ANTI-SARS-COV-2-SPIKE GLYCOPROTEIN ANTIBODIES AND ANTIGEN-BINDING FRAGMENTS

SEQUENCE LISTING

[0001] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of "10753WO01-Sequence.txt", created on June 25, 2020, and having a size of 922,462 bytes. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to antibodies and antigen-binding fragments that bind specifically to coronavirus spike proteins and methods for treating or preventing coronavirus infections with said antibodies and fragments.

BACKGROUND OF THE INVENTION

[0003] Newly identified viruses, such as coronaviruses, can be difficult to treat because they are not sufficiently characterized. The emergence of these newly identified viruses highlights the need for the development of novel antiviral strategies. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a newly-emergent coronavirus which causes a severe acute respiratory disease, COVID-19. SARS-CoV-2 was first identified from an outbreak in Wuhan, China and as of March 20, 2020, the World Health Organization has reported 209,839 confirmed cases in 168 countries, areas, or territories, resulting in 8,778 deaths. Clinical features of COVID-19 include fever, dry cough, and fatigue, and the disease can cause respiratory failure resulting in death.

[0004] Thus far, there has been no vaccine or therapeutic agent to prevent or treat SARS-CoV-2 infection. In view of the continuing threat to human health, there is an urgent need for preventive and therapeutic antiviral therapies for SARS-CoV-2 control. Because this virus uses its spike glycoprotein for interaction with the cellular receptor ACE2 and the serine protease TMPRSS2 for entry into a target cell, this spike protein represents an attractive target for antibody therapeutics. In particular, fully human antibodies that specifically bind to the SARS-

CoV-2-Spike protein (SARS-CoV-2-S) with high affinity and that inhibit virus infectivity could be important in the prevention and treatment of COVID-19.

SUMMARY OF THE INVENTION

[0005] There is a need for neutralizing therapeutic anti-SARS-CoV-2-Spike protein (SARS-CoV-2-S) antibodies and their use for treating or preventing viral infection. The present disclosure addresses this need, in part, by providing human anti-SARS-CoV-2-S antibodies, such as those of Table 1, and combinations thereof including, for example, combinations with other therapeutics (e.g., anti-inflammatory agents, antimalarial agents, antiviral agents, or other antibodies or antigen-binding fragments), and methods of use thereof for treating viral infections.

[0006] The present disclosure provides neutralizing human antigen-binding proteins that specifically bind to SARS-CoV-2-S, for example, antibodies or antigen-binding fragments thereof.

[0007] In one aspect, the present disclosure provides an isolated recombinant antibody or antigen-binding fragment thereof that specifically binds to a coronavirus spike protein (CoV-S), wherein the antibody has one or more of the following characteristics: (a) binds to CoV-S with an EC_{50} of less than about 10^{-9} M; (b) demonstrates an increase in survival in a coronavirus-infected animal after administration to said coronavirus-infected animal, as compared to a comparable coronavirus-infected animal without said administration; and/or (c) comprises three heavy chain complementarity determining regions (CDRs) (CDR-H1, CDR-H2, and CDR-H3) contained within a heavy chain variable region (HCVR) comprising an amino acid sequence having at least about 90% sequence identity to an HCVR of Table 1; and three light chain CDRs (CDR-L1, CDR-L2, and CDR-L3) contained within a light chain variable region (LCVR) comprising an amino acid sequence having at least about 90% sequence identity to an LCVR Table 1.

[0008] In some embodiments, the antibody or antigen-binding fragment comprises: (a) an immunoglobulin heavy chain variable region comprising the CDR-H1, CDR-H2, and CDR-H3 of an antibody of Table 1; and/or (b) an immunoglobulin light chain variable region comprising the CDR-L1, CDR-L2, and CDR-L3 of an antibody of Table 1.

[0009] In some embodiments, the antibody or antigen-binding fragment comprises: (a) a heavy chain immunoglobulin variable region comprising an amino acid sequence having at least 90%

amino acid sequence identity to an HCVR sequence of Table 1; and/or (b) a light chain immunoglobulin variable region comprising an amino acid sequence having at least 90% amino acid sequence identity to an LCVR sequence of Table 1.

[00010] In some embodiments, the antibody or antigen-binding fragment comprises the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 of a single antibody of Table 1. In some embodiments, the antibody or antigen-binding fragment comprises an immunoglobulin that comprises the HCVR and the LCVR of a single antibody of Table 1.

[00011] In one aspect, the present disclosure provides an antigen-binding protein that competes with any one of the antibodies or antigen-binding fragments discussed above or herein for binding to CoV-S.

[00012] In one aspect, the present disclosure provides an antigen-binding protein that binds to the same epitope as, or to an overlapping epitope on, CoV-S as any one of the antibodies or antigen-binding fragments discussed above or herein.

[00013] In any of the various embodiments, the antibody or antigen-binding fragment may be multispecific.

[00014] In any of the various embodiments, the antibody or antigen-binding fragment may comprise one or more of the following properties: a) inhibits growth of coronavirus; b) binds to the surface of a coronavirus; c) limits spread of coronavirus infection of cells *in vitro*; and d) protects mice engineered to express the human ACE2 or TMPRSS2 protein from death and/or weight loss caused by coronavirus infection.

[00015] In any of the various embodiments, CoV-S is SARS-CoV-2-S.

[00016] In one aspect, the present disclosure provides a complex comprising an antibody or antigen-binding fragment as discussed above or herein bound to a CoV-S polypeptide. In some embodiments, the CoV-S is SARS-CoV-2-S.

[00017] In one aspect, the present disclosure provides a method for making an antibody or antigen-binding fragment as discussed above or herein, comprising: (a) introducing into a host cell one or more polynucleotides encoding said antibody or antigen-binding fragment; (b) culturing the host cell under conditions favorable to expression of the one or more polynucleotides; and (c) optionally, isolating the antibody or antigen-binding fragment from the host cell and/or a medium in which the host cell is grown. In some embodiments, the host cell is a Chinese hamster ovary cell.

[00018] In one aspect, the present disclosure provides an antibody or antigen-binding fragment that is a product of the method discussed above.

[00019] In one aspect, the present disclosure provides a polypeptide comprising: (a) CDR-H1, CDR-H2, and CDR-H3 of an HCVR domain of an antibody or antigen-binding fragment that comprises an HCVR amino acid sequence set forth in Table 1; or (b) CDR-L1, CDR-L2, and CDR-L3 of an LCVR domain of an immunoglobulin chain that comprises an LCVR amino acid sequence set forth in Table 1.

[00020] In one aspect, the present disclosure provides a polynucleotide encoding the polypeptide discussed above.

[00021] In one aspect, the present disclosure provides a vector comprising the polynucleotide discussed above.

[00022] In one aspect, the present disclosure provides a host cell comprising the antibody or antigen-binding fragment or polypeptide or polynucleotide or vector as discussed above or herein.

[00023] In one aspect, the present disclosure provides a composition or kit comprising the antibody or antigen-binding fragment discussed above or herein in association with a further therapeutic agent.

[00024] In one aspect, the present disclosure provides a pharmaceutical composition comprising the antigen-binding protein, antibody or antigen-binding fragment discussed above or herein and a pharmaceutically acceptable carrier and, optionally, a further therapeutic agent. In some embodiments, the further therapeutic agent is an anti-viral drug or a vaccine. In some embodiments, the further therapeutic agent is selected from the group consisting of: an anti-inflammatory agent, an antimalarial agent, an antibody or antigen-binding fragment thereof that specifically binds TMPRSS2, and an antibody or antigen-binding fragment thereof that specifically binds to CoV-S. In some cases, the antimalarial agent is chloroquine or hydroxychloroquine. In some cases, the anti-inflammatory agent is an antibody, such as sarilumab, tocilizumab, or gimsilumab. In some embodiments, the further therapeutic agent is a second antibody or antigen-binding fragment comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 sequences of Table 1.

[00025] In one aspect, the present disclosure provides a vessel or injection device comprising the antigen-binding protein, antibody or antigen-binding fragment, or composition as discussed above or herein.

[00026] In one aspect, the present disclosure provides a method for treating or preventing infection with a coronavirus, in a subject in need thereof, comprising administering a therapeutically effective amount of an antigen-binding protein, antibody or antigen-binding fragment as discussed above or herein. In some embodiments, the coronavirus is selected from the group consisting of SARS-CoV-2, SARS-CoV, and MERS-CoV.

[00027] In some embodiments of the method for treating or preventing infection with a coronavirus, the subject is administered one or more further therapeutic agents. In some cases, the one or more further therapeutic agents is an anti-viral drug or a vaccine. In some cases, the one or more further therapeutic agents is selected from the group consisting of: an anti-inflammatory agent, an antimalarial agent, an antibody or antigen-binding fragment thereof that specifically binds TMPRSS2, and an antibody or antigen-binding fragment thereof that specifically binds to CoV-S. In some cases, the antimalarial agent is chloroquine or hydroxychloroquine. In some cases, the anti-inflammatory agent is an antibody, such as for example, sarilumab, tocilizumab, or gimsilumab. In some embodiments, the further therapeutic agent is a second antibody or antigen-binding fragment comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 sequences of Table 1. Other antibodies that can be used alone or in combination with one another or with one or more of the antibodies disclosed herein for use in the context of the methods of the present disclosure include, e.g., LY-CoV555 (Eli Lilly); 47D11 (Wang et al Nature Communications Article No. 2251); B38, H4, B5 and/or H2 (Wu et al., 10.1126/science.abc2241 (2020); STI-1499 (Sorrento Therapeutics); VIR-7831 and VIR-7832 (Vir Biotherapeutics).

[00028] In one aspect, the present disclosure provides a method for administering an antibody or antigen-binding fragment discussed above or herein into the body of a subject comprising injecting the antibody or antigen-binding fragment into the body of the subject. In some embodiments, the antibody or antigen-binding fragment is injected into the body of the subject subcutaneously, intravenously or intramuscularly.

[00029] In any of the various embodiments discussed above or herein, the antibody or antigen-binding binding fragment comprises a VH3-66 or Vk1-33 variable domain sequence.

[00030] In one aspect, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein said isolated antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID NO: 202, and three light chain complementarity determining regions (CDRs) (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 210.

[00031] In some embodiments, the HCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 204, the HCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 206, the HCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 208, the LCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 212, the LCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 55, and the LCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 214. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an HCVR that comprises the amino acid sequence set forth in SEQ ID NO: 202. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an LCVR that comprises the amino acid sequence set forth in SEQ ID NO: 210. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an HCVR that comprises the amino acid sequence set forth in SEQ ID NO: 202 and an LCVR that comprises the amino acid sequence set forth in SEQ ID NO: 210.

[00032] In one aspect, the present disclosure provides an isolated antibody that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein said isolated antibody comprises an immunoglobulin constant region, three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID NO: 202, and three light chain complementarity determining regions (CDRs) (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 210.

[00033] In some embodiments, the HCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 204, the HCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 206, the HCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 208, the LCDR1 comprises

the amino acid sequence set forth in SEQ ID NO: 212, the LCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 55, and the LCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 214. In some embodiments, the isolated antibody comprises an HCVR that comprises the amino acid sequence set forth in SEQ ID NO: 202 and an LCVR that comprises the amino acid sequence set forth in SEQ ID NO: 210. In some embodiments, the isolated antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 216 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 218. In some cases, the immunoglobulin constant region is an IgG1 constant region. In some cases, the isolated antibody is a recombinant antibody. In some cases, the isolated antibody is multispecific.

[00034] In one aspect, the present disclosure provides a pharmaceutical composition comprising an isolated antibody as discussed above or herein, and a pharmaceutically acceptable carrier or diluent.

[00035] In some embodiments, the pharmaceutical composition further comprises a second therapeutic agent. In some cases, the second therapeutic agent is selected from the group consisting of: a second antibody, or an antigen-binding fragment thereof, that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, an anti-inflammatory agent, an antimalarial agent, and an antibody or antigen-binding fragment thereof that binds TMPRSS2.

[00036] In some embodiments, the second therapeutic agent is a second antibody, or an antigen-binding fragment thereof, that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832. In some cases, the second antibody or antigen-binding fragment thereof comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within an HCVR comprising the amino acid sequence set forth in SEQ ID NO: 640, and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within an LCVR comprising the amino acid sequence set forth in SEQ ID NO: 646. In some cases, the second antibody or antigen-binding fragment thereof comprises: HCDR1, comprising the amino acid sequence set forth in SEQ ID NO: 642; HCDR2, comprising the amino acid sequence set forth in SEQ ID NO: 499; HCDR3, comprising the amino acid sequence set forth in SEQ ID NO: 644; LCDR1, comprising the amino acid sequence set forth in SEQ ID NO: 648; LCDR2, comprising the amino acid sequence set forth in SEQ ID NO: 650; and LCDR3, comprising the amino acid

sequence set forth in SEQ ID NO: 652. In some cases, the second antibody or antigen-binding fragment thereof comprises an HCVR comprising the amino acid sequence set forth in SEQ ID NO: 640 and an LCVR comprising the amino acid sequence set forth in SEQ ID NO: 646. In some cases, the second antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 654 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 656.

[00037] In one aspect, the present disclosure provides an isolated antibody or antigen-binding fragment thereof that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein said isolated antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID NO: 640, and three light chain complementarity determining regions (CDRs) (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 646.

[00038] In some embodiments, the HCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 642, the HCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 499, the HCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 644, the LCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 648, the LCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 650, and the LCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 652. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an HCVR that comprises an amino acid sequence set forth in SEQ ID NO: 640. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an LCVR that comprises an amino acid sequence set forth in SEQ ID NO: 646. In some embodiments, the isolated antibody or antigen-binding fragment thereof comprises an HCVR that comprises an amino acid sequence set forth in SEQ ID NO: 640 and an LCVR that comprises an amino acid sequence set forth in SEQ ID NO: 646.

