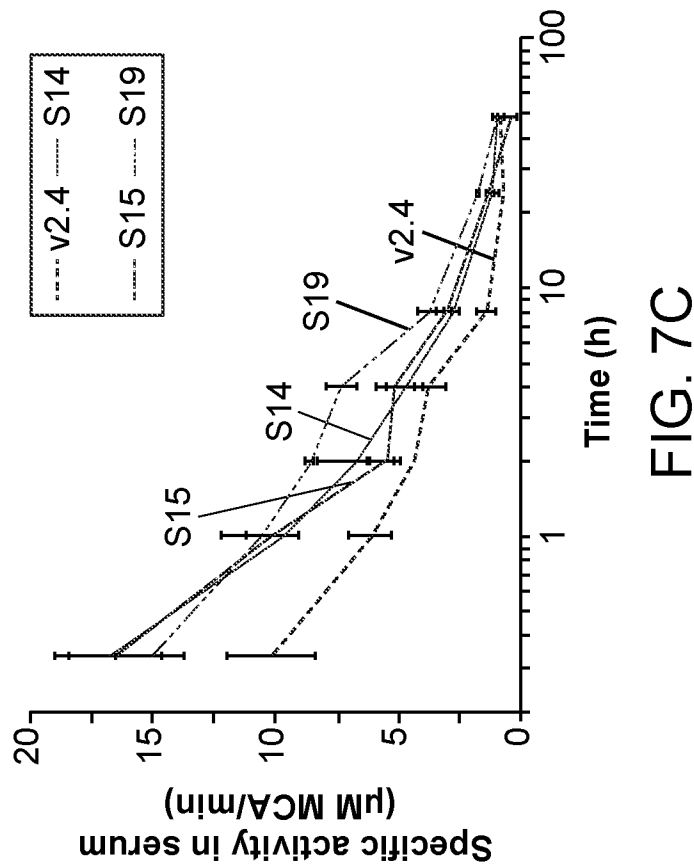
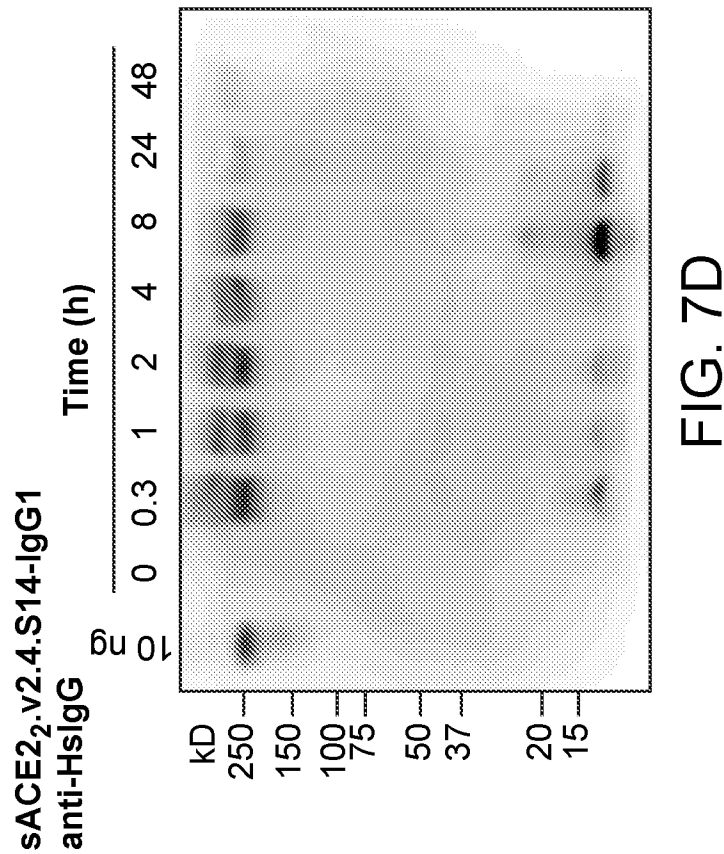


FIG. 7A



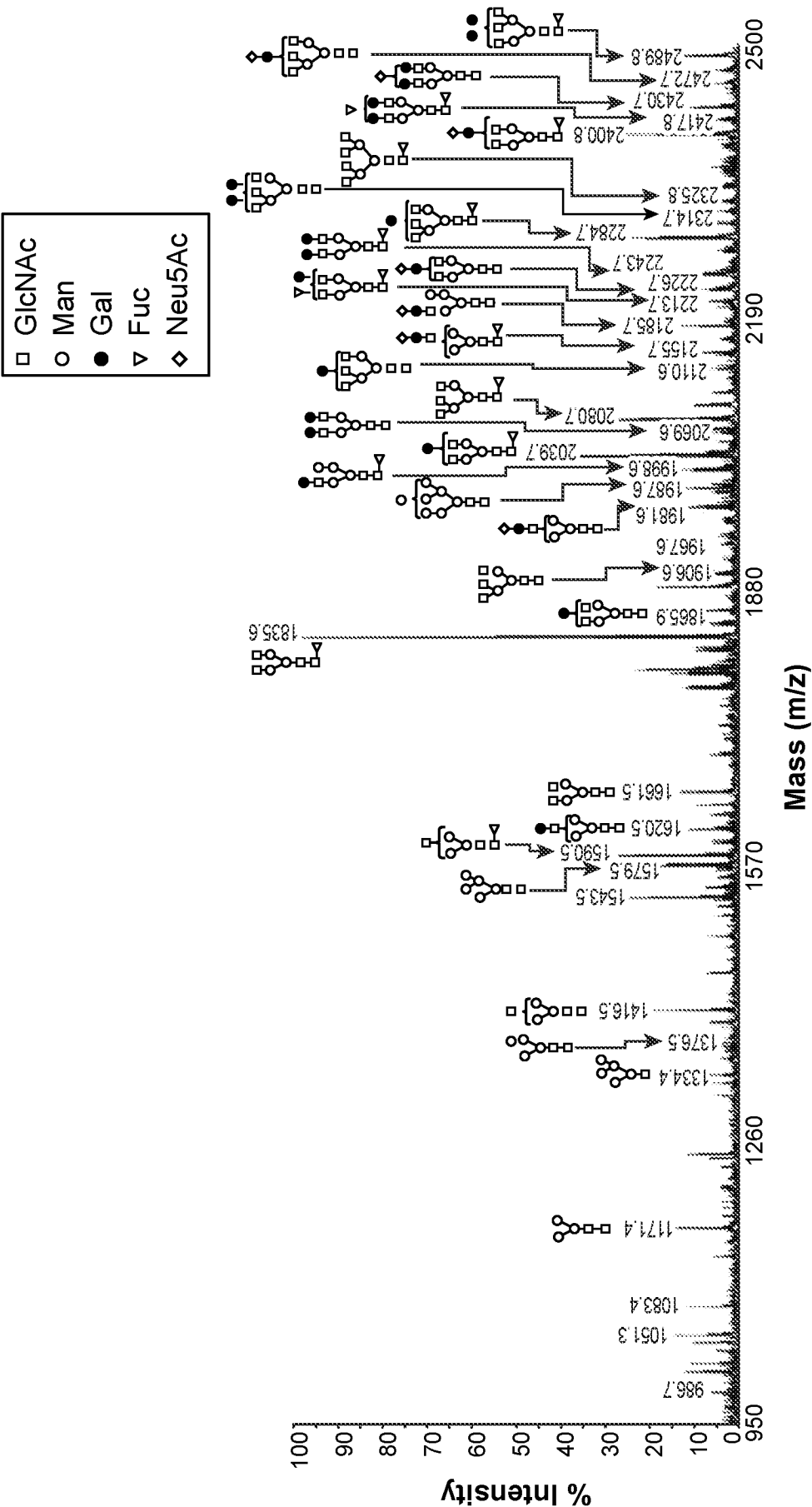


FIG. 8A

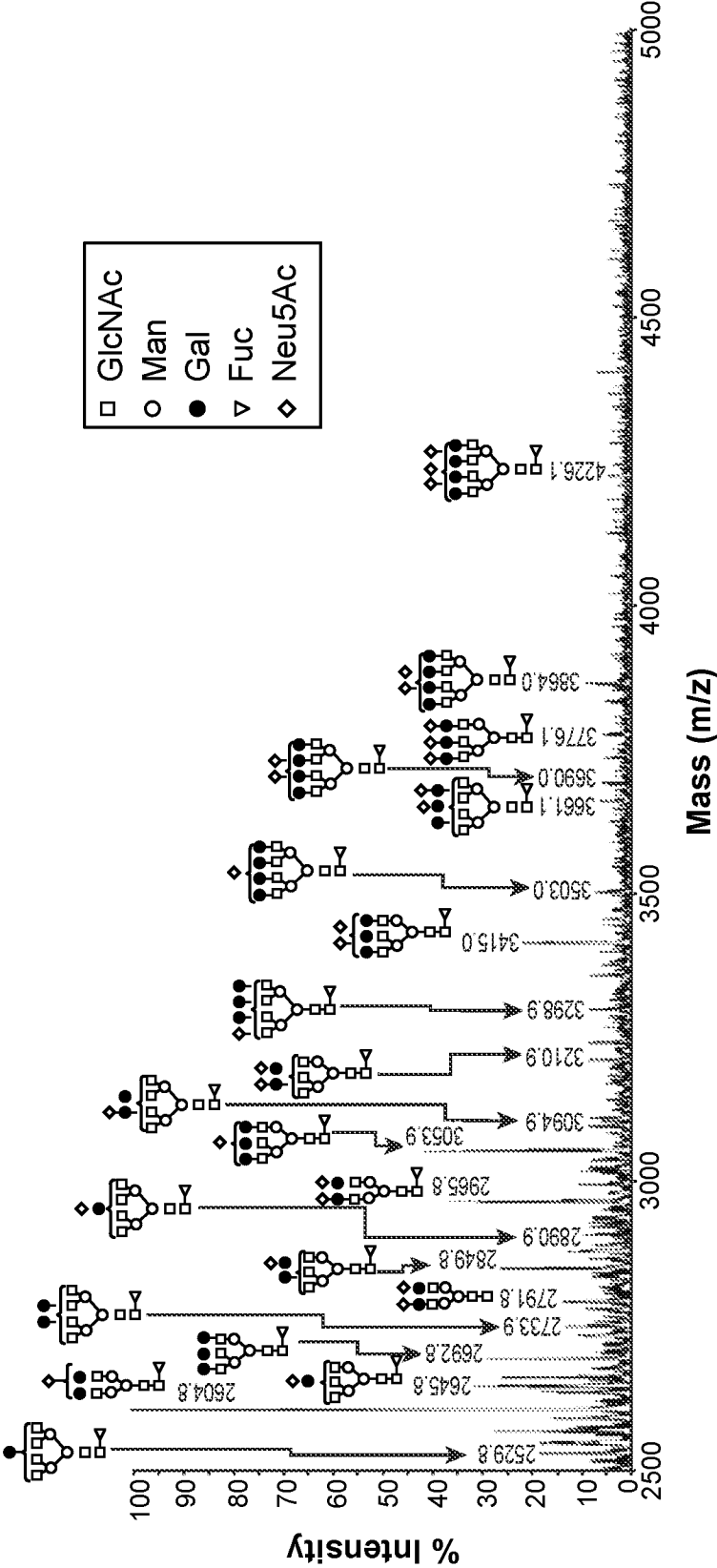


FIG. 8B

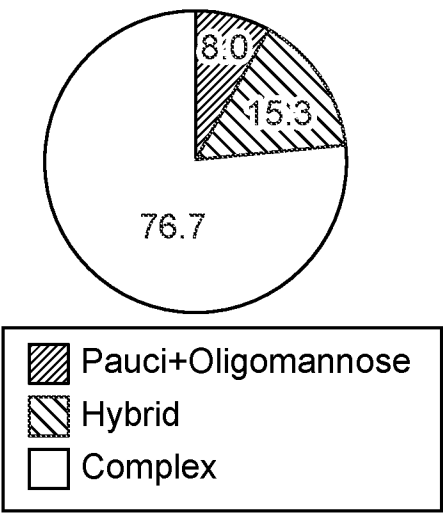


FIG. 9A

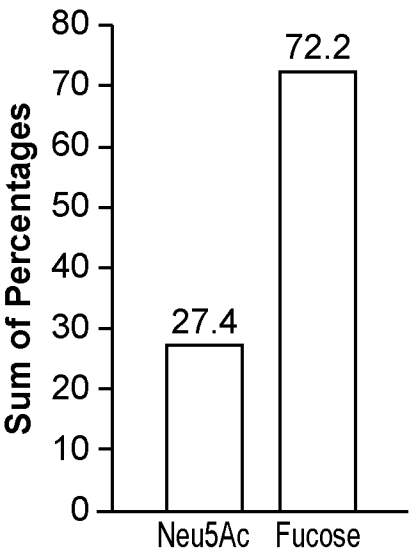


FIG. 9B

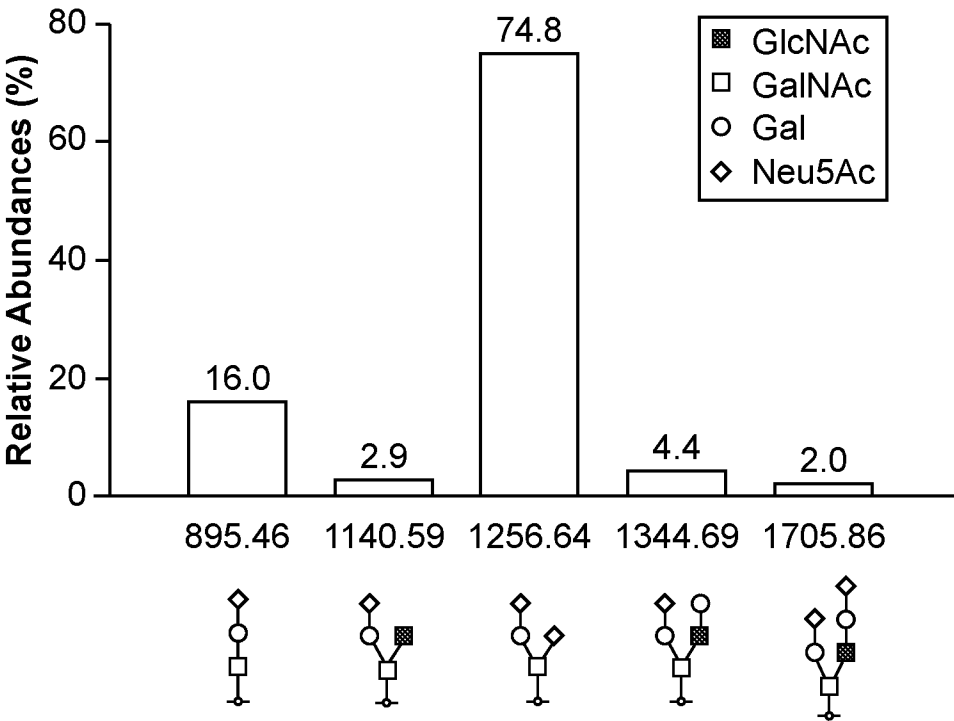


FIG. 9C

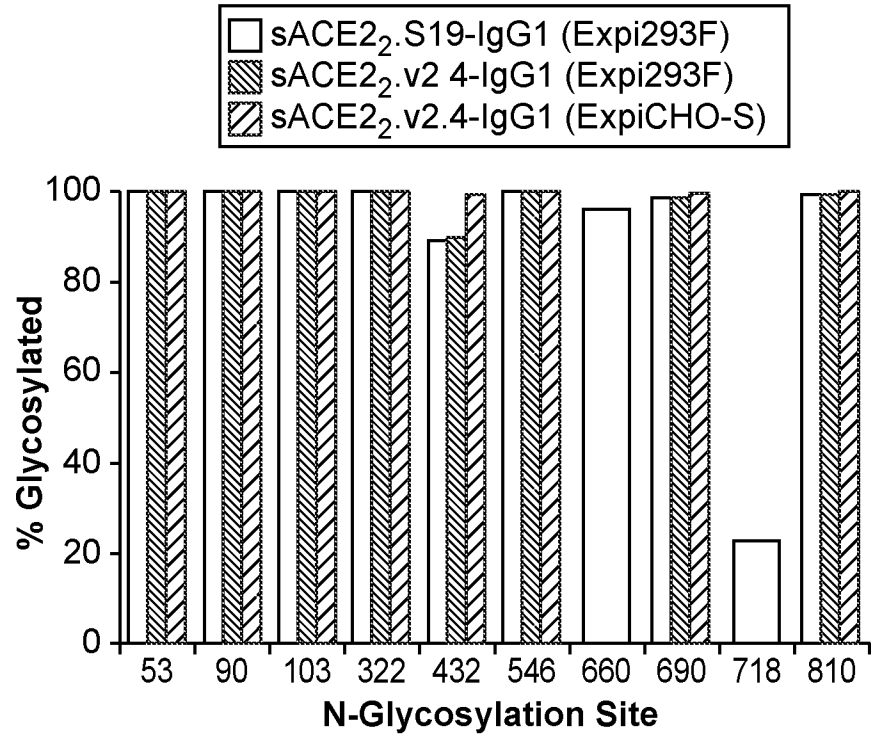


FIG. 10A