[00039] In one aspect, the present disclosure provides an isolated antibody that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein said isolated antibody comprises an immunoglobulin constant region, three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID

NO: 640, and three light chain complementarity determining regions (CDRs) (LCDR1, LCDR2 and LCDR3) contained within a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 646.

[00040] In some embodiments, the HCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 642, the HCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 499, the HCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 644, the LCDR1 comprises the amino acid sequence set forth in SEQ ID NO: 648, the LCDR2 comprises the amino acid sequence set forth in SEQ ID NO: 650, and the LCDR3 comprises the amino acid sequence set forth in SEQ ID NO: 652. In some embodiments, the isolated antibody comprises an HCVR that comprises the amino acid sequence set forth in SEQ ID NO: 640 and an LCVR that comprises the amino acid sequence set forth in SEQ ID NO: 646. In some embodiments, the isolated antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 654 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 656. In some cases, the immunoglobulin constant region is an IgG1 constant region. In some cases, the isolated antibody is a recombinant antibody. In some cases, the isolated antibody is multispecific.

[00041] In one aspect, the present disclosure provides a pharmaceutical composition comprising an isolated antibody, as discussed above or herein, and a pharmaceutically acceptable carrier or diluent.

[00042] In some embodiments, the pharmaceutical composition further comprising a second therapeutic agent. In some cases, the second therapeutic agent is selected from the group consisting of: a second antibody, or an antigen-binding fragment thereof, that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, an anti-inflammatory agent, an antimalarial agent, and an antibody or antigen-binding fragment thereof that binds TMPRSS2.

[00043] In some embodiments, the second therapeutic agent is a second antibody, or an antigen-binding fragment thereof, that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832. In some cases, the second antibody or antigen-binding fragment thereof comprises three heavy chain CDRs (HCDR1, HCDR2 and HCDR3) contained within an HCVR comprising the amino acid sequence set forth in SEQ ID NO: 202, and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within an LCVR comprising the

amino acid sequence set forth in SEQ ID NO: 210. In some cases, the second antibody or antigen-binding fragment thereof comprises: HCDR1, comprising the amino acid sequence set forth in SEQ ID NO: 204; HCDR2, comprising the amino acid sequence set forth in SEQ ID NO: 206; HCDR3, comprising the amino acid sequence set forth in SEQ ID NO: 208 LCDR1, comprising the amino acid sequence set forth in SEQ ID NO: 212; LCDR2, comprising the amino acid sequence set forth in SEQ ID NO: 55; and LCDR3, comprising the amino acid sequence set forth in SEQ ID NO: 214. In some cases, the second antibody or antigen-binding fragment thereof comprises an HCVR comprising the amino acid sequence set forth in SEQ ID NO: 202 and an LCVR comprising the amino acid sequence set forth in SEQ ID NO: 210. In some cases, the second antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 216 and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 218.

[00044] In various embodiments, any of the features or components of embodiments discussed above or herein may be combined, and such combinations are encompassed within the scope of the present disclosure. Any specific value discussed above or herein may be combined with another related value discussed above or herein to recite a range with the values representing the upper and lower ends of the range, and such ranges are encompassed within the scope of the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[00045] Figure 1 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00046] Figure 2 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00047] Figure 3 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00048] Figure 4 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00049] Figure 5 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00050] Figure 6 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00051] Figure 7 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00052] Figure 8 shows ELISA blocking data for selected anti-SARS-CoV-2-S antibodies against SARS-CoV-2 spike protein, preventing the spike protein from binding to its receptor ACE2.

[00053] Figure 9A and Figure 9B display V gene frequencies for paired Heavy (X-axis) and Light (Y-axis) chains of isolated neutralizing antibodies to SARS-CoV-2 for VelocImmune® mice (Figure 9A; N=185) and convalescent human donors (Figure 9B; N=68). The shade and size of the circle corresponds to the number of Heavy and Light chain pairs present in the repertoires of isolated neutralizing antibodies. Neutralization is defined as >70% with 1:4 dilution of antibody (~2 μg/ml) in VSV pseudoparticle neutralization assay.

[00054] Figure 10A and Figure 10B display neutralization potency. Figure 10A displays the neutralization potency of anti-SARS-CoV-2 Spike mAbs. Serial dilutions of anti-Spike mAbs, IgG1 isotype control, and recombinant dimeric ACE2 (hACE2.hFc) were added with pVSV-SARS-CoV-2-S-mNeon to Vero cells and mNeon expression was measured 24 hours post-infection as a read-out for virus infectivity. Data is graphed as percent neutralization relative to virus only infection control. Figure 10B displays neutralization potency of individual anti-Spike mAbs and combinations of mAbs against SARS-CoV-2-S virus in VeroE6 cells.

[00055] Figure 11 displays epitope bin analysis from a matrix of pre-mix binding assays for different anti-SARS-CoV-2 mAbs. Epitope binning was performed against nine anti-SARS-CoV-2 mAb as described. There were three phases (I, II, and III) for each graph. In phase I anti-

SARS-CoV-2 mAb (20ug/ml) was loaded to the anti-human Fc probe. In phase II human IgG1 blocking mAb solution (100ug/ml). In phase III a solution of 100nM SARS CoV-2 RBD-MMH pre-mix complex of each 600 nM anti-SARS-CoV-2 mAb binding site flowed over the mAb capture probe.

[00056] Figure 12 displays a 3D surface model for the structure of the Spike protein RBD domain showing the ACE2 interface and HDX-MS epitope mapping results. RBD residues protected by anti-SARS-CoV2-Spike antibodies are indicated with shading that represent the extent of protection as determined by HDX-MS experiments. The RBD structure is reproduced from PDB 6M17.

[00057] Figure 13A and Figure 13B display a complex of mAb10933 and mAb10987 with the SARS-CoV-2 RBD. Figure 13A displays a 3.9 Å cryoEM map of mAb10933 + RBD + mAb10987 complex, shaded according to the chains in the refined model of Figure 13B.RBD, mAb10933 heavy and light chains, and mAb10987 heavy and light chain are identified.

[00058] Figure 14 displays cryoEM data statistics. Data collection and refinement statistics are reported for the mAb10987 + mAb10933 + SARS-CoV-2 RBD complex structure shown in Figure 13A and Figure 13B.

DETAILED DESCRIPTION OF THE INVENTION

[00059] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[00060] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

[00061] The term "coronavirus" or "CoV" refers to any virus of the coronavirus family, including but not limited to SARS-CoV-2, MERS-CoV, and SARS-CoV. SARS-CoV-2 refers to

the newly-emerged coronavirus which was identified as the cause of a serious outbreak starting in Wuhan, China, and which is rapidly spreading to other areas of the globe. SARS-CoV-2 has also been known as 2019-nCoV and Wuhan coronavirus. It binds via the viral spike protein to human host cell receptor angiotensin-converting enzyme 2 (ACE2). The spike protein also binds to and is cleaved by TMPRSS2, which activates the spike protein for membrane fusion of the virus.

[00062] The term "CoV-S", also called "S" or "S protein" refers to the spike protein of a coronavirus, and can refer to specific S proteins such as SARS-CoV-2-S, MERS-CoV S, and SARS-CoV S. The SARS-CoV-2-Spike protein is a 1273 amino acid type I membrane glycoprotein which assembles into trimers that constitute the spikes or peplomers on the surface of the enveloped coronavirus particle. The protein has two essential functions, host receptor binding and membrane fusion, which are attributed to the N-terminal (S1) and C-terminal (S2) halves of the S protein. CoV-S binds to its cognate receptor via a receptor binding domain (RBD) present in the S1 subunit. The amino acid sequence of full-length SARS-CoV-2 spike protein is exemplified by the amino acid sequence provided in SEQ ID NO: 832. The term "CoV-S" includes protein variants of CoV spike protein isolated from different CoV isolates as well as recombinant CoV spike protein or a fragment thereof. The term also encompasses CoV spike protein or a fragment thereof coupled to, for example, a histidine tag, mouse or human Fc, or a signal sequence such as ROR1.

[00063] The term "coronavirus infection" or "CoV infection," as used herein, refers to infection with a coronavirus such as SARS-CoV-2, MERS-CoV, or SARS-CoV. The term includes coronavirus respiratory tract infections, often in the lower respiratory tract. Symptoms can include high fever, dry cough, shortness of breath, pneumonia, gastro-intestinal symptoms such as diarrhea, organ failure (kidney failure and renal dysfunction), septic shock, and death in severe cases.

Viruses

[00064] The present invention includes methods for treating or preventing a viral infection in a subject. The term "virus" includes any virus whose infection in the body of a subject is treatable or preventable by administration of an anti-CoV-S antibody or antigen-binding fragment thereof (*e.g.*, wherein infectivity of the virus is at least partially dependent on CoV-S). In an

embodiment of the invention, a "virus" is any virus that expresses spike protein (e.g., CoV-S). The term "virus" also includes a CoV-S-dependent respiratory virus which is a virus that infects the respiratory tissue of a subject (*e.g.*, upper and/or lower respiratory tract, trachea, bronchi, lungs) and is treatable or preventable by administration of an anti-CoV-S antibody or antigen-binding fragment thereof. For example, in an embodiment of the invention, virus includes coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), SARS-CoV (severe acute respiratory syndrome coronavirus), and MERS-CoV (Middle East respiratory syndrome (MERS) coronavirus). Coronaviruses can include the genera of alphacoronaviruses, betacoronaviruses, gammacoronaviruses, and deltacoronaviruses. In some embodiments, the antibodies or antigen-binding fragments provided herein can bind to and/or neutralize an alphacoronavirus, a betacoronavirus, a gammacoronavirus, and/or a deltacoronavirus. In certain embodiments, this binding and/or neutralization can be specific for a particular genus of coronavirus or for a particular subgroup of a genus. "Viral infection" refers to the invasion and multiplication of a virus in the body of a subject.

[00065] Coronavirus virions are spherical with diameters of approximately 125 nm. The most prominent feature of coronaviruses is the club-shape spike projections emanating from the surface of the virion. These spikes are a defining feature of the virion and give them the appearance of a solar corona, prompting the name, coronaviruses. Within the envelope of the virion is the nucleocapsid. Coronaviruses have helically symmetrical nucleocapsids, which is uncommon among positive-sense RNA viruses, but far more common for negative-sense RNA viruses. SARS-CoV-2, MERS-CoV, and SARS-CoV belong to the coronavirus family. The initial attachment of the virion to the host cell is initiated by interactions between the S protein and its receptor. The sites of receptor binding domains (RBD) within the S1 region of a coronavirus S protein vary depending on the virus, with some having the RBD at the C-terminus of S1. The S-protein/receptor interaction is the primary determinant for a coronavirus to infect a host species and also governs the tissue tropism of the virus. Many coronaviruses utilize peptidases as their cellular receptor. Following receptor binding, the virus must next gain access to the host cell cytosol. This is generally accomplished by acid-dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes.

Anti-CoV-S Antibodies and Antigen-Binding Fragments

[00066] The present invention provides antigen-binding proteins, such as antibodies and antigen-binding fragments thereof, that specifically bind to CoV spike protein or an antigenic fragment thereof.

[00067] The term "antibody", as used herein, refers to immunoglobulin molecules comprising four polypeptide chains, two heavy chains (HCs) and two light chains (LCs) inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM). Exemplary antibodies include, for example, those listed in Table 1. Each heavy chain comprises a heavy chain variable region ("HCVR" or "V_H") and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region ("LCVR or "V_L") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Heavy chain CDRs can also be referred to as HCDRs or CDR-Hs, and numbered as described above (e.g., HCDR1, HCDR2, and HCDR3 or CDR-H1, CDR-H2, and CDR-H3). Likewise, light chain CDRs can be referred to as LCDRs or CDR-Ls, and numbered LCDR1, LCDR2, and LCDR3, or CDR-L1, CDR-L2, and CDR-L3. In certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment thereof) are identical to the human germline sequences, or are naturally or artificially modified. Exemplary human germline sequences include, but are not limited to, VH3-66 and Vk1-33. Thus, the present disclosure provides anti-CoV-S antibodies or antigen-binding fragments thereof (*e.g.*, anti-SARS-CoV-2-S antibodies or antigen-binding fragments thereof) comprising HCDR and LCDR sequences of Table 1 within a VH3-66 or Vk1-33 variable heavy chain or light chain region. The present disclosure further provides anti-CoV-S antibodies or antigen-binding fragments thereof (*e.g.*, anti-SARS-CoV-2-S antibodies or antigen-binding fragments thereof) comprising HCDR and LCDR sequences of Table 1 within a combination of a light chain selected from IgKV4-1, IgKV 1-5, IgKV1-9, IgKV1-12, IgKV3-15, IgKV1-16, IgKV1-17, IgKV3-20, IgLV3-21, IgKV2-24, IgKV1-33, IgKV1-39, IgLV1-40, IgLV1-44, IgLV1-51, IgLV3-1, IgKV1-6, IgLV2-8, IgKV3-11, IgLV2-11, IgLV2-14, IgLV2-23, or IgLV6-57, and a heavy chain selected from IgHV1-69, IgHV3-64, IgHV4-59, IgHV3-53,

IgHV3-48, IgHV4-34, IgHV3-33, IgHV3-30, IgHV3-23, IgHV3-20, IgHV1-18, IgHV3-15, IgHV3-11, IgHV3-9, IgHV1-8, IgHV3-7, IgHV2-5, IgHV1-2, IgHV2-70, IgHV3-66, IgHV5-51, IgHV1-46, IgHV4-39, IgHV4-31, IgHV3-30-3, IgHV2-26, or IgHV7-4-1. The present disclosure further provides anti-CoV-S antibodies or antigen-binding fragments thereof (*e.g.*, anti-SARS-CoV-2-S antibodies or antigen-binding fragments thereof) comprising HCVR and LCVR sequences of Table 1 within a combination of a light chain selected from IgKV4-1, IgKV 1-5, IgKV1-9, IgKV1-12, IgKV3-15, IgKV1-16, IgKV1-17, IgKV3-20, IgLV3-21, IgKV2-24, IgKV1-33, IgKV1-39, IgLV1-40, IgLV1-44, IgLV1-51, IgLV3-1, IgKV1-6, IgLV2-8, IgKV3-11, IgLV2-11, IgLV2-14, IgLV2-23, or IgLV6-57, and a heavy chain selected from IgHV1-69, IgHV3-64, IgHV4-59, IgHV3-53, IgHV3-48, IgHV4-34, IgHV3-33, IgHV3-30, IgHV3-23, IgHV3-20, IgHV1-18, IgHV3-15, IgHV3-11, IgHV3-9, IgHV1-8, IgHV3-7, IgHV2-5, IgHV1-2, IgHV2-70, IgHV3-66, IgHV5-51, IgHV1-46, IgHV4-39, IgHV4-31, IgHV3-30-3, IgHV2-26, or IgHV7-4-1.