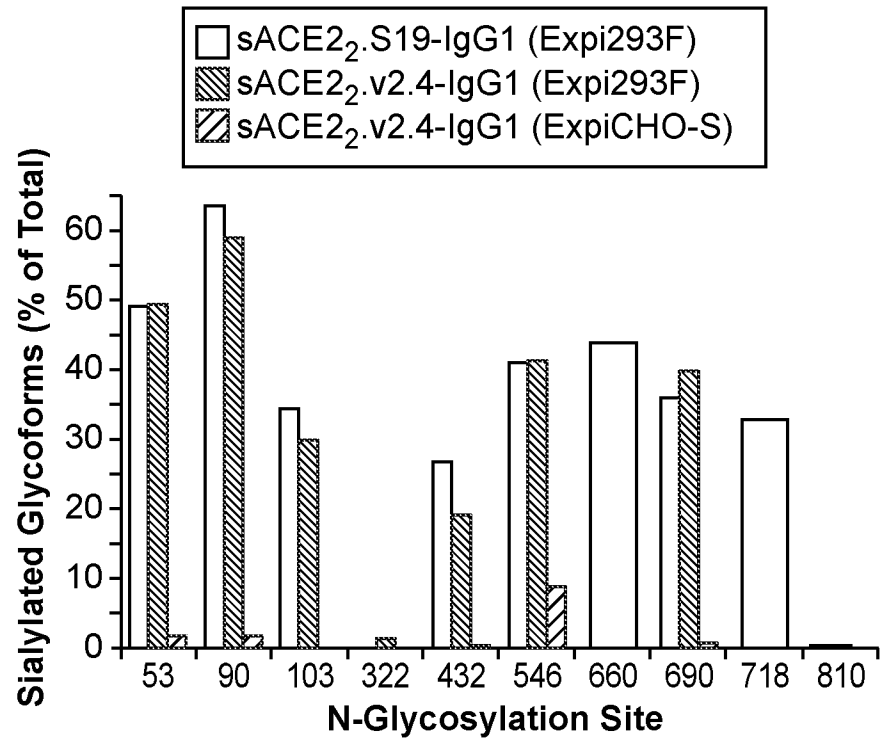


FIG. 10B

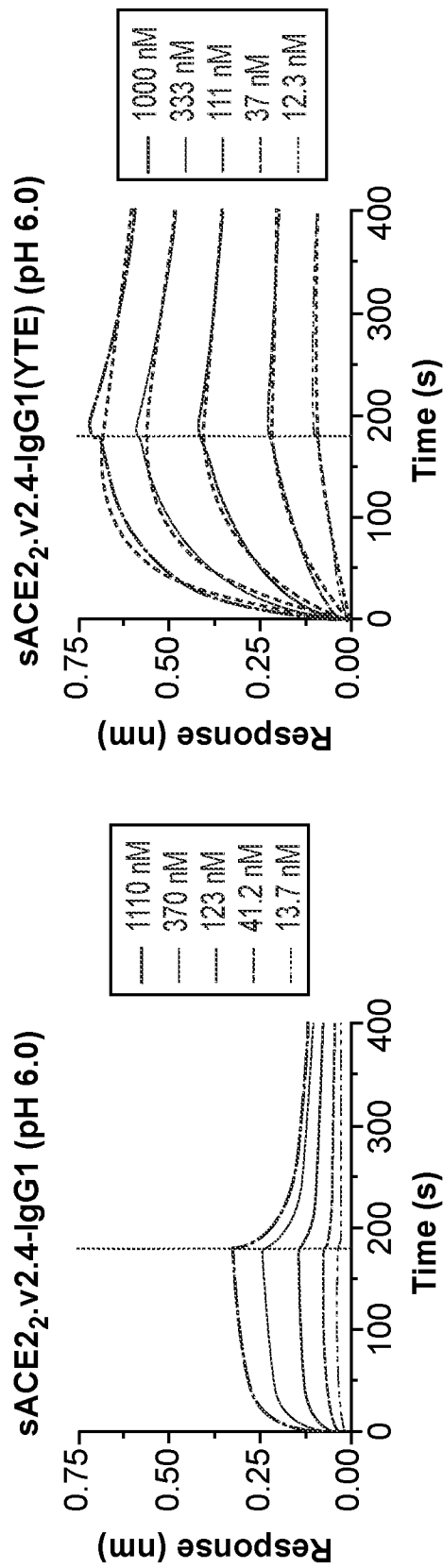


FIG. 11A

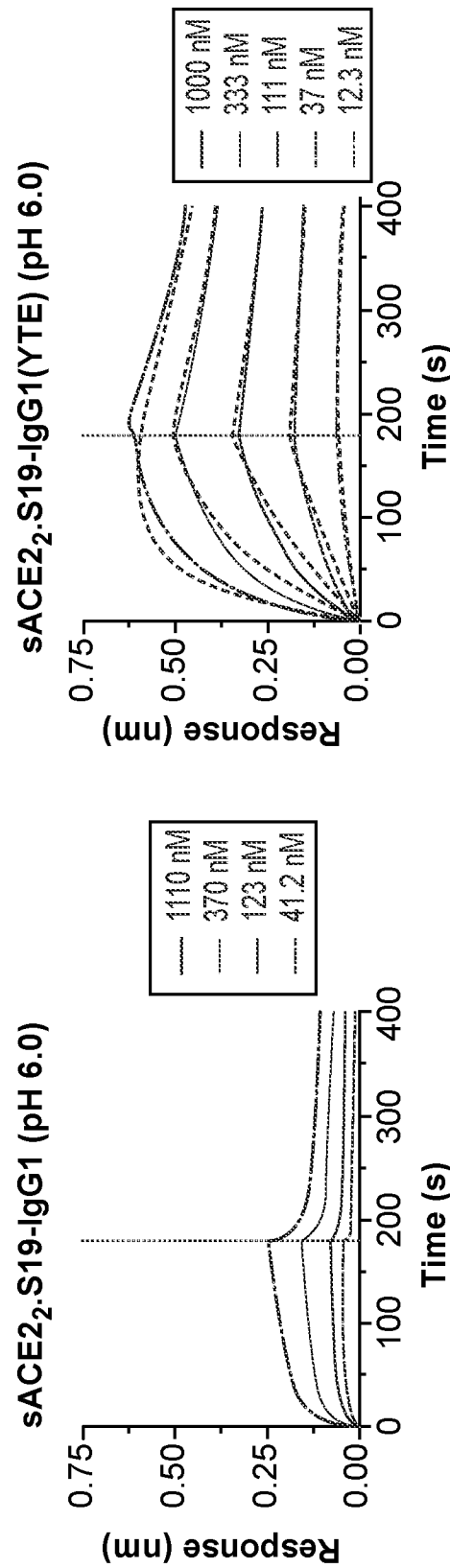


FIG. 11C

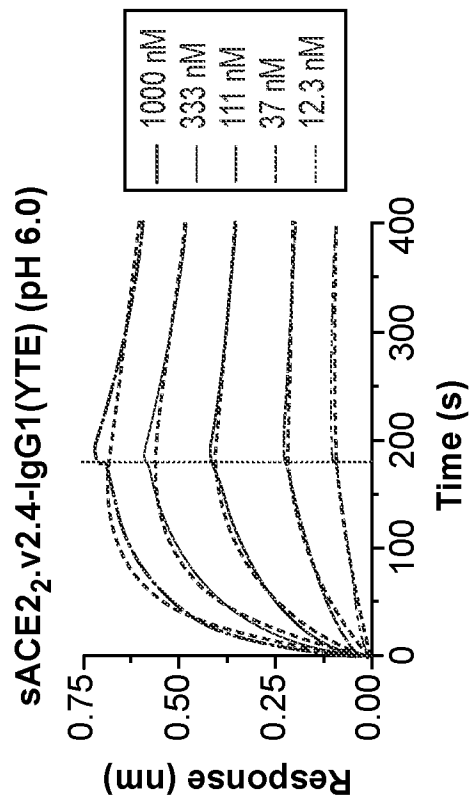


FIG. 11B

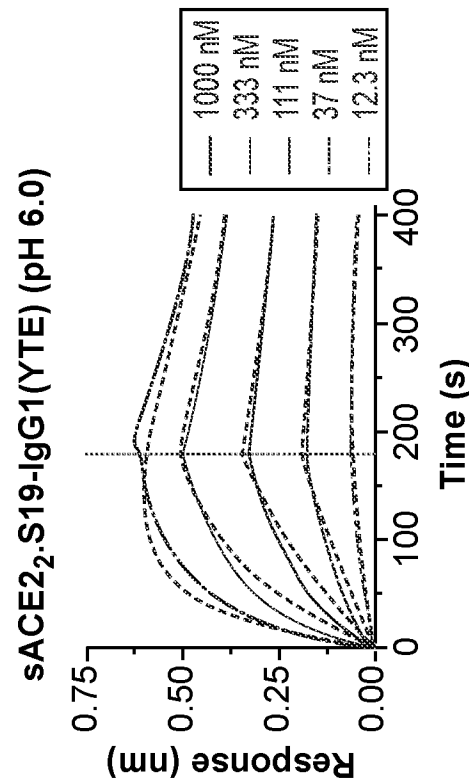


FIG. 11D

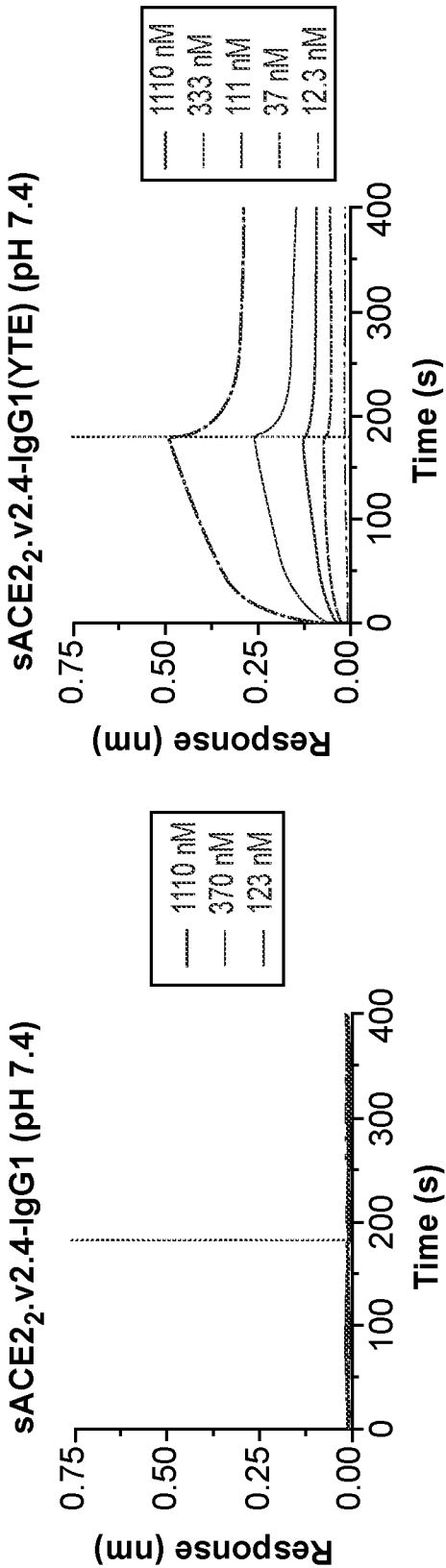


FIG. 11F