[00068] Typically, the variable domains of both the heavy and light immunoglobulin chains comprise three hypervariable regions, also called complementarity determining regions (CDRs), located within relatively conserved framework regions (FR). In general, from N-terminal to C-terminal, both light and heavy chains variable domains comprise FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. In an embodiment of the invention, the assignment of amino acids to each domain is in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, *et al.*; National Institutes of Health, Bethesda, Md.; 5th ed.; NIH Publ. No. 91-3242 (1991); Kabat (1978) Adv. Prot. Chem. 32:1-75; Kabat, *et al.*, (1977) J. Biol. Chem. 252:6609-6616; Chothia, *et al.*, (1987) J Mol. Biol. 196:901-917 or Chothia, *et al.*, (1989) Nature 342:878-883.

[00069] The present invention includes monoclonal anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof, as well as monoclonal compositions comprising a plurality of isolated monoclonal antigen-binding proteins. The term "monoclonal antibody", as used herein, refers to a population of substantially homogeneous antibodies, *i.e.*, the antibody molecules comprising the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. A "plurality" of such monoclonal antibodies and fragments in a composition refers to a concentration of identical (*i.e.*, as discussed above, in amino acid sequence except for possible naturally occurring

mutations that may be present in minor amounts) antibodies and fragments which is above that which would normally occur in nature, *e.g.*, in the blood of a host organism such as a mouse or a human.

[00070] In an embodiment of the invention, an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment comprises a heavy chain constant domain, *e.g.*, of the type IgA (*e.g.*, IgA1 or IgA2), IgD, IgE, IgG (*e.g.*, IgG1, IgG2, IgG3 and IgG4) or IgM. In an embodiment of the invention, an antigen-binding protein, *e.g.*, antibody or antigen-binding fragment comprises a light chain constant domain, *e.g.*, of the type kappa or lambda.

[00071] The term "human" antigen-binding protein, such as an antibody, as used herein, includes antibodies having variable and constant regions derived from human germline immunoglobulin sequences whether in a human cell or grafted into a non-human cell, *e.g.*, a mouse cell. See *e.g.*, US8502018, US6596541 or US5789215. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and, in particular, CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse) have been grafted onto human FR sequences. The term includes antibodies recombinantly produced in a non-human mammal or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject. See below.

[00072] The present invention includes anti-CoV-S chimeric antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof, and methods of use thereof. As used herein, a "chimeric antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, where the first and second antibodies are from different species. (US4816567; and Morrison *et al.*, (1984) Proc. Natl. Acad. Sci. USA 81: 6851-6855).

[00073] The present invention includes anti-CoV-S hybrid antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof, and methods of use thereof. As used herein, a "hybrid antibody" is an antibody having the variable domain from a first antibody and the constant domain from a second antibody, wherein the first and second antibodies are from different animals, or wherein the variable domain, but not the constant region, is from a first animal. For example, a variable domain can be taken from an antibody isolated from a human

and expressed with a fixed constant region not isolated from that antibody. Exemplary hybrid antibodies are described in Example 1, which refers to antibody heavy chain variable region and light chain variable region derived PCR products that were cloned into expression vectors containing a heavy constant region and a light constant region, respectively. Hybrid antibodies are synthetic and non-natrually occurring because the variable and constant regions they contain are not isolated from a single natural source.

[00074] The term "recombinant" antigen-binding proteins, such as antibodies or antigen-binding fragments thereof, refers to such molecules created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term includes antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system, or a non-human cell expression system, or isolated from a recombinant combinatorial human antibody library. In some embodiments, a recombinant antibody shares a sequence with an antibody isolated from an organism (*e.g.*, a mouse or a human), but has been expressed via recombinant DNA technology. Such antibodies may have post-translational modifications (*e.g.*, glycosylation) that differ from the antibody as isolated from the organism.

[00075] Recombinant anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments, disclosed herein may also be produced in an *E. coli*/T7 expression system. In this embodiment, nucleic acids encoding the anti-CoV-S antibody immunoglobulin molecules of the invention (*e.g.*, as found in Table 1) may be inserted into a pET-based plasmid and expressed in the *E. coli*/T7 system. For example, the present invention includes methods for expressing an antibody or antigen-binding fragment thereof or immunoglobulin chain thereof in a host cell (*e.g.*, bacterial host cell such as *E. coli* such as BL21 or BL21DE3) comprising expressing T7 RNA polymerase in the cell which also includes a polynucleotide encoding an immunoglobulin chain that is operably linked to a T7 promoter. For example, in an embodiment of the invention, a bacterial host cell, such as an *E. coli*, includes a polynucleotide encoding the T7 RNA polymerase gene operably linked to a *lac* promoter and expression of the polymerase and the chain is induced by incubation of the host cell with IPTG (isopropyl-beta-D-thiogalactopyranoside). See US4952496 and US5693489 or Studier & Moffatt, Use of

bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes, J. Mol. Biol. 1986 May 5;189(1): 113-30.

[00076] There are several methods by which to produce recombinant antibodies which are known in the art. One example of a method for recombinant production of antibodies is disclosed in US4816567.

[00077] Transformation can be by any known method for introducing polynucleotides (e.g., DNA or RNA, including mRNA) into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, lipid nanoparticle technology, biolistic injection and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors such as lentivirus or adeno-associated virus. Methods of transforming cells are well known in the art. See, for example, U.S. Pat. Nos. 4,399,216; 4,912,040; 4,740,461 and 4,959,455. In some embodiments, an antibody or antigen-binding fragment thereof of the present disclosure can be introduced to a subject in nucleic acid form (e.g, DNA or RNA, including mRNA), such that the subject's own cells produce the antibody. The present disclosure further provides modifications to nucleotide sequences encoding the anti-CoV-S antibodies described herein that result in increased antibody expression, increased antibody stability, increased nucleic acid (e.g., mRNA) stability, or improved affinity or specificity of the antibodies for the CoV spike protein.

[00078] Thus, the present invention includes recombinant methods for making an anti-CoV-S antigen-binding protein, such as an antibody or antigen-binding fragment thereof of the present invention, or an immunoglobulin chain thereof, comprising (i) introducing one or more polynucleotides (*e.g.*, including the nucleotide sequence of any one or more of the sequences of Table 2) encoding light and/or heavy immunoglobulin chains, or CDRs, of the antigen-binding protein, *e.g.*, of Table 1, for example, wherein the polynucleotide is in a vector; and/or integrated into a host cell chromosome and/or is operably linked to a promoter; (ii) culturing the host cell (*e.g.*, CHO or *Pichia* or *Pichia pastoris*) under condition favorable to expression of the polynucleotide and, (iii) optionally, isolating the antigen-binding protein, (*e.g.*, antibody or fragment) or chain from the host cell and/or medium in which the host cell is grown. For example, a polynucleotide can be integrated into a host cell chromosome through targeted

insertion with a vector such as adeno-associated virus (AAV), e.g., after cleavage of the chromosome using a gene editing system (e.g., CRISPR (for example, CRISPR-Cas9), TALEN, megaTAL, zinc finger, or Argonaute). Targeted insertions can take place, for example, at host cell loci such as an albumin or immunoglopbulin genomic locus. Alternatively, insertion can be at a random locus, e.g., using a vector such as lentivirus. When making an antigen-binding protein (*e.g.*, antibody or antigen-binding fragment) comprising more than one immunoglobulin chain, *e.g.*, an antibody that comprises two heavy immunoglobulin chains and two light immunoglobulin chains, co-expression of the chains in a single host cell leads to association of the chains, *e.g.*, in the cell or on the cell surface or outside the cell if such chains are secreted, so as to form the antigen-binding protein (*e.g.*, antibody or antigen-binding fragment). The methods include those wherein only a heavy immunoglobulin chain or only a light immunoglobulin chain (*e.g.*, any of those discussed herein including mature fragments and/or variable domains thereof) is expressed. Such chains are useful, for example, as intermediates in the expression of an antibody or antigen-binding fragment that includes such a chain. For example, the present invention also includes anti-CoV-S antigen-binding proteins, such as antibodies and antigen-binding fragments thereof, comprising a heavy chain immunoglobulin (or variable domain thereof or comprising the CDRs thereof) encoded by a polynucleotide comprising a nucleotide sequence set forth in Table 2 and a light chain immunoglobulin (or variable domain thereof or comprising the CDRs thereof) encoded by a nucleotide sequence set forth in Table 2 which are the product of such production methods, and, optionally, the purification methods set forth herein. For example, in some embodiments, the product of the method is an anti-CoV-S antigen-binding protein which is an antibody or fragment comprising an HCVR comprising an amino acid sequence set forth in Table 1 and an LCVR comprising an amino acid sequence set forth in Table 1, wherein the HCVR and LCVR sequences are selected from a single antibody listed in Table 1. In some embodiments, the product of the method is an anti-CoV-S antigen-binding protein which is an antibody or fragment comprising HCDR1, HCDR2, and HCDR3 comprising amino acid sequences set forth in Table 1 and LCDR1, LCDR2, and LCDR3 comprising amino acid sequences set forth in Table 1, wherein the six CDR sequences are selected from a single antibody listed in Table 1. In some embodiments, the product of the method is an anti-CoV-S antigen-binding protein which is an antibody or fragment

comprising a heavy chain comprising an HC amino acid sequence set forth in Table 1 and a light chain comprising an LC amino acid sequence set forth in Table 1.

[00079] Eukaryotic and prokaryotic host cells, including mammalian cells, may be used as hosts for expression of an anti-CoV-S antigen-binding protein. Such host cells are well known in the art and many are available from the American Type Culture Collection (ATCC). These host cells include, *inter alia*, Chinese hamster ovary (CHO) cells, NS0, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), A549 cells, 3T3 cells, HEK-293 cells and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Other cell lines that may be used are insect cell lines (*e.g.*, *Spodoptera frugiperda* or *Trichoplusia ni*), amphibian cells, bacterial cells, plant cells and fungal cells. Fungal cells include yeast and filamentous fungus cells including, for example, *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia minuta* (*Ogataea minuta*, *Pichia lindneri*), *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia* sp., *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Hansenula polymorpha*, *Kluyveromyces* sp., *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium* sp., *Fusarium gramineum*, *Fusarium venenatum*, *Physcomitrella patens* and *Neurospora crassa*. The present invention includes an isolated host cell (*e.g.*, a CHO cell) comprising an antigen-binding protein, such as those of Table 1; or a polynucleotide encoding such a polypeptide thereof.

[00080] The term "specifically binds" refers to those antigen-binding proteins (*e.g.*, mAbs) having a binding affinity to an antigen, such as a CoV-S protein (*e.g.*, SARS-CoV-2-S), expressed as K_D, of at least about 10^{-8} M, as measured by real-time, label free bio-layer interferometry assay, for example, at 25°C or 37°C, *e.g.*, an Octet® HTX biosensor, or by surface plasmon resonance, *e.g.*, BIACORE™, or by solution-affinity ELISA. The present invention includes antigen-binding proteins that specifically bind to a CoV-S protein.

[00081] The terms "antigen-binding portion" or "antigen-binding fragment" of an antibody or antigen-binding protein, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Non-limiting examples of antigen-binding

fragments include: (i) Fab fragments; (ii) $F(ab')_2$ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.*, as defined in WO08/020079 or WO09/138519) (*e.g.*, monovalent nanobodies, bivalent nanobodies, *etc.*), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein. In an embodiment of the invention, the antigen-binding fragment comprises three or more CDRs of an antibody of Table 1 (*e.g.*, CDR-H1, CDR-H2 and CDR-H3; or CDR-L1, CDR-L2 and CDR-L3).

[00082] An antigen-binding fragment of an antibody will, in an embodiment of the invention, comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[00083] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide

molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[00084] Antigen-binding proteins (*e.g.*, antibodies and antigen-binding fragments) may be mono-specific or multi-specific (*e.g.*, bi-specific). Multispecific antigen-binding proteins are discussed further herein.

[00085] In specific embodiments, antibody or antibody fragments of the invention may be conjugated to a moiety such a ligand or a therapeutic moiety ("immunoconjugate"), such as an anti-viral drug, a second anti-influenza antibody, or any other therapeutic moiety useful for treating a viral infection, *e.g.*, influenza viral infection. See below.

[00086] The present invention also provides a complex comprising an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment, discussed herein complexed with CoV-S polypeptide or an antigenic fragment thereof and/or with a secondary antibody or antigen-binding fragment thereof (*e.g.*, detectably labeled secondary antibody) that binds specifically to the anti-CoV-S antibody or fragment. In an embodiment of the invention, the antibody or fragment is *in vitro* (*e.g.*, is immobilized to a solid substrate) or is in the body of a subject. In an embodiment of the invention, the CoV-S is *in vitro* (*e.g.*, is immobilized to a solid substrate) or is on the surface of a virus or is in the body of a subject. Immobilized anti-CoV-S antibodies and antigen-binding fragments thereof which are covalently linked to an insoluble matrix material (*e.g.*, glass or polysaccharide such as agarose or sepharose, *e.g.*, a bead or other particle thereof) are also part of the present invention; optionally, wherein the immobilized antibody is complexed with CoV-S or antigenic fragment thereof or a secondary antibody or fragment thereof.

[00087] "Isolated" antigen-binding proteins, antibodies or antigen-binding fragments thereof, polypeptides, polynucleotides and vectors, are at least partially free of other biological molecules from the cells or cell culture from which they are produced. Such biological molecules include nucleic acids, proteins, other antibodies or antigen-binding fragments, lipids, carbohydrates, or other material such as cellular debris and growth medium. An isolated antibody or antigen-binding fragment may further be at least partially free of expression system components such as biological molecules from a host cell or of the growth medium thereof. Generally, the term

"isolated" is not intended to refer to a complete absence of such biological molecules or to an absence of water, buffers, or salts or to components of a pharmaceutical formulation that includes the antibodies or fragments.

[00088] The term "epitope" refers to an antigenic determinant (*e.g.*, a CoV-S polypeptide) that interacts with a specific antigen-binding site of an antigen-binding protein, *e.g.*, a variable region of an antibody molecule, known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may be linear or conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[00089] Methods for determining the epitope of an antigen-binding protein, *e.g.*, antibody or fragment or polypeptide, include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antigen-binding protein (*e.g.*, antibody or fragment or polypeptide) (*e.g.*, coversin) interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antigen-binding protein, *e.g.*, antibody or fragment or polypeptide, to the deuterium-labeled protein. Next, the CoV-S protein/ antigen-binding protein complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/ antigen-binding protein interface may retain deuterium and

therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antigen-binding protein (*e.g.*, antibody or fragment or polypeptide), the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antigen-binding protein interacts. See, *e.g.*, Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-265A.

[00090] The term "competes" as used herein, refers to an antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) that binds to an antigen (*e.g.*, CoV-S) and inhibits or blocks the binding of another antigen-binding protein (*e.g.*, antibody or antigen-binding fragment thereof) to the antigen. The term also includes competition between two antigen-binding proteins *e.g.*, antibodies, in both orientations, *i.e.*, a first antibody that binds and blocks binding of second antibody and *vice versa*. In certain embodiments, the first antigen-binding protein (*e.g.*, antibody) and second antigen-binding protein (*e.g.*, antibody) may bind to the same epitope. Alternatively, the first and second antigen-binding proteins (*e.g.*, antibodies) may bind to different, but, for example, overlapping epitopes, wherein binding of one inhibits or blocks the binding of the second antibody, *e.g.*, via steric hindrance. Competition between antigen-binding proteins (*e.g.*, antibodies) may be measured by methods known in the art, for example, by a real-time, label-free bio-layer interferometry assay. Epitope mapping (e.g., via alanine scanning or hydrogen-deuterium exchange (HDX)) can be used to determine whether two or more antibodies are non-competing (e.g., on a spike protein receptor binding domain (RBD) monomer), competing for the same epitope, or competing but with diverse micro-epitopes (e.g., identified through HDX). In an embodiment of the invention, competition between a first and second anti-CoV-S antigen-binding protein (*e.g.*, antibody) is determined by measuring the ability of an immobilized first anti-CoV-S antigen-binding protein (*e.g.*, antibody) (not initially complexed with CoV-S protein) to bind to soluble CoV-S protein complexed with a second anti-CoV-S antigen-binding protein (*e.g.*, antibody). A reduction in the ability of the first anti-CoV-S antigen-binding protein (*e.g.*, antibody) to bind to the complexed CoV-S protein, relative to uncomplexed CoV-S protein, indicates that the first and second anti-CoV-S antigen-binding proteins (*e.g.*, antibodies) compete. The degree of competition can be expressed as a percentage of the reduction in binding. Such competition can be measured using a real time, label-free bio-

layer interferometry assay, *e.g.*, on an Octet RED384 biosensor (Pall ForteBio Corp.), ELISA (enzyme-linked immunosorbent assays) or SPR (surface plasmon resonance).

[00091] Binding competition between anti-CoV-S antigen-binding proteins (*e.g.*, monoclonal antibodies (mAbs)) can be determined using a real time, label-free bio-layer interferometry assay on an Octet RED384 biosensor (Pall ForteBio Corp.). For example, to determine competition between two anti-CoV-S monoclonal antibodies, the anti-CoV-S mAb can be first captured onto anti-hFc antibody coated Octet biosensor tips (Pall ForteBio Corp., # 18-5060) by submerging the tips into a solution of anti-CoV-S mAb (subsequently referred to as "mAb1"). As a positive-control for blocking, the antibody captured biosensor tips can then be saturated with a known blocking isotype control mAb (subsequently referred to as "blocking mAb") by dipping into a solution of blocking mAb. To determine if mAb2 competes with mAb1, the biosensor tips can then be subsequently dipped into a co-complexed solution of CoV-S polypeptide and a second anti-CoV-S mAb (subsequently referred to as "mAb2"), that had been pre-incubated for a period of time and binding of mAb1 to the CoV-S polypeptide can be determined. The biosensor tips can be washed in buffer in between every step of the experiment. The real-time binding response can be monitored during the course of the experiment and the binding response at the end of every step can be recorded.

[00092] For example, in an embodiment of the invention, the competition assay is conducted at 25 °C and pH about 7, *e.g.*, 7.4, *e.g.*, in the presence of buffer, salt, surfactant and a non-specific protein (*e.g.*, bovine serum albumin).

[00093] Typically, an antibody or antigen-binding fragment of the invention which is modified in some way retains the ability to specifically bind to CoV-S, *e.g.*, retains at least 10% of its CoV-S binding activity (when compared to the parental antibody) when that activity is expressed on a molar basis. Preferably, an antibody or antigen-binding fragment of the invention retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the CoV-S binding affinity as the parental antibody. It is also intended that an antibody or antigen-binding fragment of the invention can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

[00094] A "variant" of a polypeptide, such as an immunoglobulin chain (*e.g.*, mAb8021 V_H, V_L, HC, or LC, mAb8028 V_H, V_L, HC, or LC, or mAb8029 V_H, V_L, HC, or LC), refers to a

polypeptide comprising an amino acid sequence that is at least about 70-99.9% (*e.g.*, 70, 72, 74, 75, 76, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, 99.9%) identical or similar to a referenced amino acid sequence that is set forth herein (*e.g.*, SEQ ID NO: 2, 10, 18, 20, 22, 30, 38, 40, 42, 50, 58, or 60); when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences (*e.g.*, expect threshold: 10; word size: 3; max matches in a query range: 0; BLOSUM 62 matrix; gap costs: existence 11, extension 1; conditional compositional score matrix adjustment).

[00095] A "variant" of a polynucleotide refers to a polynucleotide comprising a nucleotide sequence that is at least about 70-99.9% (*e.g.*, at least about 70, 72, 74, 75, 76, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or 99.9%) identical to a referenced nucleotide sequence that is set forth herein (*e.g.*, SEQ ID NO: 1, 9, 17, 19, 21, 29, 37, 39, 41, 49, 57, or 59); when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences (*e.g.*, expect threshold: 10; word size: 28; max matches in a query range: 0; match/mismatch scores: 1, -2; gap costs: linear).

[00096] Anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof of the present invention, in an embodiment of the invention, include a heavy chain immunoglobulin variable region having at least 70% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater) amino acid sequence identity to the HCVR amino acid sequences set forth in Table 1; and/or a light chain immunoglobulin variable region having at least 70% (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater) amino acid sequence identity to the LCVR amino acid sequences set forth in Table 1.

[00097] In addition, a variant anti-CoV-S antigen-binding protein may include a polypeptide comprising an amino acid sequence that is set forth herein except for one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) mutations such as, for example, missense mutations (*e.g.*, conservative substitutions), non-sense mutations, deletions, or insertions. For example, the present invention includes antigen-binding proteins which include an immunoglobulin light chain variant comprising an LCVR amino acid sequence set forth in Table 1 but having one or more of such mutations and/or an immunoglobulin heavy chain variant comprising an HCVR amino acid sequence set forth in Table 1 but having one or more of such mutations. In an embodiment of

the invention, a variant anti-CoV-S antigen-binding protein includes an immunoglobulin light chain variant comprising CDR-L1, CDR-L2 and CDR-L3 wherein one or more (*e.g.*, 1 or 2 or 3) of such CDRs has one or more of such mutations (*e.g.*, conservative substitutions) and/or an immunoglobulin heavy chain variant comprising CDR-H1, CDR-H2 and CDR-H3 wherein one or more (*e.g.*, 1 or 2 or 3) of such CDRs has one or more of such mutations (*e.g.*, conservative substitutions). Substitutions can be in a CDR, framework, or constant region.

[00098] The invention further provides variant anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments thereof, comprising one or more variant CDRs (*e.g.*, any one or more of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and/or CDR-H3) that are set forth herein with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% sequence identity or similarity to, *e.g.*, the heavy chain and light chain CDRs of Table 1.

[00099] Embodiments of the present invention also include variant antigen-binding proteins, *e.g.*, anti-CoV-S antibodies and antigen-binding fragments thereof, that comprise immunoglobulin V_{HS} and V_{LS}; or HCs and LCs, which comprise an amino acid sequence having 70% or more (*e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater) overall amino acid sequence identity or similarity to the amino acid sequences of the corresponding V_{HS}, V_{LS}, HCs or LCs specifically set forth herein, but wherein the CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 of such immunoglobulins are not variants and comprise CDR amino acid sequence set forth in Table 1. Thus, in such embodiments, the CDRs within variant antigen-binding proteins are not, themselves, variants.

[000100] Conservatively modified variant anti-CoV-S antibodies and antigen-binding fragments thereof are also part of the present invention. A "conservatively modified variant" or a "conservative substitution" refers to a variant wherein there is one or more substitutions of amino acids in a polypeptide with other amino acids having similar characteristics (*e.g.* charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.). Such changes can frequently be made without significantly disrupting the biological activity of the antibody or fragment. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al.* (1987) Molecular Biology of the Gene, The

Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to significantly disrupt biological activity.

[000101] Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45.

[000102] Function-conservative variants of the anti-CoV-S antibodies and antigen-binding fragments thereof are also part of the present invention. Any of the variants of the anti-CoV-S antibodies and antigen-binding fragments thereof (as discussed herein) may be "function-conservative variants". Such function-conservative variants may, in some cases, also be characterized as conservatively modified variants. "Function-conservative variants," as used herein, refers to variants of the anti-CoV-S antibodies or antigen-binding fragments thereof in which one or more amino acid residues have been changed without significantly altering one or more functional properties of the antibody or fragment. In an embodiment of the invention, a function-conservative variant anti-CoV-S antibody or antigen-binding fragment thereof of the present invention comprises a variant amino acid sequence and exhibits one or more of the following functional properties:

- Inhibits growth of coronavirus (*e.g.*, SARS-CoV-2, SARS-CoV, and/or MERS-CoV) in ACE2- and/or TMPRSS2-expressing cells (*e.g.*, Calu-3 cells);
- Does not significantly bind to MDCK/Tet-on cells which do not express ACE2 and/or TMPRSS2;
- Limits spread of coronavirus infection (*e.g.*, by SARS-CoV-2, SARS-CoV, and/or MERS-CoV) of cells, *e.g.*, Calu-3, *in vitro*; and/or
- Protects a mouse engineered to express the human TMPRSS2 and/or ACE2 protein from death caused by coronavirus infection (*e.g.*, SARS-CoV-2, SARS-CoV, or

MERS-CoV), for example, wherein the mice are infected with an otherwise lethal dose of the virus, optionally when combined with a second therapeutic agent.

- Protects a mouse engineered to express the human TMPRSS2 and/or ACE2 protein from weight loss caused by coronavirus infection (*e.g.*, SARS-CoV-2, SARS-CoV, or MERS-CoV), for example, wherein the mice are infected with a dose of the virus that would otherwise cause weighht loss, optionally when combined with a second therapeutic agent.

[000103] A "neutralizing" or "antagonist" anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment, refers to a molecule that inhibits an activity of CoV-S to any detectable degree, *e.g.*, inhibits the ability of CoV-S to bind to a receptor such as ACE2, to be cleaved by a protease such as TMPRSS2, or to mediate viral entry into a host cell or viral reproduction in a host cell.

[000104] Table 1 refers to antigen-binding proteins, such as antibodies and antigen-binding fragments thereof, that comprise the heavy chain or V_H (or a variant thereof) and light chain or V_L (or a variant thereof) as set forth below; or that comprise a V_H that comprises the CDRs thereof (CDR-H1 (or a variant thereof), CDR-H2 (or a variant thereof) and CDR-H3 (or a variant thereof)) and a V_L that comprises the CDRs thereof (CDR-L1 (or a variant thereof), CDR-L2 (or a variant thereof) and CDR-L3 (or a variant thereof)), *e.g.*, wherein the immunoglobulin chains, variable regions and/or CDRs comprise the specific amino acid sequences described below.

[000105] The antibodies described herein also include embodiments wherein the V_H is fused to a wild-type IgG4 (*e.g.*, wherein residue 108 is S) or to IgG4 variants (*e.g.*, wherein residue 108 is P).

[000106] Antibodies and antigen-binding fragments of the present invention comprise immunoglobulin chains including the amino acid sequences set forth herein as well as cellular and *in vitro* post-translational modifications to the antibody. For example, the present invention includes antibodies and antigen-binding fragments thereof that specifically bind to CoV-S comprising heavy and/or light chain amino acid sequences set forth herein (*e.g.*, CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and/or CDR-L3) as well as antibodies and fragments wherein one or more amino acid residues is glycosylated, one or more Asn residues is deamidated, one or more residues (*e.g.*, Met, Trp and/or His) is oxidized, the N-terminal Gln is pyroglutamate (pyroE) and/or the C-terminal Lysine is missing.

[000107] The amino acid and nucleotide sequences of exemplary anti-SARS-CoV-2-Spike protein (SARS-CoV-2-S) antibodies are shown in the Table of Exemparly Sequences, below.