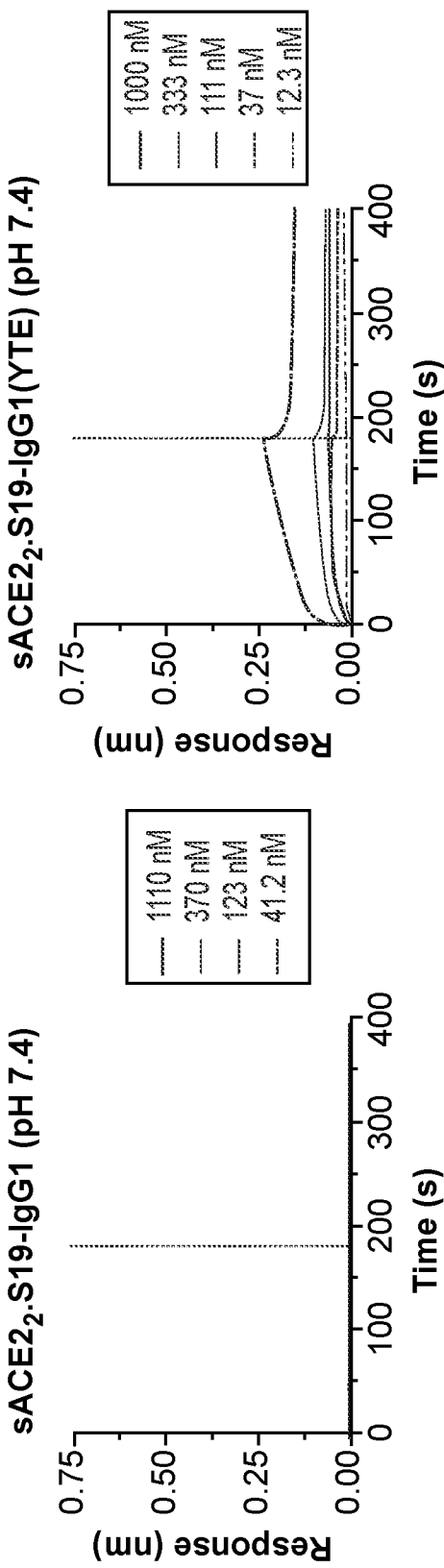


FIG. 11H

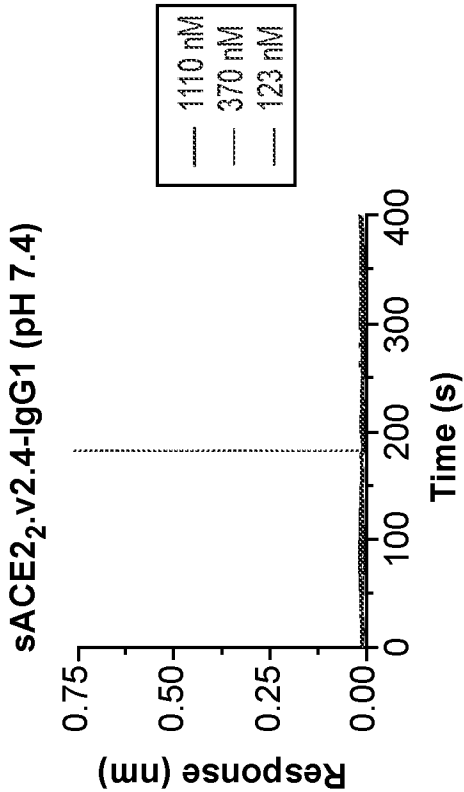


FIG. 11E

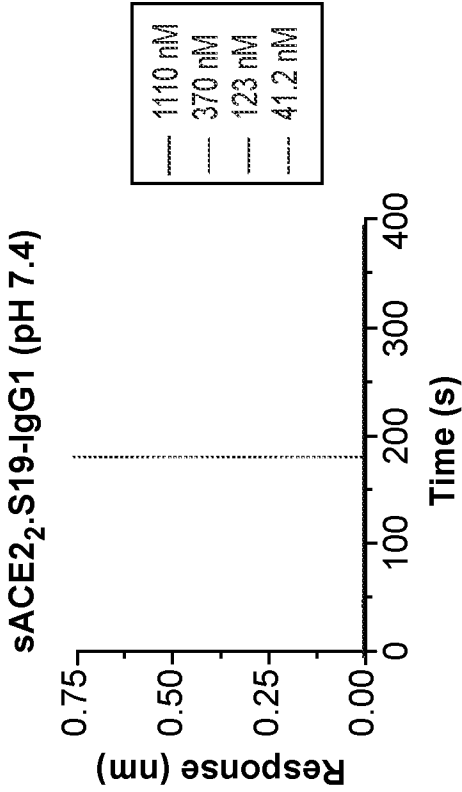


FIG. 11G

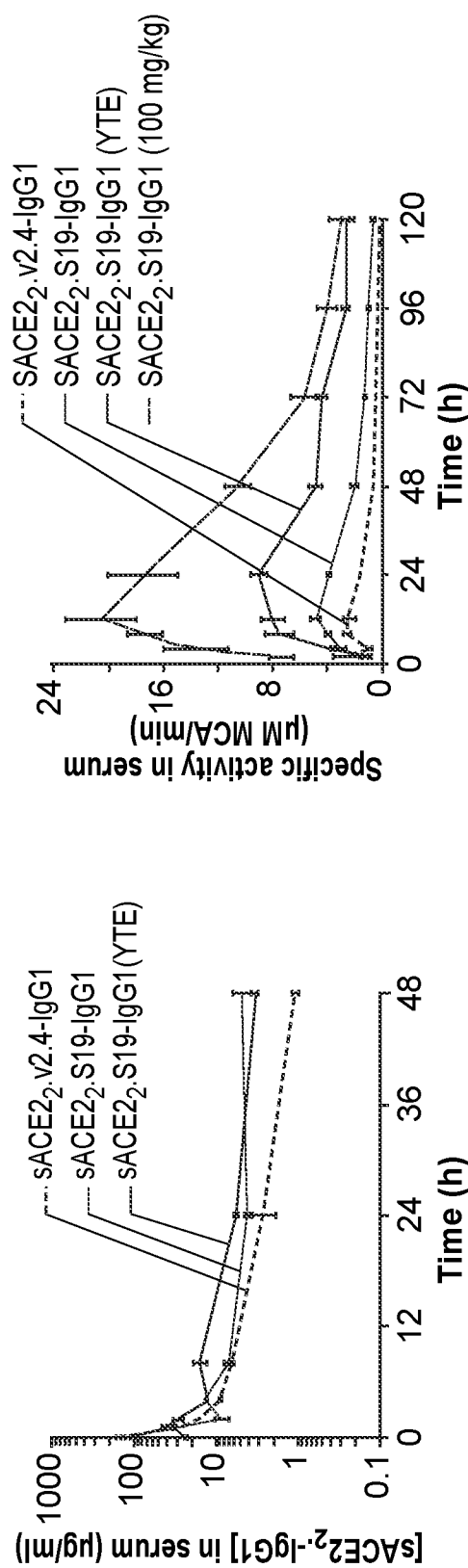


FIG. 12B

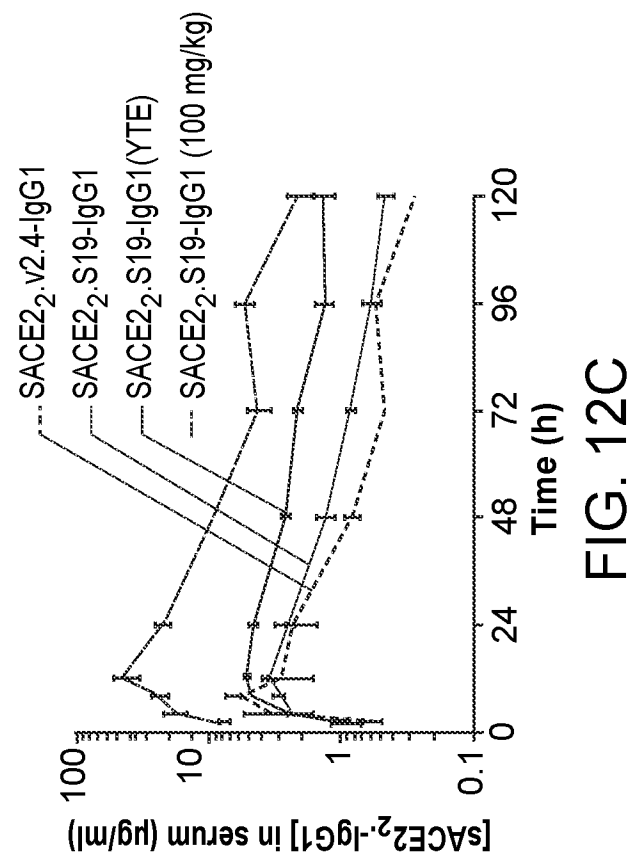


FIG. 12C

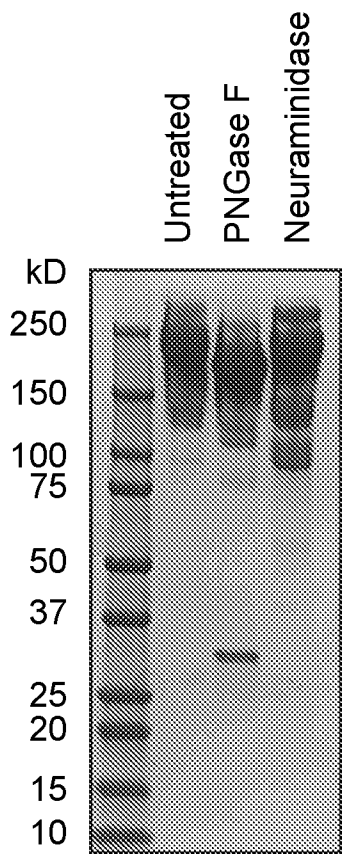


FIG. 13A

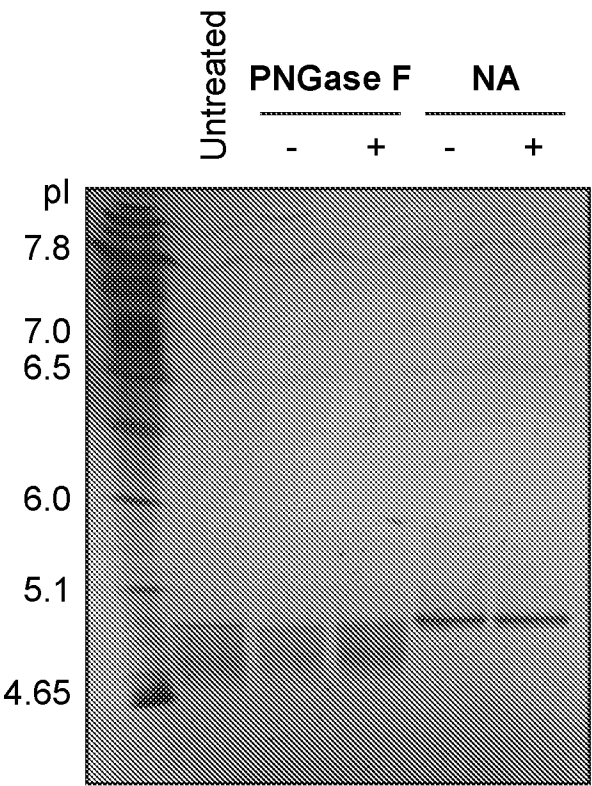