Table of Exemplary Sequences

Antibody Designation	Component Part	Sequence	SEQ ID NO
mAb10933	**Amino Acids**		
	HCVR	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYM SWIRQAPGKGLEWVSYITYSGSTIYYADSVKGRF TISRDNAKSSLYLQMNSLRAEDTAVYYCARDRGT TMVPFDYWGQGTLVTVSS	202
	HCDR1	GFTFSDYY	204
	HCDR2	ITYSGSTI	206
	HCDR3	ARDRGTTMVPFDY	208
	LCVR	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLN WYQQKPGKAPKLLIYAASNLETGVPSRFSGSGSG TDFTFTISGLQPEDIATYYCQQYDNLPLTFGGGT KVEIK	210
	LCDR1	QDITNY	212
	LCDR2	AAS	55
	LCDR3	QQYDNLPLT	214
	HC	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYM SWIRQAPGKGLEWVSYITYSGSTIYYADSVKGRF TISRDNAKSSLYLQMNSLRAEDTAVYYCARDRGT TMVPFDYWGQGTLVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	216
	LC	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLN WYQQKPGKAPKLLIYAASNLETGVPSRFSGSGSG TDFTFTISGLQPEDIATYYCQQYDNLPLTFGGGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD	218

		STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC	
	Nucleic Acids		
	HCVR	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGG TCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTCAGTGACTACTACATG AGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGG AGTGGGTTTCATACATTACTTATAGTGGTAGTAC CATATACTACGCAGACTCTGTGAAGGGCCGATTC ACCATCTCCAGGGACAACGCCAAGAGCTCACTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACAC GGCCGTGTATTACTGTGCGAGAGATCGCGGTACA ACTATGGTCCCCTTTGACTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCA	201
	HCDR1	GGATTCACCTTCAGTGACTACTAC	203
	HCDR2	ATTACTTATAGTGGTAGTACCATA	205
	HCDR3	GCGAGAGATCGCGGTACAACTATGGTCCCCTTTG ACTAC	207
	LCVR	GACATCCAGATGACCCAGTCTCCATCCTCCCTGT CTGCATCTGTAGGAGACAGAGTCACCATCACTTG CCAGGCGAGTCAGGACATTACCAACTATTTAAAT TGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGC TCCTGATCTACGCTGCATCCAATTTGGAAACAGG GGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG ACAGATTTTACTTTCACCATCAGCGGCCTGCAGC CTGAAGATATTGCAACATATTACTGTCAACAGTA TGATAATCTCCCTCTCACTTTCGGCGGAGGGACC AAGGTGGAGATCAAA	209
	LCDR1	CAGGACATTACCAACTAT	211
	LCDR2	GCTGCATCC	54
	LCDR3	CAACAGTATGATAATCTCCCTCTCACT	213
	HC	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGG TCAAGCCTGGAGGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTCAGTGACTACTACATG AGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGG AGTGGGTTTCATACATTACTTATAGTGGTAGTAC CATATACTACGCAGACTCTGTGAAGGGCCGATTC ACCATCTCCAGGGACAACGCCAAGAGCTCACTGT ATCTGCAAATGAACAGCCTGAGAGCCGAGGACAC GGCCGTGTATTACTGTGCGAGAGATCGCGGTACA ACTATGGTCCCCTTTGACTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC	215

		TGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC CAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCA CACATGCCCACCGTGCCCAGCACCTGAACTCCTG GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAAC CCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT ACACCCTGCCCCCATCCCGGGATGAGCTGACCAA GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCAC GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGT GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACGCAGAAG TCCCTCTCCCTGTCTCCGGGTAAATGA	
	LC	GACATCCAGATGACCCAGTCTCCATCCTCCCTGT CTGCATCTGTAGGAGACAGAGTCACCATCACTTG CCAGGCGAGTCAGGACATTACCAACTATTTAAAT TGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGC TCCTGATCTACGCTGCATCCAATTTGGAAACAGG GGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG ACAGATTTTACTTTCACCATCAGCGGCCTGCAGC CTGAAGATATTGCAACATATTACTGTCAACAGTA TGATAATCTCCCTCTCACTTTCGGCGGAGGGACC AAGGTGGAGATCAAACGAACTGTGGCTGCACCAT CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTT GAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG AATAACTTCTATCCCAGAGAGGCCAAAGTACAGT GGAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTGA GCAAAGCAGACTACGAGAAACACAAAGTCTACGC CTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG	217
mAb10934	**Amino Acids**		

	HCVR	EVQLVESGGGLVKPGGSLRLSCAASGITFSNAWM SWVRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKG RFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTAR WDWYFDLWGRGTLVTVSS	220
	HCDR1	GITFSNAW	222
	HCDR2	IKSKTDGGTT	224
	HCDR3	TTARWDWYFDL	226
	LCVR	DIQMTQSPSSLSASVGDRVTITCQASQDIWNYIN WYQQKPGKAPKLLIYDASNLKTGVPSRFSGSGSG TDFTFTISSLQPEDIATYYCQQHDDLPPTFGQGT KVEIK	228
	LCDR1	QDIWNY	230
	LCDR2	DAS	194
	LCDR3	QQHDDLPPT	232
	HC	EVQLVESGGGLVKPGGSLRLSCAASGITFSNAWM SWVRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKG RFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTAR WDWYFDLWGRGTLVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	234
	LC	DIQMTQSPSSLSASVGDRVTITCQASQDIWNYIN WYQQKPGKAPKLLIYDASNLKTGVPSRFSGSGSG TDFTFTISSLQPEDIATYYCQQHDDLPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC	236
	Nucleic Acids		
	HCVR	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGG TAAAGCCTGGGGGGTCCCTTAGACTCTCCTGTGC AGCCTCTGGAATCACTTTCAGTAACGCCTGGATG AGTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGG AGTGGGTTGGCCGTATTAAAAGCAAAACTGATGG TGGGACAACAGACTACGCCGCACCCGTGAAAGGC AGATTCACCATCTCAAGAGATGATTCAAAAAACA CGCTGTATCTACAAATGAACAGCCTGAAAACCGA	219

		GGACACAGCCGTGTATTACTGTACCACAGCGAGG TGGGACTGGTACTTCGATCTCTGGGGCCGTGGCA CCCTGGTCACTGTCTCCTCA	
	HCDR1	GGAATCACTTTCAGTAACGCCTGG	221
	HCDR2	ATTAAAAGCAAAACTGATGGTGGGACAACA	223
	HCDR3	ACCACAGCGAGGTGGGACTGGTACTTCGATCTC	225
	LCVR	GACATCCAGATGACCCAGTCTCCATCCTCCCTGT CTGCATCTGTAGGAGACAGAGTCACCATCACTTG CCAGGCGAGTCAGGACATTTGGAATTATATAAAT TGGTATCAGCAGAAACCAGGGAAGGCCCCTAAGC TCCTGATCTACGATGCATCCAATTTGAAAACAGG GGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG ACAGATTTTACTTTCACCATCAGCAGCCTGCAGC CTGAAGATATTGCAACATATTACTGTCAACAGCA TGATGATCTCCCTCCGACCTTCGGCCAAGGGACC AAGGTGGAAATCAAA	227
	LCDR1	CAGGACATTTGGAATTAT	229
	LCDR2	GATGCATCC	193
	LCDR3	CAACAGCATGATGATCTCCCTCCGACC	231
	HC	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGG TAAAGCCTGGGGGGTCCCTTAGACTCTCCTGTGC AGCCTCTGGAATCACTTTCAGTAACGCCTGGATG AGTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGG AGTGGGTTGGCCGTATTAAAAGCAAAACTGATGG TGGGACAACAGACTACGCCGCACCCGTGAAAGGC AGATTCACCATCTCAAGAGATGATTCAAAAAACA CGCTGTATCTACAAATGAACAGCCTGAAAACCGA GGACACAGCCGTGTATTACTGTACCACAGCGAGG TGGGACTGGTACTTCGATCTCTGGGGCCGTGGCA CCCTGGTCACTGTCTCCTCAGCCTCCACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC CAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCA CACATGCCCACCGTGCCCAGCACCTGAACTCCTG GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAAC CCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCG	233

		GGAGGAGCAGTACAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT ACACCCTGCCCCCATCCCGGGATGAGCTGACCAA GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCAC GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGT GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACGCAGAAG TCCCTCTCCCTGTCTCCGGGTAAATGA	
	LC	GACATCCAGATGACCCAGTCTCCATCCTCCCTGT CTGCATCTGTAGGAGACAGAGTCACCATCACTTG CCAGGCGAGTCAGGACATTTGGAATTATATAAAT TGGTATCAGCAGAAACCAGGGAAGGCCCCTAAGC TCCTGATCTACGATGCATCCAATTTGAAAACAGG GGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG ACAGATTTTACTTTCACCATCAGCAGCCTGCAGC CTGAAGATATTGCAACATATTACTGTCAACAGCA TGATGATCTCCCTCCGACCTTCGGCCAAGGGACC AAGGTGGAAATCAAACGAACTGTGGCTGCACCAT CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTT GAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTG AATAACTTCTATCCCAGAGAGGCCAAAGTACAGT GGAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC AGCACCTACAGCCTCAGCAGCACCCTGACGCTGA GCAAAGCAGACTACGAGAAACACAAAGTCTACGC CTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG	235
mAb10987	**Amino Acids**		
	HCVR	QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAM YWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRF TISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDY GDYLLVYWGQGTLVTVSS	640
	HCDR1	GFTFSNYA	642
	HCDR2	ISYDGSNK	499
	HCDR3	ASGSDYGDYLLVY	644
	LCVR	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNY VSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSK SGNTASLTISGLQSEDEADYYCNSLTSISTWVFG GGTKLTVL	646

	LCDR1	SSDVGGYNY	648
	LCDR2	DVS	650
	LCDR3	NSLTSISTWV	652
	HC	QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYAM YWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRF TISRDNSKNTLYLQMNSLRTEDTAVYYCASGSDY GDYLLVYWGQGTLVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	654
	LC	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNY VSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSK SGNTASLTISGLQSEDEADYYCNSLTSISTWVFG GGTKLTVLGQPKAAPSVTLFPPSSEELQANKATL VCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGS TVEKTVAPTECS	656
	Nucleic Acids		
	HCVR	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGG TCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTCAGTAACTATGCTATG TACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGG AGTGGGTGGCAGTTATATCATATGATGGAAGTAA TAAATACTATGCAGACTCCGTGAAGGGCCGATTC ACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAAATGAACAGCCTGAGAACTGAGGACAC GGCTGTGTATTACTGTGCGAGTGGCTCCGACTAC GGTGACTACTTATTGGTTTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCA	639
	HCDR1	GGATTCACCTTCAGTAACTATGCT	641
	HCDR2	ATATCATATGATGGAAGTAATAAA	498
	HCDR3	GCGAGTGGCTCCGACTACGGTGACTACTTATTGG TTTAC	643
	LCVR	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTG GGTCTCCTGGACAGTCGATCACCATCTCCTGCAC TGGAACCAGCAGTGACGTTGGTGGTTATAACTAT GTCTCCTGGTACCAACAACACCCAGGCAAAGCCC	645

		CCAAACTCATGATTTATGATGTCAGTAAGCGGCC CTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAG TCTGGCAACACGGCCTCCCTGACCATCTCTGGGC TCCAGTCTGAGGACGAGGCTGATTATTACTGCAA CTCTTTGACAAGCATCAGCACTTGGGTGTTCGGC GGAGGGACCAAGCTGACCGTCCTA	
	LCDR1	AGCAGTGACGTTGGTGGTTATAACTAT	647
	LCDR2	GATGTCAGT	649
	LCDR3	AACTCTTTGACAAGCATCAGCACTTGGGTG	651
	HC	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGG TCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTCAGTAACTATGCTATG TACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGG AGTGGGTGGCAGTTATATCATATGATGGAAGTAA TAAATACTATGCAGACTCCGTGAAGGGCCGATTC ACCATCTCCAGAGACAATTCCAAGAACACGCTGT ATCTGCAAATGAACAGCCTGAGAACTGAGGACAC GGCTGTGTATTACTGTGCGAGTGGCTCCGACTAC GGTGACTACTTATTGGTTTACTGGGGCCAGGGAA CCCTGGTCACCGTCTCCTCAGCCTCCACCAAGGG CCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGTGACGGT GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCGGCTGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC CAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACA AGAAAGTTGAGCCCAAATCTTGTGACAAAACTCA CACATGCCCACCGTGCCCAGCACCTGAACTCCTG GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAAC CCAAGGACACCCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACG GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA AGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGT ACACCCTGCCCCCATCCCGGGATGAGCTGACCAA GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC TTCTATCCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCAC GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGT	653

		GGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACGCAGAAG TCCCTCTCCCTGTCTCCGGGTAAATGA	
	LC	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTG GGTCTCCTGGACAGTCGATCACCATCTCCTGCAC TGGAACCAGCAGTGACGTTGGTGGTTATAACTAT GTCTCCTGGTACCAACAACACCCAGGCAAAGCCC CCAAACTCATGATTTATGATGTCAGTAAGCGGCC CTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAG TCTGGCAACACGGCCTCCCTGACCATCTCTGGGC TCCAGTCTGAGGACGAGGCTGATTATTACTGCAA CTCTTTGACAAGCATCAGCACTTGGGTGTTCGGC GGAGGGACCAAGCTGACCGTCCTAGGCCAGCCCA AGGCCGCCCCCTCCGTGACCCTGTTCCCCCCCTC CTCCGAGGAGCTGCAGGCCAACAAGGCCACCCTG GTGTGCCTGATCTCCGACTTCTACCCCGGCGCCG TGACCGTGGCCTGGAAGGCCGACTCCTCCCCCGT GAAGGCCGGCGTGGAGACCACCACCCCCTCCAAG CAGTCCAACAACAAGTACGCCGCCTCCTCCTACC TGTCCCTGACCCCCGAGCAGTGGAAGTCCCACCG GTCCTACTCCTGCCAGGTGACCCACGAGGGCTCC ACCGTGGAGAAGACCGTGGCCCCCACCGAGTGCT CCTGA	655
mAb10989	**Amino Acids**		
	HCVR	QVQLVQSGAEVKKPGASVKVSCKASGYIFTGYYM HWVRQAPGQGLEWMGWINPNSGGANYAQKFQGRV TLTRDTSITTVYMELSRLRFDDTAVYYCARGSRY DWNQNNWFDPWGQGTLVTVSS	678
	HCDR1	GYIFTGYY	680
	HCDR2	INPNSGGA	682
	HCDR3	ARGSRYDWNQNNWFDP	684
	LCVR	QSALTQPASVSGSPGQSITISCTGTSSDVGTYNY VSWYQQHPGKAPKLMIFDVSNRPSGVSDRFSGSK SGNTASLTISGLQAEDEADYYCSSFTTSSTVVFG GGTKLTVL	686
	LCDR1	SSDVGTYNY	688
	LCDR2	DVS	650
	LCDR3	SSFTTSSTVV	690
	HC	QVQLVQSGAEVKKPGASVKVSCKASGYIFTGYYM HWVRQAPGQGLEWMGWINPNSGGANYAQKFQGRV TLTRDTSITTVYMELSRLRFDDTAVYYCARGSRY DWNQNNWFDPWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY	692

		ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	
	LC	QSALTQPASVSGSPGQSITISCTGTSSDVGTYNY VSWYQQHPGKAPKLMIFDVSNRPSGVSDRFSGSK SGNTASLTISGLQAEDEADYYCSSFTTSSTVVFG GGTKLTVLGQPKAAPSVTLFPPSSEELQANKATL VCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGS TVEKTVAPTECS	694
	Nucleic Acids		
	HCVR	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGA AGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAA GGCTTCTGGATACATCTTCACCGGCTACTATATG CACTGGGTGCGACAGGCCCCTGGACAGGGGCTTG AGTGGATGGGATGGATCAACCCTAACAGTGGTGG CGCAAACTATGCACAGAAGTTTCAGGGCAGGGTC ACCCTGACCAGGGACACGTCCATCACCACAGTCT ACATGGAACTGAGCAGGCTGAGATTTGACGACAC GGCCGTGTATTACTGTGCGAGAGGATCCCGGTAT GACTGGAACCAGAACAACTGGTTCGACCCCTGGG GCCAGGGAACCCTGGTCACCGTCTCCTCA	677
	HCDR1	GGATACATCTTCACCGGCTACTAT	679
	HCDR2	ATCAACCCTAACAGTGGTGGCGCA	681
	HCDR3	GCGAGAGGATCCCGGTATGACTGGAACCAGAACA ACTGGTTCGACCCC	683
	LCVR	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTG GGTCTCCTGGACAGTCGATCACCATCTCCTGCAC TGGAACCAGCAGTGACGTTGGTACTTATAACTAT GTCTCCTGGTACCAACAACACCCAGGCAAAGCCC CCAAACTCATGATTTTTGATGTCAGTAATCGGCC CTCAGGGGTTTCTGATCGCTTCTCTGGCTCCAAG TCTGGCAACACGGCCTCCCTGACCATCTCTGGGC TCCAGGCTGAGGACGAGGCTGATTATTACTGCAG CTCATTTACAACCAGCAGCACTGTGGTTTTCGGC GGAGGGACCAAGCTGACCGTCCTA	685
	LCDR1	AGCAGTGACGTTGGTACTTATAACTAT	687
	LCDR2	GATGTCAGT	649
	LCDR3	AGCTCATTTACAACCAGCAGCACTGTGGTT	689

	HC	CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGA AGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAA GGCTTCTGGATACATCTTCACCGGCTACTATATG CACTGGGTGCGACAGGCCCCTGGACAGGGGCTTG AGTGGATGGGATGGATCAACCCTAACAGTGGTGG CGCAAACTATGCACAGAAGTTTCAGGGCAGGGTC ACCCTGACCAGGGACACGTCCATCACCACAGTCT ACATGGAACTGAGCAGGCTGAGATTTGACGACAC GGCCGTGTATTACTGTGCGAGAGGATCCCGGTAT GACTGGAACCAGAACAACTGGTTCGACCCCTGGG GCCAGGGAACCCTGGTCACCGTCTCCTCAGCCTC CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCC TCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCC TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC GGTGACGGTGTCGTGGAACTCAGGCGCCCTGACC AGCGGCGTGCACACCTTCCCGGCTGTCCTACAGT CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC ATCTGCAACGTGAATCACAAGCCCAGCAACACCA AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGA CAAAACTCACACATGCCCACCGTGCCCAGCACCT GAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCC CCCCAAAACCCAAGGACACCCTCATGATCTCCCG GACCCCTGAGGTCACATGCGTGGTGGTGGACGTG AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGG ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGT CTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGATGA GCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG AGTGGGAGAGCAATGGGCAGCCGGAGAACAACTA CAAGACCACGCCTCCCGTGCTGGACTCCGACGGC TCCTTCTTCCTCTACAGCAAGCTCACCGTGGACA AGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG CTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGTCCCTCTCCCTGTCTCCGGGTAAAT GA	691
	LC	CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTG GGTCTCCTGGACAGTCGATCACCATCTCCTGCAC TGGAACCAGCAGTGACGTTGGTACTTATAACTAT GTCTCCTGGTACCAACAACACCCAGGCAAAGCCC CCAAACTCATGATTTTTGATGTCAGTAATCGGCC CTCAGGGGTTTCTGATCGCTTCTCTGGCTCCAAG TCTGGCAACACGGCCTCCCTGACCATCTCTGGGC	693

		TCCAGGCTGAGGACGAGGCTGATTATTACTGCAG CTCATTTACAACCAGCAGCACTGTGGTTTTCGGC GGAGGGACCAAGCTGACCGTCCTAGGCCAGCCCA AGGCCGCCCCCTCCGTGACCCTGTTCCCCCCCTC CTCCGAGGAGCTGCAGGCCAACAAGGCCACCCTG GTGTGCCTGATCTCCGACTTCTACCCCGGCGCCG TGACCGTGGCCTGGAAGGCCGACTCCTCCCCCGT GAAGGCCGGCGTGGAGACCACCACCCCCTCCAAG CAGTCCAACAACAAGTACGCCGCCTCCTCCTACC TGTCCCTGACCCCCGAGCAGTGGAAGTCCCACCG GTCCTACTCCTGCCAGGTGACCCACGAGGGCTCC ACCGTGGAGAAGACCGTGGCCCCCACCGAGTGCT CCTGA	

Administration of Antibodies

[000108] The present invention provides methods for administering an anti-CoV-S antigen-binding protein of the present invention, *e.g.*, those of Table 1, comprising introducing the antigen-binding protein into the body of a subject (*e.g.*, a human). For example, the method comprises piercing the body of the subject with a needle of a syringe and injecting the antigen-binding protein into the body of the subject, *e.g.*, into the vein, artery, tumor, muscular tissue or subcutis of the subject.

[000109] The present invention provides a vessel (*e.g.*, a plastic or glass vial, *e.g.*, with a cap or a chromatography column, hollow bore needle or a syringe cylinder) comprising an anti-CoV-S antigen-binding protein of the present invention, *e.g.*, those of Table 1.

[000110] The present invention also provides an injection device comprising one or more antigen-binding proteins (*e.g.,* antibody or antigen-binding fragment) that bind specifically to CoV-S, *e.g.*, those of Table 1, or a pharmaceutical composition thereof. The injection device may be packaged into a kit. An injection device is a device that introduces a substance into the body of a subject via a parenteral route, *e.g.*, intramuscular, subcutaneous or intravenous. For example, an injection device may be a syringe (*e.g.*, pre-filled with the pharmaceutical composition, such as an auto-injector) which, for example, includes a cylinder or barrel for holding fluid to be injected (*e.g.*, comprising the antibody or fragment or a pharmaceutical composition thereof), a needle for piecing skin and/or blood vessels for injection of the fluid; and a plunger for pushing the fluid out of the cylinder and through the needle bore. In an embodiment of the invention, an injection device that comprises an antigen-binding protein, *e.g.*, an antibody or antigen-binding fragment thereof, from a combination of the present invention, or

a pharmaceutical composition thereof is an intravenous (IV) injection device. Such a device can include the antigen-binding protein or a pharmaceutical composition thereof in a cannula or trocar/needle which may be attached to a tube which may be attached to a bag or reservoir for holding fluid (*e.g.*, saline) introduced into the body of the subject through the cannula or trocar/needle. The antibody or fragment or a pharmaceutical composition thereof may, in an embodiment of the invention, be introduced into the device once the trocar and cannula are inserted into the vein of a subject and the trocar is removed from the inserted cannula. The IV device may, for example, be inserted into a peripheral vein (*e.g.*, in the hand or arm); the superior *vena cava* or inferior *vena cava*, or within the right atrium of the heart (*e.g.*, a central IV); or into a subclavian, internal jugular, or a femoral vein and, for example, advanced toward the heart until it reaches the superior vena cava or right atrium (*e.g.*, a central venous line). In an embodiment of the invention, an injection device is an autoinjector; a jet injector or an external infusion pump. A jet injector uses a high-pressure narrow jet of liquid which penetrate the epidermis to introduce the antibody or fragment or a pharmaceutical composition thereof to a subject's body. External infusion pumps are medical devices that deliver the antibody or fragment or a pharmaceutical composition thereof into a subject's body in controlled amounts. External infusion pumps may be powered electrically or mechanically. Different pumps operate in different ways, for example, a syringe pump holds fluid in the reservoir of a syringe, and a moveable piston controls fluid delivery, an elastomeric pump holds fluid in a stretchable balloon reservoir, and pressure from the elastic walls of the balloon drives fluid delivery. In a peristaltic pump, a set of rollers pinches down on a length of flexible tubing, pushing fluid forward. In a multi-channel pump, fluids can be delivered from multiple reservoirs at multiple rates.

Preparation of Human Antibodies

[000111] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to CoV-S. An immunogen comprising any one of the following can be used to generate antibodies to CoV-S. In certain embodiments of the invention, the antibodies of the invention are obtained from mice immunized with a full length, native CoV-S, or with a live attenuated or inactivated virus, or with DNA encoding the protein or fragment thereof. Alternatively, the CoV-S protein or a fragment thereof may be produced using standard

biochemical techniques and modified and used as immunogen. In one embodiment of the invention, the immunogen is a recombinantly produced CoV-S protein or fragment thereof. In certain embodiments of the invention, the immunogen may be a CoV-S polypeptide vaccine. In certain embodiments, one or more booster injections may be administered. In certain embodiments, the immunogen may be a recombinant CoV-S polypeptide expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells.

[000112] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to CoV-S can be initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[000113] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[000114] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, *etc.* The mouse constant regions are replaced with a desired human constant region to generate

the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Anti-Coronavirus Spike Protein Antibodies Comprising Fc Variants

[000115] According to certain embodiments of the present invention, anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments, are provided comprising an Fc domain comprising one or more mutations, which, for example, enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-CoV-S antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T); or a modification at position 428 and/or 433 (*e.g.*, H/L/R/S/P/Q or K) and/or 434 (*e.g.*, A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.*, 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.*, M428L) and 434S (*e.g.*, N434S) modification; a 428L, 259I (*e.g.*, V259I), and 308F (*e.g.*, V308F) modification; a 433K (*e.g.*, H433K) and a 434 (*e.g.*, 434Y) modification; a 252, 254, and 256 (*e.g.*, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.*, T250Q and M428L); and a 307 and/or 308 modification (*e.g.*, 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[000116] For example, the present invention includes anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments, comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F).

[000117] Anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof, that comprise a V_H and/or V_L as set forth herein comprising any possible combinations of the foregoing Fc domain mutations, are contemplated within the scope of the present invention.

[000118] The present invention also includes anti-CoV-S antigen-binding proteins, antibodies or antigen-binding fragments, comprising a V_H set forth herein and a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (See, *e.g.*, WO2014/022540).

Immunoconjugates

[000119] The invention encompasses an anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments, conjugated to another moiety, *e.g.*, a therapeutic moiety (an "immunoconjugate"), such as a toxoid or an anti-viral drug to treat influenza virus infection. In an embodiment of the invention, an anti-CoV-S antibody or fragment is conjugated to any of the further therapeutic agents set forth herein. As used herein, the term "immunoconjugate" refers to an antigen-binding protein, *e.g.*, an antibody or antigen-binding fragment, which is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety,

an enzyme, a peptide or protein or a therapeutic agent. The antigen-binding protein may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target (CoV-S). Examples of immunoconjugates include antibody-drug conjugates and antibody-toxin fusion proteins. In one embodiment of the invention, the agent may be a second, different antibody that binds specifically to CoV-S. The type of therapeutic moiety that may be conjugated to the anti-CoV-S antigen-binding protein (*e.g.*, antibody or fragment) will take into account the condition to be treated and the desired therapeutic effect to be achieved. See, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", Monoclonal Antibodies 1984: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62: 119-58 (1982).

Multi-specific Antibodies

[000120] The present invention includes anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof, as well as methods of use thereof and methods of making such antigen-binding proteins. The term "anti-CoV-S" antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments, includes multispecific (*e.g.*, bispecific or biparatopic) molecules that include at least one first antigen-binding domain that specifically binds to CoV-S (*e.g.*, an antigen-binding domain from an antibody of Table 1) and at least one second antigen-binding domain that binds to a different antigen or to an epitope in CoV-S which is different from that of the first antigen-binding domain. In some embodiments, the first antigen-binding domain and the second antigen-binding domain are both selected from the antigen-binding domains of Table 1. In an embodiment of the invention, the first and second epitopes overlap. In another embodiment of the invention, the first and second epitopes do not overlap. For example, in an

embodiment of the invention, a multispecific antibody is a bispecific IgG antibody (*e.g.*, IgG1 or IgG4) that includes a first antigen-binding domain that binds specifically to CoV-S including the heavy and light immunoglobulin chain of an antibody of Table 1, and a second antigen-binding domain that binds specifically to a different epitope of CoV-S. In some embodiments, a bispecific IgG antibody (*e.g.*, IgG1 or IgG4) includes a first antigen-binding domain that binds specifically to CoV-S and a second binding domain that binds to a host cell protein, e.g., ACE2 or TMPRSS2.