FIG. 13B

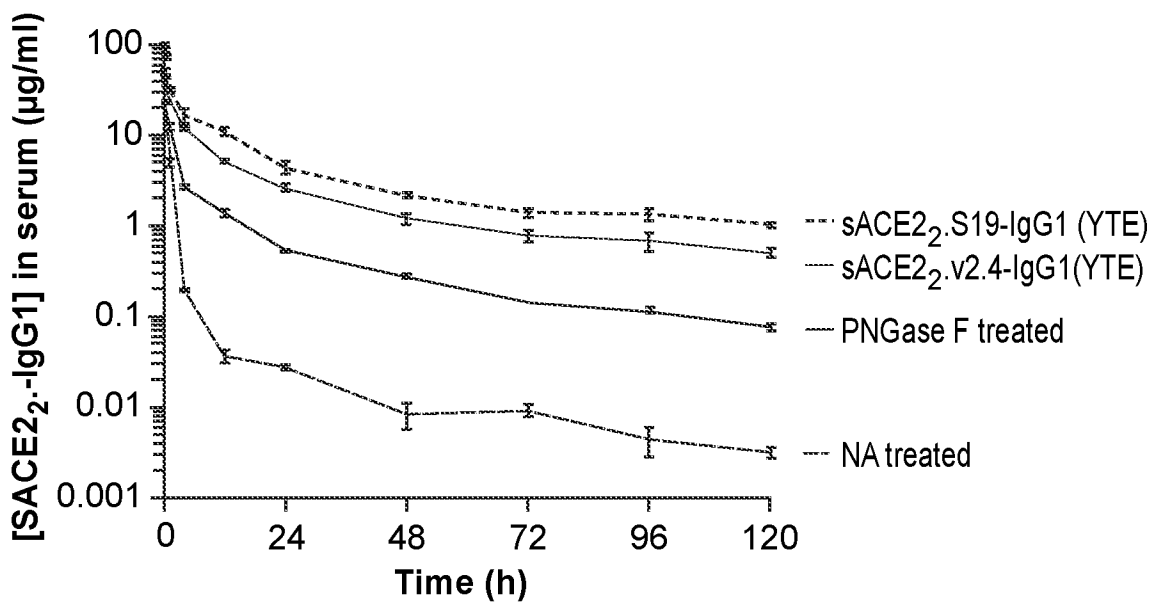


FIG. 13C

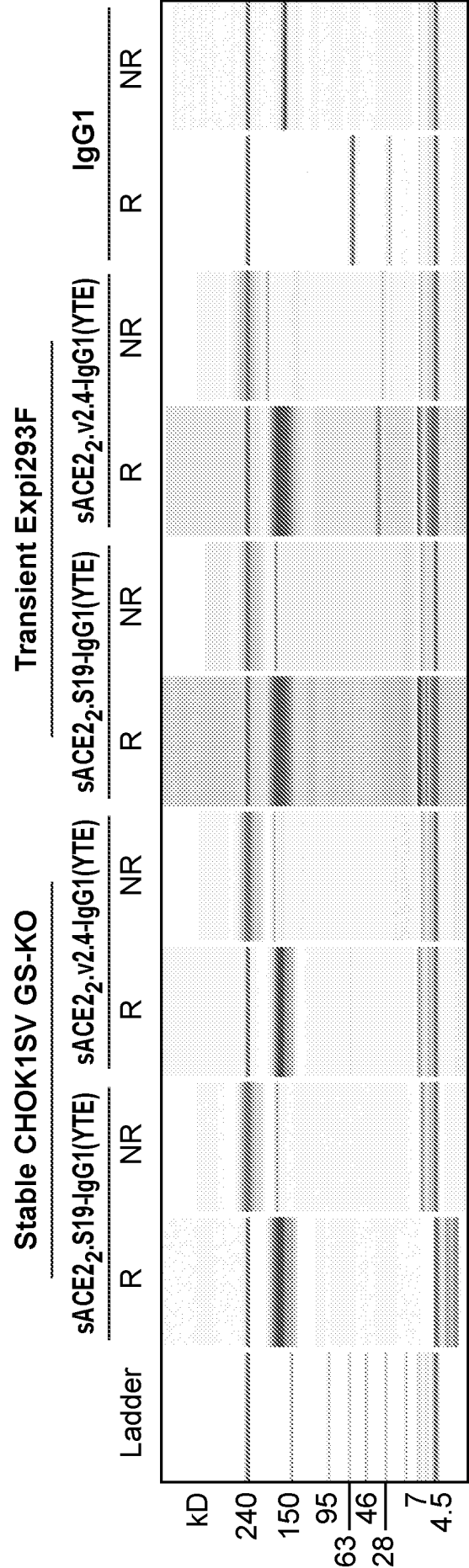


FIG. 14A

sACE2₂. v2.4-IgG1(YTE) from Expi293F

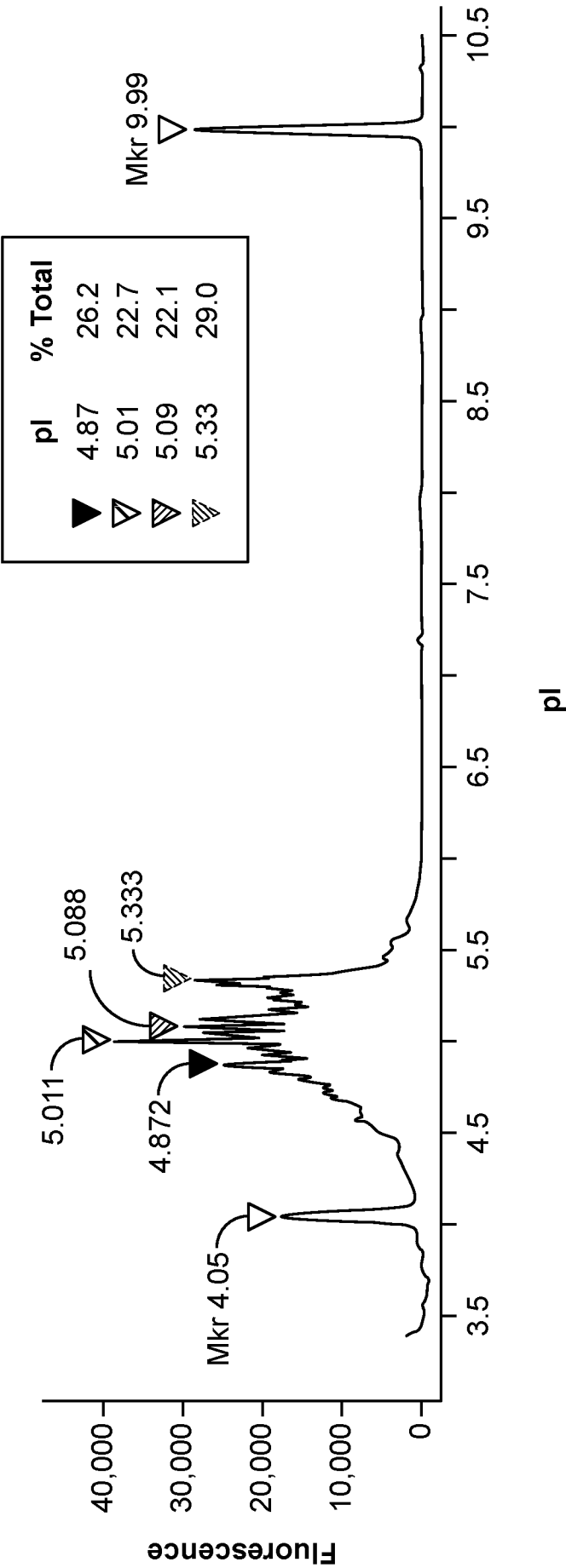


FIG. 14B

sACE2₂.S19-IgG1(YTE) from Expi293F

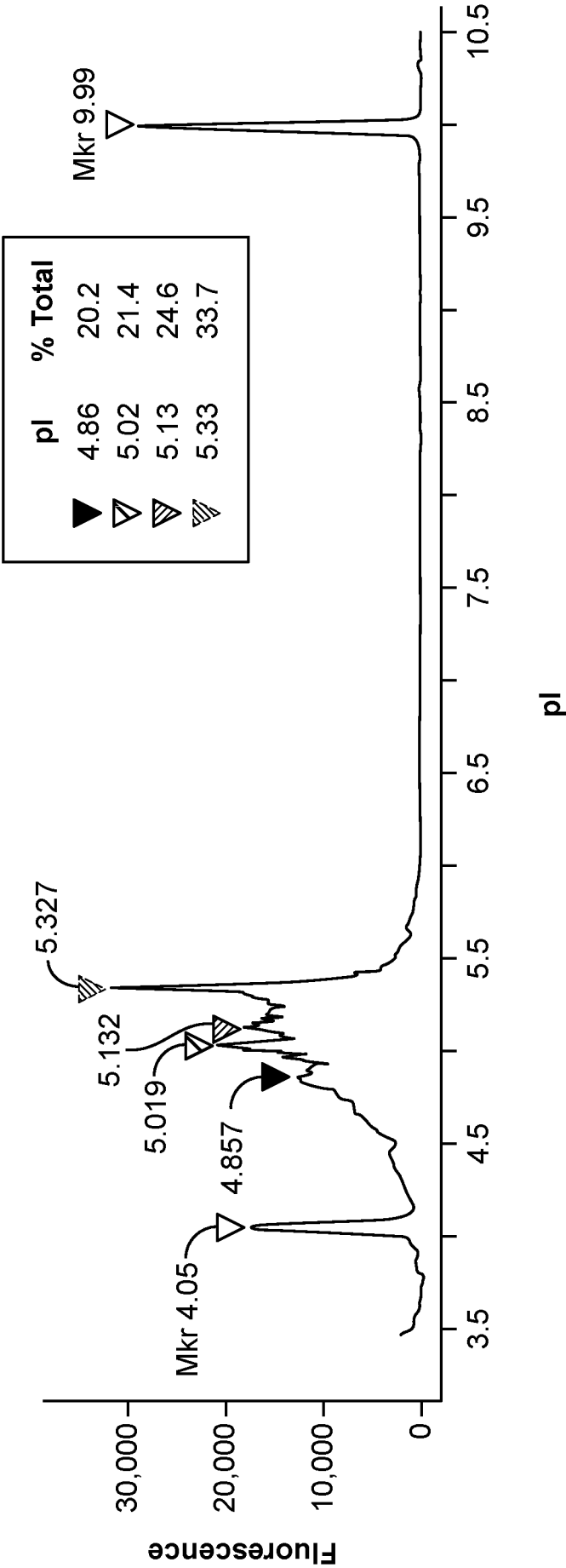


FIG. 14C