[000121] The antibodies of Table 1 include multispecific molecules, *e.g.*, antibodies or antigen-binding fragments, that include the CDR-Hs and CDR-Ls, V_H and V_L, or HC and LC of those antibodies, respectively (including variants thereof as set forth herein).

[000122] In an embodiment of the invention, an antigen-binding domain that binds specifically to CoV-S, which may be included in a multispecific molecule, comprises:

(1)

(i) a heavy chain variable domain sequence that comprises CDR-H1, CDR-H2, and CDR-H3 amino acid sequences set forth in Table 1, and

(ii) a light chain variable domain sequence that comprises CDR-L1, CDR-L2, and CDR-L3 amino acid sequences set forth in Table 1;

or,

(2)

(i) a heavy chain variable domain sequence comprising an amino acid sequence set forth in Table 1, and

(ii) a light chain variable domain sequence comprising an amino acid sequence set forth in Table 1;

or,

(3)

(i) a heavy chain immunoglobulin sequence comprising an amino acid sequence set forth in Table 1, and

(ii) a light chain immunoglobulin sequence comprising an amino acid sequence set forth in Table 1.

[000123] In an embodiment of the invention, the multispecific antibody or fragment includes more than two different binding specificities (*e.g.*, a trispecific molecule), for example, one or

more additional antigen-binding domains which are the same or different from the first and/or second antigen-binding domain.

[000124] In one embodiment of the invention, a bispecific antigen-binding fragment comprises a first scFv (*e.g.*, comprising V_H and V_L sequences of Table 1) having binding specificity for a first epitope (*e.g.*, CoV-S) and a second scFv having binding specificity for a second, different epitope. For example, in an embodiment of the invention, the first and second scFv are tethered with a linker, *e.g.*, a peptide linker (*e.g.*, a GS linker such as $(GGGGS)_n$ (SEQ ID NO: 834) wherein n is, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10). Other bispecific antigen-binding fragments include an $F(ab)_2$ of a bispecific IgG antibody which comprises the heavy and light chain CDRs of Table 1 and of another antibody that binds to a different epitope.

Therapeutic Methods

[000125] The present invention provides methods for treating or preventing viral infection (*e.g.*, coronavirus infection) by administering a therapeutically effective amount of anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment, (*e.g.*, of Table 1) to a subject (*e.g.*, a human) in need of such treatment or prevention.

[000126] Coronavirus infection may be treated or prevented, in a subject, by administering an anti-CoV-S antigen-binding protein of the present invention to a subject.

[000127] An effective or therapeutically effective dose of anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment (*e.g.*, of Table 1), for treating or preventing a viral infection refers to the amount of the antibody or fragment sufficient to alleviate one or more signs and/or symptoms of the infection in the treated subject, whether by inducing the regression or elimination of such signs and/or symptoms or by inhibiting the progression of such signs and/or symptoms. The dose amount may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. In an embodiment of the invention, an effective or therapeutically effective dose of antibody or antigen-binding fragment thereof of the present invention, for treating or preventing viral infection, *e.g.*, in an adult human subject, is about 0.01 to about 200 mg/kg, *e.g.*, up to about 150 mg/kg. In an embodiment of the invention, the dosage is up to about 10.8 or 11 grams (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 grams). Depending on the severity of the infection, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the

antigen-binding protein of the present invention can be administered at an initial dose, followed by one or more secondary doses. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[000128] As used herein, the term "subject" refers to a mammal (*e.g.*, rat, mouse, cat, dog, cow, pig, sheep, horse, goat, rabbit), preferably a human, for example, in need of prevention and/or treatment of a disease or disorder such as viral infection or cancer. The subject may have a viral infection, *e.g.*, an influenza infection, or be predisposed to developing an infection. Subjects predisposed to developing an infection, or subjects who may be at elevated risk for contracting an infection (*e.g.*, of coronavirus or influenza virus), include subjects with compromised immune systems because of autoimmune disease, subjects receiving immunosuppressive therapy (for example, following organ transplant), subjects afflicted with human immunodeficiency syndrome (HIV) or acquired immune deficiency syndrome (AIDS), subjects with forms of anemia that deplete or destroy white blood cells, subjects receiving radiation or chemotherapy, or subjects afflicted with an inflammatory disorder. Additionally, subjects of very young (*e.g.*, 5 years of age or younger) or old age (*e.g.*, 65 years of age or older) are at increased risk. Moreover, a subject may be at risk of contracting a viral infection due to proximity to an outbreak of the disease, *e.g.* subject resides in a densely-populated city or in close proximity to subjects having confirmed or suspected infections of a virus, or choice of employment, *e.g.* hospital worker, pharmaceutical researcher, traveler to infected area, or frequent flier.

[000129] "Treat" or "treating" means to administer an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment of the present invention (*e.g.*, of Table 1), to a subject having one or more signs or symptoms of a disease or infection, *e.g.*, viral infection, for which the antigen-binding protein is effective when administered to the subject at an effective or therapeutically effective amount or dose (as discussed herein).

[000130] The present invention also encompasses prophylactically administering an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof of the present invention (*e.g.*, of Table 1), to a subject who is at risk of viral infection so as to prevent such infection. Passive antibody-based immunoprophylaxis has proven an effective strategy for preventing subject from viral infection. See *e.g.*, Berry *et al.*, Passive broad-spectrum influenza immunoprophylaxis. Influenza Res Treat. 2014; 2014:267594. Epub 2014 Sep 22; and Jianqiang *et al.*, Passive immune neutralization strategies for prevention and control of influenza A infections, Immunotherapy. 2012 February; 4(2): 175–186; Prabhu *et al.*, Antivir Ther. 2009;14(7):911-21, Prophylactic and therapeutic efficacy of a chimeric monoclonal antibody specific for H5 hemagglutinin against lethal H5N1 influenza. "Prevent" or "preventing" means to administer an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment of the present invention (*e.g.*, of Table 1), to a subject to inhibit the manifestation of a disease or infection (*e.g.*, viral infection) in the body of a subject, for which the antigen-binding protein is effective when administered to the subject at an effective or therapeutically effective amount or dose (as discussed herein).

[000131] In an embodiment of the invention, a sign or symptom of a viral infection in a subject is survival or proliferation of virus in the body of the subject, *e.g.*, as determined by viral titer assay (*e.g.*, coronavirus propagation in embryonated chicken eggs or coronavirus spike protein assay). Other signs and symptoms of viral infection are discussed herein.

[000132] As noted above, in some embodiments the subject may be a non-human animal, and the antigen-binding proteins (*e.g.*, antibodies and antigen-binding fragments) discussed herein may be used in a veterinary context to treat and/or prevent disease in the non-human animals (*e.g.*, cats, dogs, pigs, cows, horses, goats, rabbits, sheep, and the like).

[000133] The present invention provides a method for treating or preventing viral infection (*e.g.*, coronavirus infection) or for inducing the regression or elimination or inhibiting the progression of at least one sign or symptom of viral infection such as:

- fever or feeling feverish/chills;
- cough;
- sore throat;
- runny or stuffy nose;
- sneezing;

- muscle or body aches;
- headaches;
- fatigue (tiredness);
- vomiting;
- diarrhea;
- respiratory tract infection;
- chest discomfort;
- shortness of breath;
- bronchitis; and/or
- pneumonia,

which sign or symptom is secondary to viral infection, in a subject in need thereof (*e.g.*, a human), by administering a therapeutically effective amount of anti-CoV-S antigen-binding protein (*e.g.*, of Table 1) to the subject, for example, by injection of the protein into the body of the subject.

Combinations and Pharmaceutical Compositions

[000134] To prepare pharmaceutical compositions of the anti-CoV-S antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof (*e.g.*, of Table 1), antigen-binding protein is admixed with a pharmaceutically acceptable carrier or excipient. See, *e.g.*, Remington's Pharmaceutical Sciences and U.S. Pharmacopeia: National Formulary, Mack Publishing Company, Easton, Pa. (1984); Hardman, *et al.* (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, *et al.* (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y. In an embodiment of the invention, the pharmaceutical composition is sterile. Such compositions are part of the present invention.

[000135] The scope of the present invention includes desiccated, *e.g.*, freeze-dried, compositions comprising an anti-CoV-S antigen-binding proteins, *e.g.*, antibody or antigen-

binding fragment thereof (*e.g.*, of Table 1), or a pharmaceutical composition thereof that includes a pharmaceutically acceptable carrier but substantially lacks water.

[000136] In a further embodiment of the invention, a further therapeutic agent that is administered to a subject in association with an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof (*e.g.*, of Table 1), disclosed herein is administered to the subject in accordance with the Physicians' Desk Reference 2003 (Thomson Healthcare; 57th edition (Nov. 1, 2002)).

[000137] The mode of administration can vary. Routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal or intra-arterial.

[000138] The present invention provides methods for administering an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof (*e.g.*, of Table 1), comprising introducing the protein into the body of a subject. For example, the method comprises piercing the body of the subject with a needle of a syringe and injecting the antigen-binding protein into the body of the subject, *e.g.*, into the vein, artery, tumor, muscular tissue or subcutis of the subject.

[000139] The present invention provides a vessel (*e.g.*, a plastic or glass vial, *e.g.*, with a cap or a chromatography column, hollow bore needle or a syringe cylinder) comprising any of the anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments thereof (*e.g.*, of Table 1), polypeptides (*e.g.*, an HC, LC, V_H or V_L of Table 1) or polynucleotides (*e.g.*, of Table 2) or vectors set forth herein or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier.

[000140] In an embodiment of the present disclosure, an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof of the present invention (*e.g.*, of Table 1), is administered in association with one or more further therapeutic agents. A further therapeutic agent includes, but is not limited to: an anti-inflammatory agent, an antimalarial agent, a second antibody or antigen-binding fragment thereof that specifically binds TMPRSS2, and a second antibody or antigen-binding fragment thereof that specifically binds to CoV-S. In some embodiments, an antimalarial agent is chloroquine or hydroxychloroquine. In some embodiments, an anti-inflammatory agent is an antibody such as sarilumab, tocilizumab, or

gimsilumab. In some embodiments, the further therapeutic agent is a second antibody or antigen-binding fragment disclosed herein, *e.g.*, of Table 1. In certain embodiments, one, two, three, four, or more antibodies, or antigen-binding fragments thereof, of Table 1 can be administered in combination (e.g., concurrently or sequentially). Particular combinations of antibodies of Table 1 are listed in the Table of Exemplary Antibody Combinations, below (each number representing a specific combination, e.g., mAb10989 and mAb10987 is Combination 1, mAb10989 and mAb10934 is Combination 2, and so on). In some embodiments, a combination of antibodies can be selected from among those binding to different epitope clusters. For example, certain antibodies described herein belong to epitope clusters as follows: Cluster 1, mAb10987, mAb10922, mAb10936, and mAb10934; Cluster 2, mAb10989, mAb10977, and mAb10933; Cluster 3, mAb10920; Cluster 4, mAb10954, mAb10986, and mAb10964; and Cluster 5, mAb10984. Thus, a combination of two antibodies can be selected from, for example, Cluster 1 and Cluster 2, Cluster 1 and Cluster 3, Cluster 1 and Cluster 4, Cluster 1 and Cluster 5, Cluster 2 and Cluster 3, Cluster 2 and Cluster 4, Cluster 2 and Cluster 5, Cluster 3 and Cluster 4, Cluster 3 and Cluster 5, and Cluster 4 and Cluster 5. In some embodiments, an antibody that specifically binds TMPRSS2 is H1H7017N, as described in International Patent Pub. No. WO/2019/147831.

Table of Exemplary Antibody Combinations

	mAb 10989	mAb 10987	mAb 10934	mAb 10933	mAb 10920	mAb 10922	mAb 10936	mAb 10954	mAb 10964	mAb 10977	mAb 10984	mAb 10986
mAb 10989	X	1	2	3	4	5	6	7	8	9	10	11
mAb 10987	12	X	13	14	15	16	17	18	19	20	21	22
mAb 10934	23	24	X	25	26	27	28	29	30	31	32	33
mAb 10933	34	35	36	X	37	38	39	40	41	42	43	44
mAb 10920	45	46	47	48	X	49	50	51	52	53	54	55
mAb 10922	56	57	58	59	60	X	61	62	63	64	65	66
mAb 10936	67	68	69	70	71	72	X	73	74	75	76	77
mAb 10954	78	79	80	81	82	83	84	X	85	86	87	88
mAb 10964	89	90	91	92	93	94	95	96	X	97	98	99
mAb 10977	100	101	102	103	104	105	106	107	108	X	109	110
mAb 10984	111	112	113	114	115	116	117	118	119	120	X	121
mAb 10986	122	123	124	125	126	127	128	129	130	131	132	X

[000141] In some embodiments, anti-CoV-S antigen-binding proteins (*e.g.*, anti-SARS-CoV-2-S antibodies or antigen-binding fragments thereof) from different human donors may be combined. The present invention includes a composition comprising two (or more) anti-SARS-CoV-2-S antibodies or antigen-binding fragments comprising variable domains from human subjects, wherein the two (or more) antibodies or antigen-binding fragments are derived from different subjects (*e.g.*, two different human subjects). Antibody variable regions derived from human B cells are discussed, *e.g.*, in Examples 1 and 2 (Table 3), which describes that variable domains cloned from such B cells are combined with a constant region not from those B cells to produce hybrid antibodies. The source (Donor) of such antibody variable regions is shown in the Table of Exemplary Human-Derived Antibody Variable Regions, below. In some embodiments, a composition may comprise a combination of an antibody or antigen-binding fragment thereof with variable domains derived from donor 1 and an antibody or antigen-binding fragment thereof with variable domains derived from donor 2. In some embodiments, a composition may comprise a combination of an antibody or antigen-binding fragment thereof with variable domains derived from donor 1 and an antibody or antigen-binding fragment thereof with variable domains derived from donor 3. In some embodiments, a composition may comprise a combination of an antibody or antigen-binding fragment thereof with variable domains derived from donor 2 and an antibody or antigen-binding fragment thereof with variable domains derived from donor 3. In some embodiments, a composition may comprise a combination of mAb10987 (*e.g.*, an antibody comprising the CDRs, the variable regions, or the heavy and light chain sequences shown in Table 1) from Donor 1, and mAb10989 (*e.g.*, an antibody comprising the CDRs, the variable regions, or the heavy and light chain sequences shown in Table 1) from Donor 3.