sACE2₂. v2.4-IgG1(YTE) from CHOK1SV GS-KO

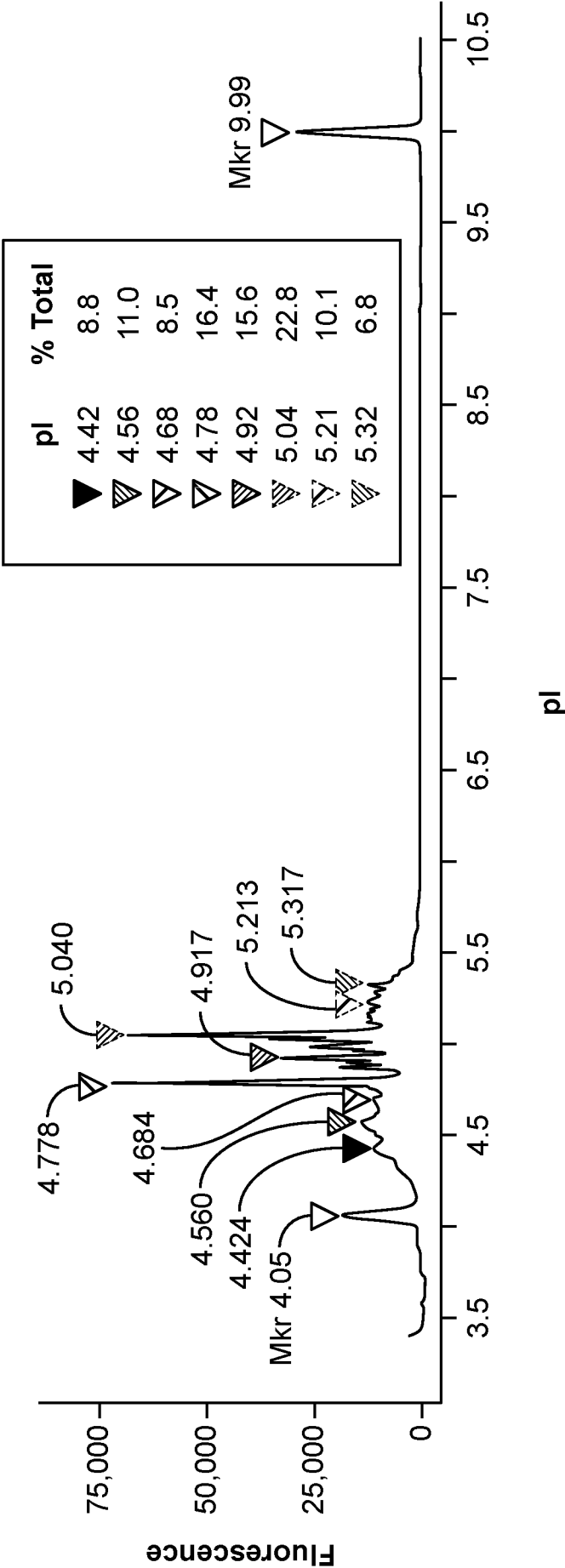


FIG. 14D

sACE2₂.S19-IgG1(YTE) from CHOK1SV GS-KO

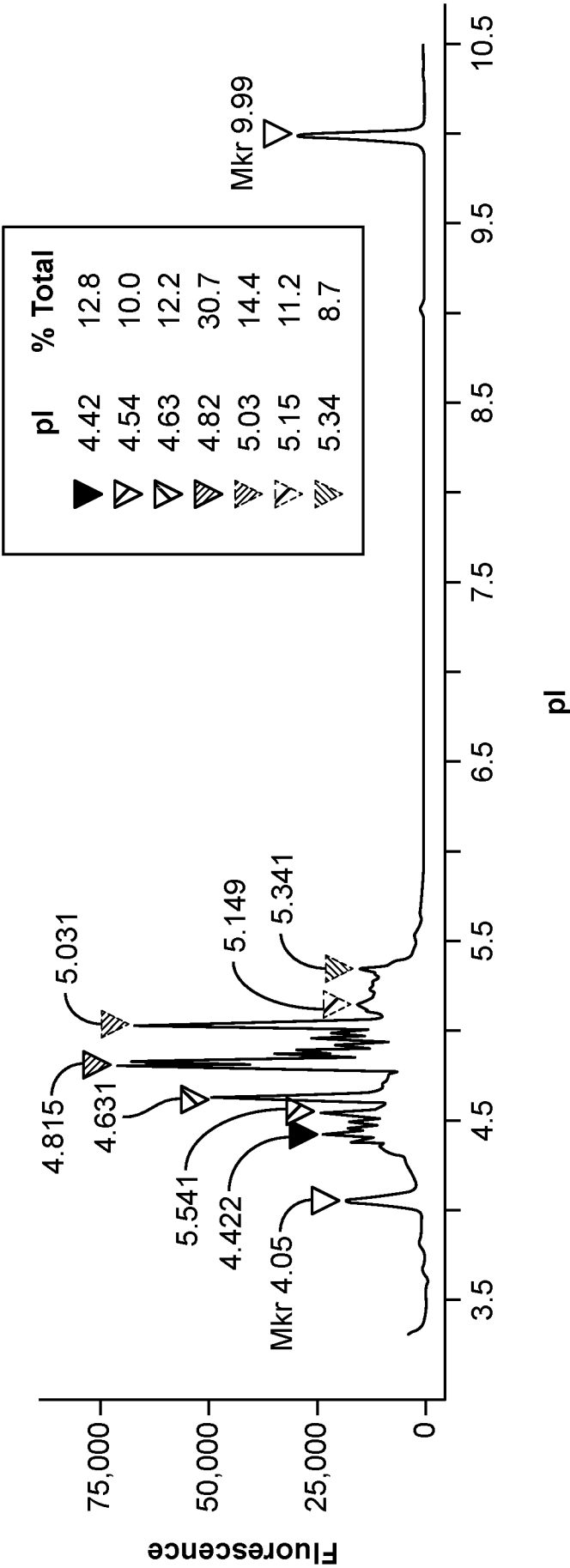


FIG. 14E

sACE2₂.v2.4-IgG1(YTE)

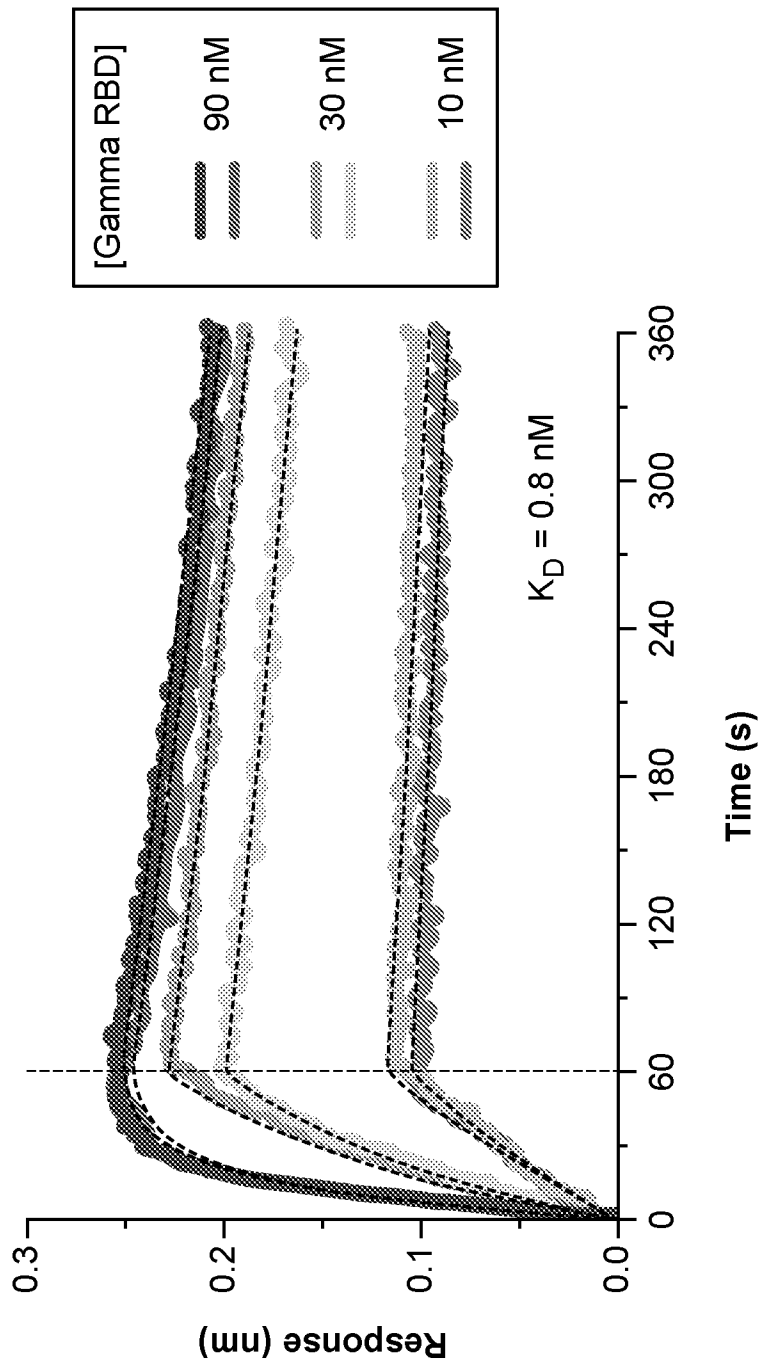


FIG. 15A

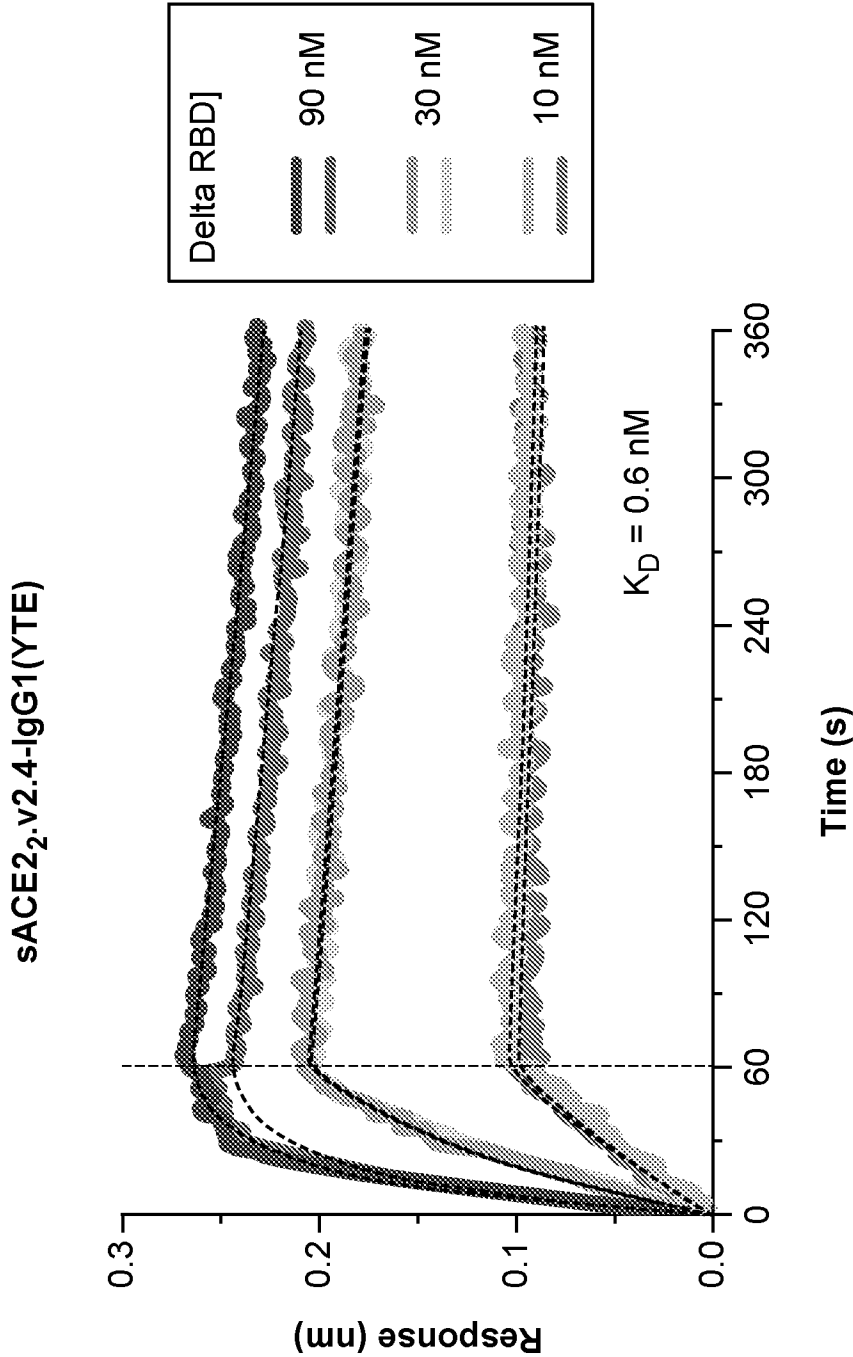


FIG. 15B

sACE2₂.S19-IgG1(YTE)

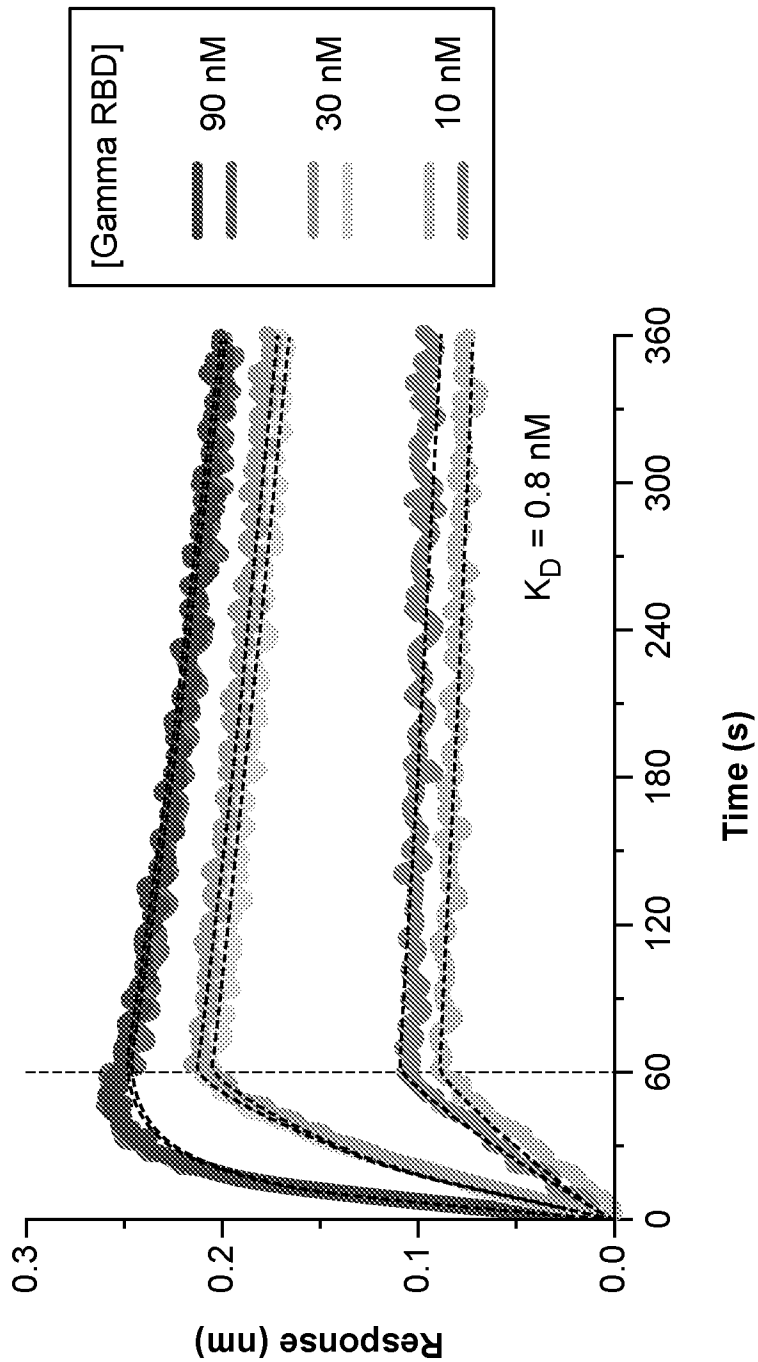


FIG. 15C

sACE2₂.S19-IgG1(YTE)

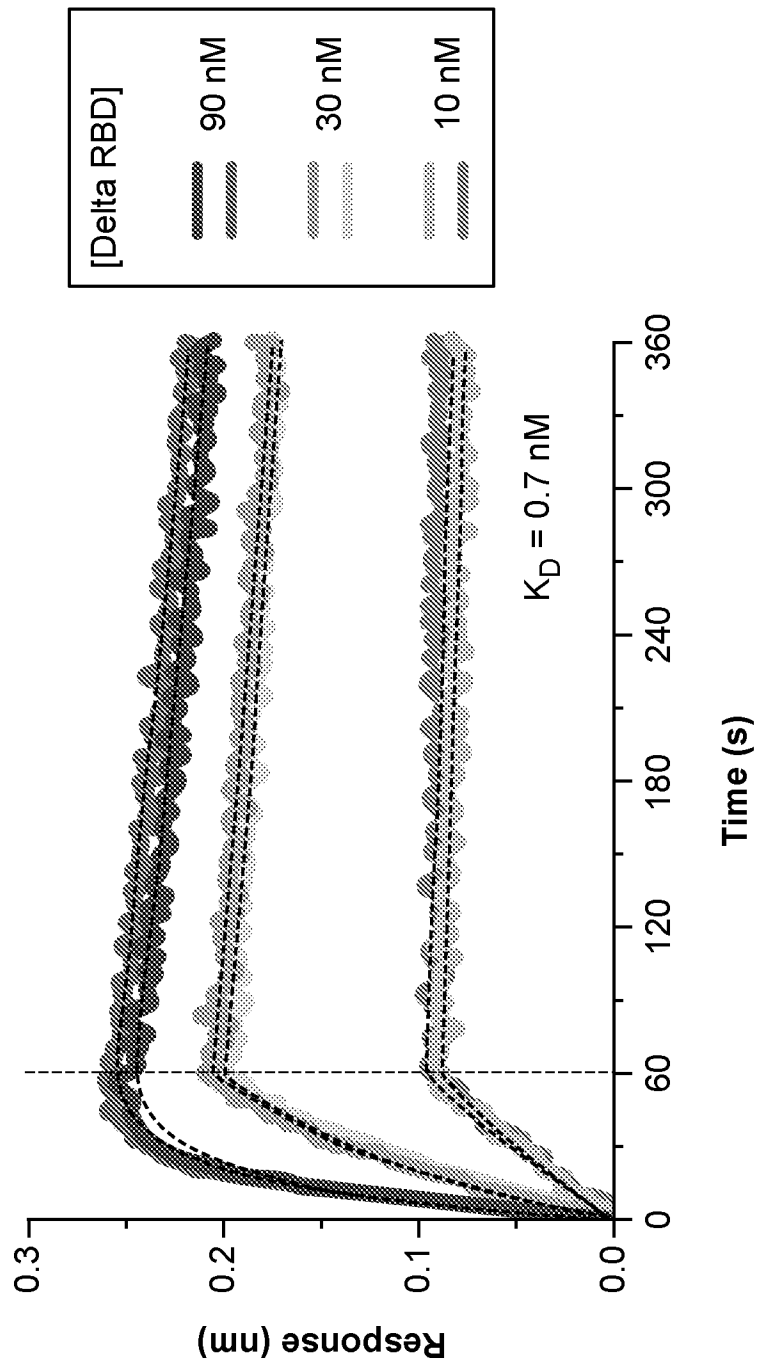


FIG. 15D

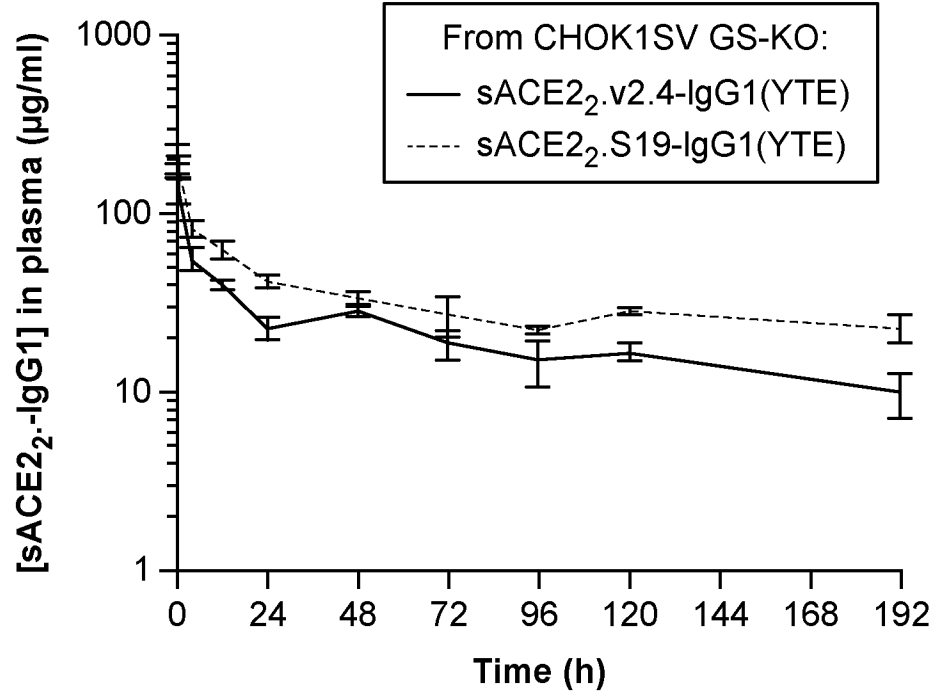


FIG. 16A

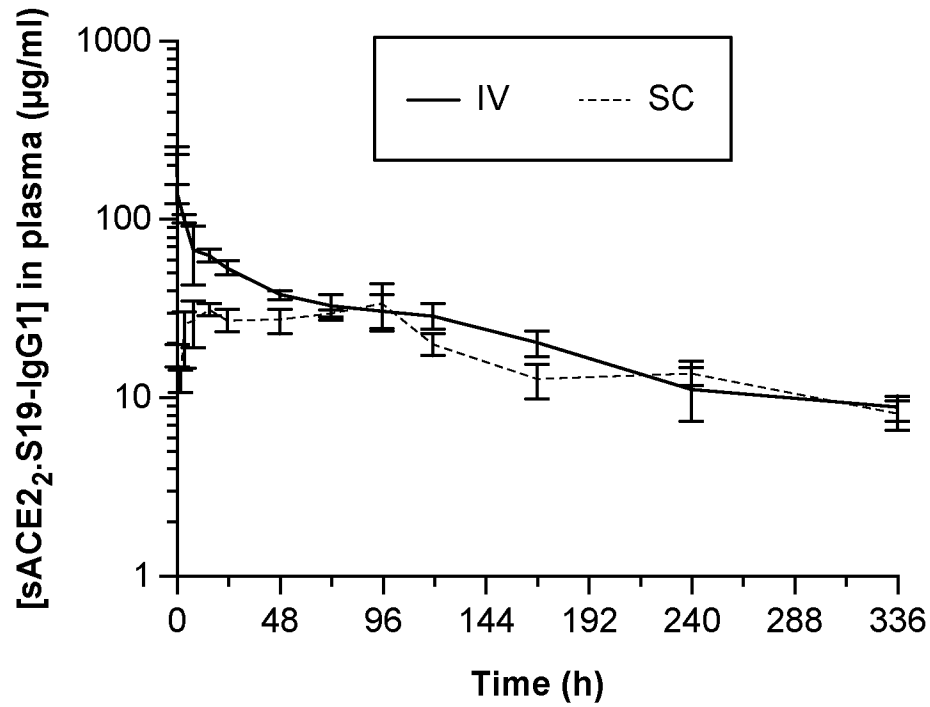


FIG. 16B

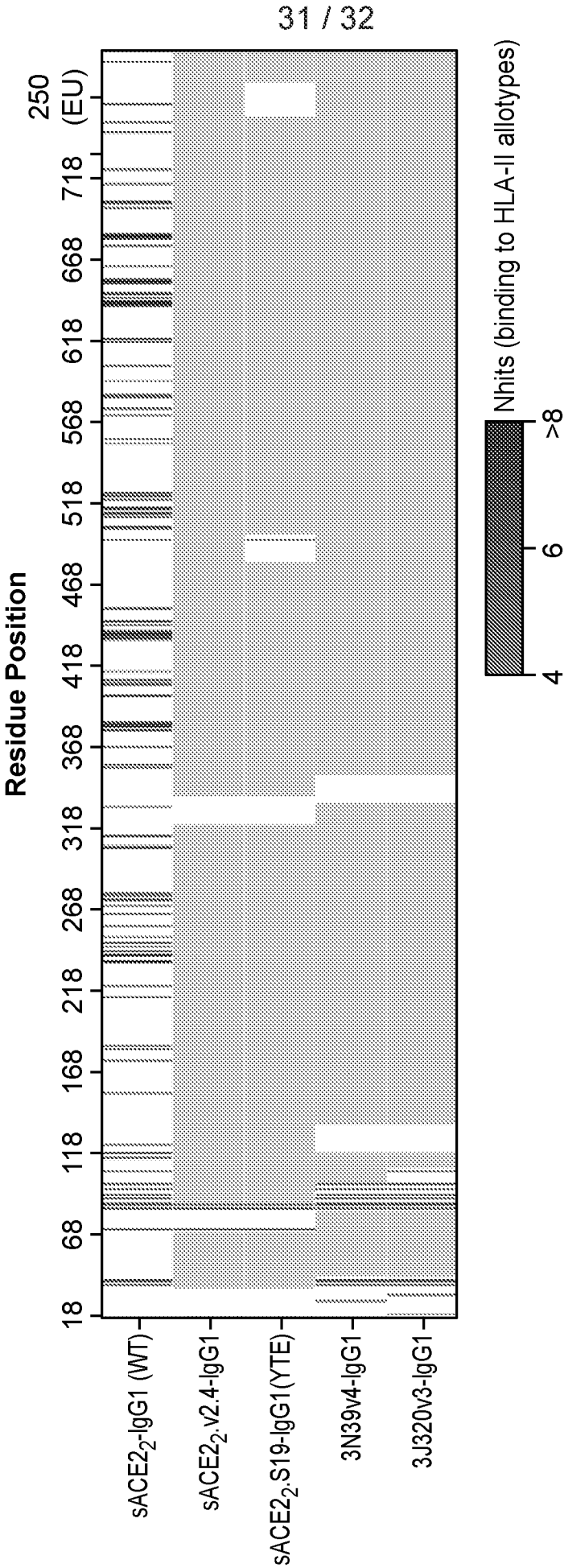


FIG. 17

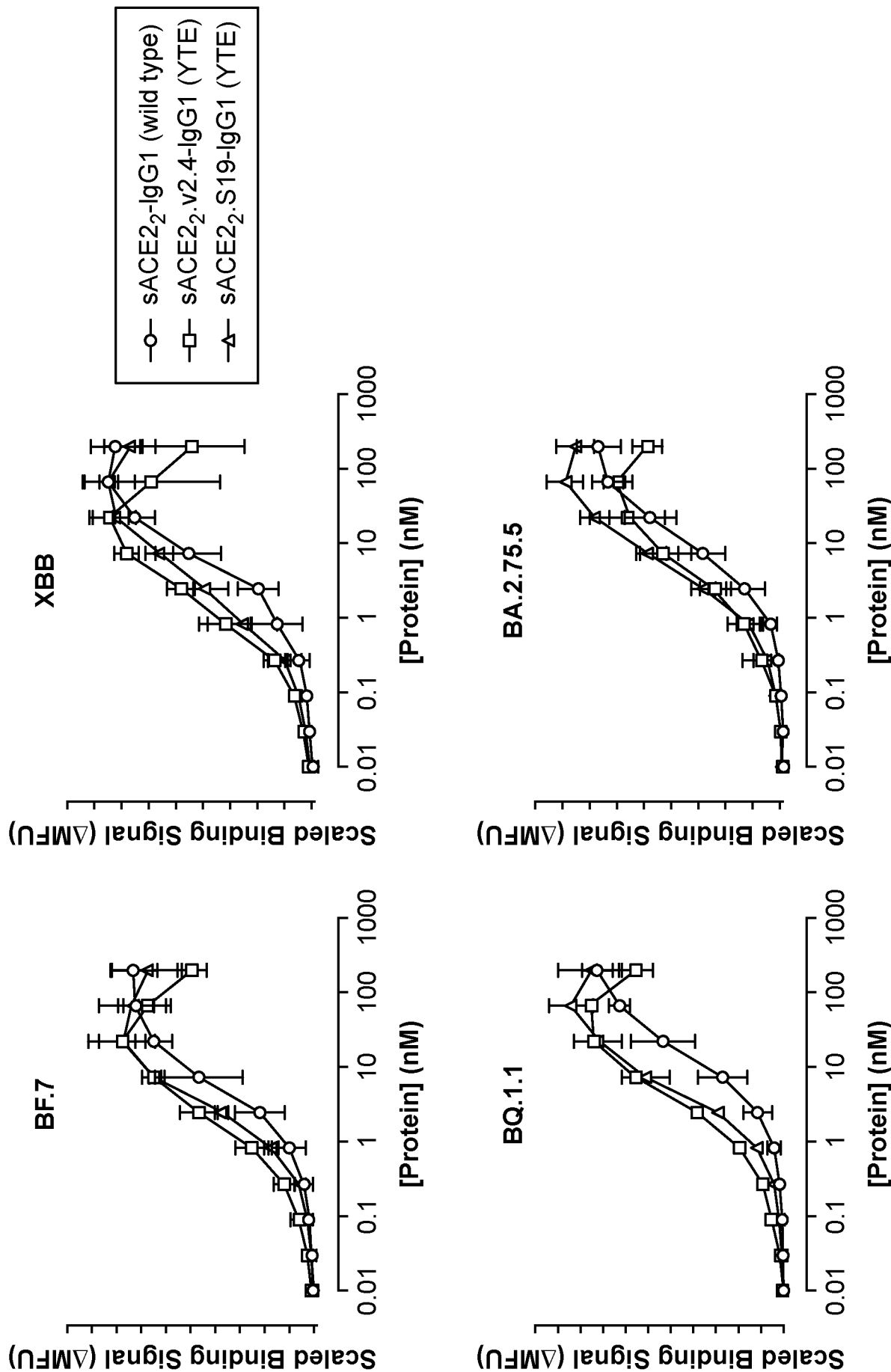


FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082361**A. CLASSIFICATION OF SUBJECT MATTER**IPC: *A61K 38/48* (2024.01); *A61K 39/00* (2024.01); *A61P 31/14* (2024.01)CPC: *A61K 39/0005*; *A61K 38/4813*; *A61P 31/14*

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.
A	US 2022/0332769 A1 (ACHELOIS BIOPHARMA INC.) 20 October 2022 (20.10.2022) entire document	1, 2
A	BERNARDI et al. "Development and simulation of fully glycosylated molecular models of ACE2-Fc fusion proteins and their interaction with the SARS-CoV-2 spike protein binding domain," Plos One, 05 August 2020 (05.08.2020), Vol. 15, Iss. 8, Pgs. 1-12. entire document	1, 2
A	US 2021/0275664 A1 (THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVIC et al.) 09 September 2021 (09.09.2021) entire document	1, 2
A	ZHANG et al. "Engineered ACE2 decoy mitigates lung injury and death induced by SARS-CoV-2 variants," Nature Chemical Biology, 19 January 2022 (19.01.2022), Vol. 18, Iss. 3, Pgs. 342-351. entire document	1, 2

☒ 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

23 February 2024 (23.02.2024)

Date of mailing of the international search report

08 April 2024 (08.04.2024)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Authorized officer

MATOS
TAINA

Facsimile No. **571-273-8300**Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082361

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG et al. "An ACE2 decoy can be administered by inhalation and potently targets omicron variants of SARS#CoV#2," EMBO Molecular Medicine, 23 September 2022 (23.09.2022), Vol. 14, No. 11, Pgs. 1-17. entire document	1, 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082361**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 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.: **4-43**
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 1-3 are drawn to modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising substitution(s) relative to wild-type human ACE2.

The first invention of Group I+ is restricted to a modified angiotensin-converting enzyme 2 (ACE2) polypeptide selected to be SEQ ID NO:2, further comprising an isoleucine at position 491, a serine at position 662, and a serine at position 720. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the mutations listed in instant claim 2. It is believed that claims 1 and 2 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NO:2 comprising V491I, M662S, and N720S.

Applicant is invited to elect additional ACE2 polypeptides and their respective, corresponding, SEQ ID NOs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a modified angiotensin-converting enzyme 2 (ACE2) polypeptide selected to be SEQ ID NO:2, further comprising a tryptophan at position 663 and a tyrosine at position 673. Additional ACE2 polypeptides and their respective, corresponding, SEQ ID NOs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element responsible for enhancing ACE2 activity, requiring the selection of alternative ACE2 enzymes where "A protein comprising: a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising at least one substitution relative to wild-type human ACE2 of SEQ ID NO: 1, wherein the at least one substitution introduces at least one glycosylation site not present in wild-type human ACE2."

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

Additionally, even if Groups I+ were considered to share the technical features of a protein comprising: a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising at least one substitution relative to wild-type human ACE2 of SEQ ID NO: 1, wherein the at least one substitution introduces at least one glycosylation site not present in wild-type human ACE2; a protein comprising: a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising a substitution or combination of substitutions relative to wild-type human ACE2 of SEQ ID NO: 1. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2022/0332769 A1 to Achelois Biopharma Inc. discloses a protein (multivalent particle comprising a fusion protein, Para. [0004]) comprising: a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising at least one substitution relative to wild-type human ACE2 of SEQ ID NO: 1 (mutations that disrupt the ACE2 catalytic function, such as H374A and H378A, also significantly reduce ACE2 binding to CoV-2 spike protein, Para. [0201]; the fusion protein or second fusion protein comprises an amino acid sequence of at least 75% sequence identity to an amino acid sequence according to SEQ ID NO: 30, Para. [0146]; where SEQ ID NO:30 of Achelois Biopharma Inc. comprises a polypeptide with 100% identity to SEQ ID NO:1 of the instant application); a protein comprising: a modified angiotensin-converting enzyme 2 (ACE2) polypeptide comprising a substitution or combination of substitutions relative to wild-type human ACE2 of SEQ ID NO: 1 (mutations that disrupt the ACE2 catalytic function, such as H374A and H378A, also significantly reduce ACE2 binding to CoV-2 spike protein, Para. [0201]; the fusion protein or second fusion protein comprises an amino acid sequence of at least 75% sequence identity to an amino acid sequence according to SEQ ID NO: 30, Para. [0146]; where SEQ ID NO:30 of Achelois Biopharma Inc. comprises a polypeptide with 100% identity to SEQ ID NO:1 of the instant application).

Further US 2021/0275664 A1 to The United States Of America As Represented By The Secretary Department Of Health And Human Service et al. teaches wherein the at least one substitution introduces at least one glycosylation site not present in wild-type human ACE2 (amino acid sequence on the surface of a polypeptide, such as a protein, which accommodates the attachment of a glycan. An N-linked glycosylation site is triplet sequence of NX(S/T) in which N is asparagine, X is any residues except proline, and (S/T) is a serine or threonine residue, Para. [0066]; insertions, substitutions, or deletions of one or more amino acid residues, glycosylation variants, Para. [0236]).

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/082361

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying 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, 2**

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.