Table of Exemplary Human-Derived Antibody Variable Regions

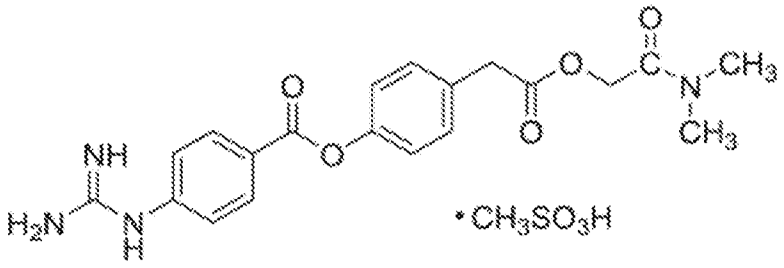
mAb	Donor
mAb10954	Donor 3
mAb10955	Donor 3
mAb10956	Donor 3
mAb10957	Donor 3
mAb10964	Donor 1
mAb10965	Donor 2

mAb10966	Donor 3
mAb10967	Donor 3
mAb10970	Donor 1
mAb10971	Donor 1
mAb10977	Donor 1
mAb10984	Donor 1
mAb10985	Donor 1
mAb10986	Donor 1
mAb10987	Donor 1
mAb10988	Donor 3
mAb10989	Donor 3
mAb10969	Donor 1

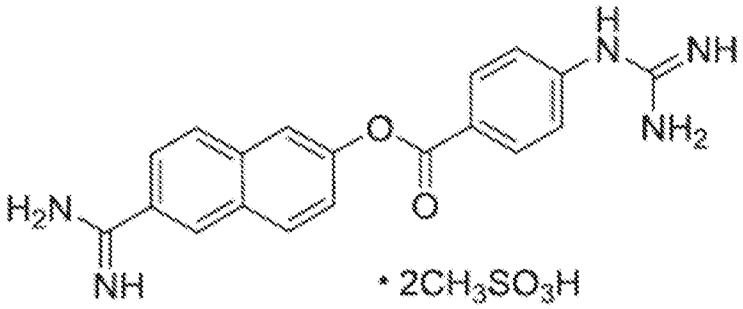
[000142] In some embodiments, the further therapeutic agent is an anti-viral drug and/or a vaccine. As used herein, the term "anti-viral drug" refers to any anti-infective drug or therapy used to treat, prevent, or ameliorate a viral infection in a subject. The term "anti-viral drug" includes, but is not limited to a cationic steroid antimicrobial, leupeptin, aprotinin, ribavirin, or interferon-alpha2b. Methods for treating or preventing virus (*e.g.*, coronavirus) infection in a subject in need of said treatment or prevention by administering an antibody or antigen-binding fragment of Table 1 in association with a further therapeutic agent are part of the present invention.

[000143] For example, in an embodiment of the invention, the further therapeutic agent is a vaccine, *e.g.*, a coronavirus vaccine. In an embodiment of the invention, a vaccine is an inactivated/killed virus vaccine, a live attenuated virus vaccine or a virus subunit vaccine.

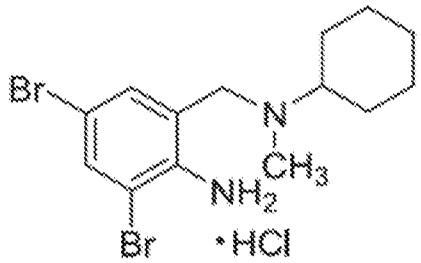
[000144] For example, in an embodiment of the invention, the further therapeutic agent is:



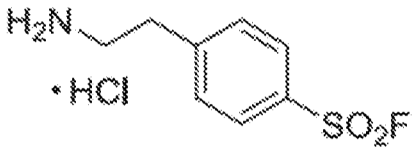
(camostat mesylate);



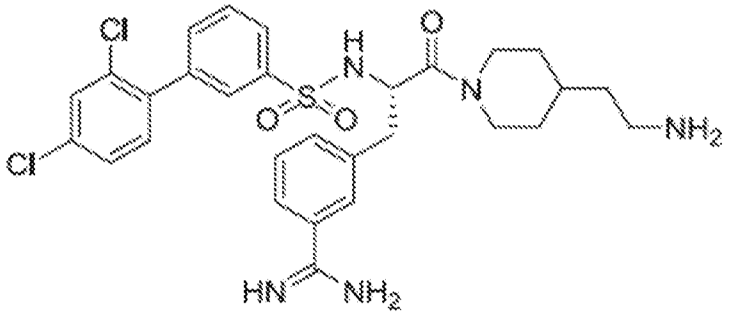
(nafamostat mesylate);



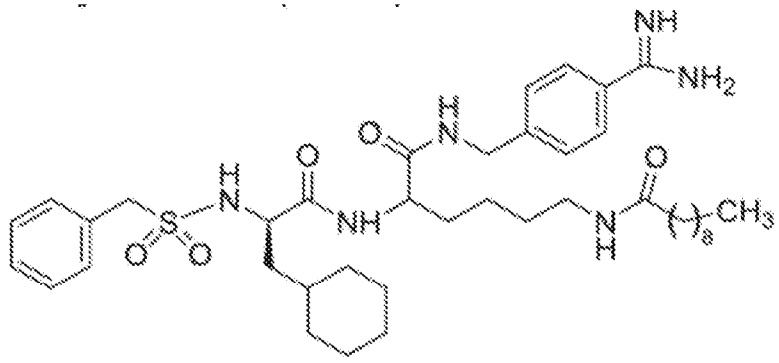
(bromhexine hydrochloride (BHH));



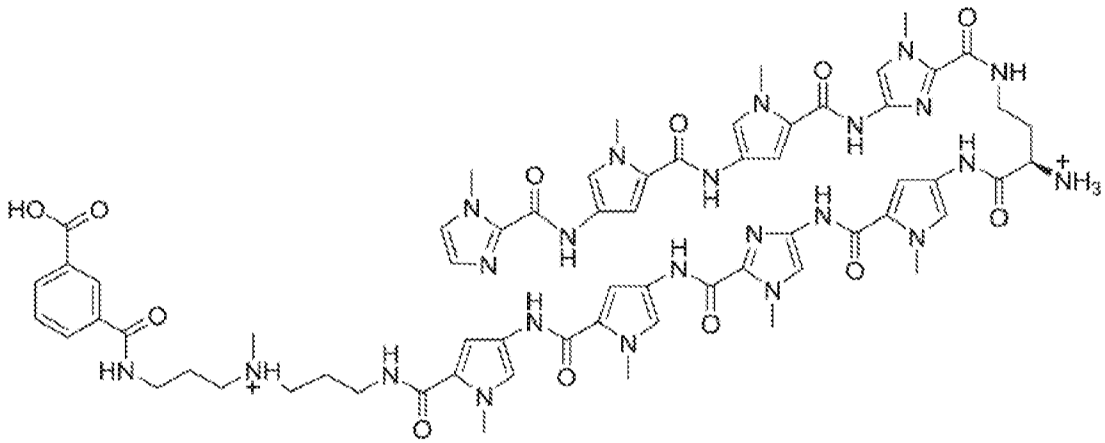
(4-(2-aminomethyl)benzenesulfonyl fluoride hydrochloride (AEBSF));



;



; or



(polyamide). See Shen *et al.* Biochimie 142: 1-10 (2017).

[000145] In an embodiment of the invention, the anti-viral drug is an antibody or antigen-binding fragment that binds specifically to coronavirus, *e.g.*, SARS-CoV-2, SARS-CoV, or MERS-CoV. Exemplary anti-CoV-S antibodies include, but are not limited to: H4sH15188P; H1H15188P; H1H15211P; H1H15177P; H4sH15211P; H1H15260P2; H1H15259P2; H1H15203P; H4sH15260P2; H4sH15231P2; H1H15237P2; H1H15208P; H1H15228P2; H1H15233P2; H1H15264P2; H1H15231P2; H1H15253P2; H1H15215P; and H1H15249P2, as set forth in International patent application publication no. WO/2015/179535, or an antigen-binding fragment thereof, *e.g.*, wherein the antibody or fragment comprises a light chain immunoglobulin that includes CDR-L1, CDR-L2 and CDR-L3 (*e.g.*, the V_L or light chain thereof); and a heavy chain that includes CDR-H1, CDR-H2 and CDR-H3 (*e.g.*, the V_H or heavy chain thereof) of any of the foregoing anti-CoV-S antibodies.

[000146] In a certain embodiment of the invention, the further therapeutic agent is not aprotinin, leupeptin, a cationic steroid antimicrobial, an influenza vaccine (*e.g.*, killed, live,

attenuated whole virus or subunit vaccine), or an antibody against influenza virus (*e.g.*, an anti-hemagglutinin antibody).

[000147] The term "in association with" indicates that the components, an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof of the present invention, along with another agent, can be formulated into a single composition, *e.g.*, for simultaneous delivery, or formulated separately into two or more compositions (*e.g.*, a kit). Each component can be administered to a subject at a different time than when the other component is administered; for example, each administration may be given non-simultaneously (*e.g.*, separately or sequentially) at intervals over a given period of time. Moreover, the separate components may be administered to a subject by the same or by a different route (*e.g.*, wherein an anti-CoV-S antibody or antigen-binding fragment thereof.

Kits

[000148] Further provided are kits comprising one or more components that include, but are not limited to, an anti-CoV-S antigen-binding protein, *e.g.*, an antibody or antigen-binding fragment as discussed herein (*e.g.*, of Table 1), in association with one or more additional components including, but not limited to, a further therapeutic agent, as discussed herein. The antigen-binding protein and/or the further therapeutic agent can be formulated as a single composition or separately in two or more compositions, *e.g.*, with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

[000149] In one embodiment of the invention, the kit includes an anti-CoV-S antigen-binding protein, *e.g.*, an antibody or antigen-binding fragment thereof of the invention (*e.g.*, of Table 1), or a pharmaceutical composition thereof in one container (*e.g.*, in a sterile glass or plastic vial) and a further therapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial).

[000150] In another embodiment, the kit comprises a combination of the invention, including an anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof of the invention (*e.g.*, of Table 1), or pharmaceutical composition thereof in combination with one or more further therapeutic agents formulated together, optionally, in a pharmaceutical composition, in a single, common container.

[000151] If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device (*e.g.*, an injection device) for performing such

administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above containing the anti-CoV-S antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof of the present invention (*e.g.*, of Table 1).

[000152] The kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

Diagnostic Uses of the Antibodies

[000153] The anti-CoV-S antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments thereof of the present invention (*e.g.*, of Table 1), may be used to detect and/or measure CoV-S in a sample. Exemplary assays for CoV-S may include, *e.g.*, contacting a sample with an anti-CoV-S antigen-binding protein of the invention, wherein the anti-CoV-S antigen-binding protein is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate CoV-S from samples. The presence of an anti-CoV-S antigen-binding protein complexed with CoV-S indicates the presence of CoV-S in the sample. Alternatively, an unlabeled anti-CoV-S antibody can be used in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure CoV-S in a sample include neutralization assays, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS). Thus, the present invention includes a method for detecting the presence of spike protein polypeptide in a sample comprising contacting the sample with an anti-CoV-S antigen-binding protein and detecting the presence of a CoV-S/anti-CoV-S antigen-binding protein wherein the presence of the complex indicates the presence of CoV-S.

[000154] An anti-CoV-S antigen-binding protein of the invention (*e.g.*, of Table 1) may be used in a Western blot or immune-protein blot procedure for detecting the presence of CoV-S or a fragment thereof in a sample. Such a procedure forms part of the present invention and includes the steps of *e.g.*:

(1) providing a membrane or other solid substrate comprising a sample to be tested for the presence of CoV-S, *e.g.*, optionally including the step of transferring proteins from a sample to be tested for the presence of CoV-S (*e.g.*, from a PAGE or SDS-PAGE electrophoretic separation of the proteins in the sample) onto a membrane or other solid substrate using a method known in the art (*e.g.*, semi-dry blotting or tank blotting); and contacting the membrane or other solid substrate to be tested for the presence of CoV-S or a fragment thereof with an anti-CoV-S antigen-binding protein of the invention.

[000155] Such a membrane may take the form, for example, of a nitrocellulose or vinyl-based (*e.g.*, polyvinylidene fluoride (PVDF)) membrane to which the proteins to be tested for the presence of CoV-S in a non-denaturing PAGE (polyacrylamide gel electrophoresis) gel or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) gel have been transferred (*e.g.*, following electrophoretic separation in the gel). Before contacting the membrane with the anti-CoV-S antigen-binding protein, the membrane is optionally blocked, *e.g.*, with non-fat dry milk or the like so as to bind non-specific protein binding sites on the membrane.

(2) washing the membrane one or more times to remove unbound anti-CoV-S antigen-binding protein and other unbound substances; and

(3) detecting the bound anti-CoV-S antigen-binding protein.

[000156] Detection of the bound antigen-binding protein indicates that the CoV-S protein is present on the membrane or substrate and in the sample. Detection of the bound antigen-binding protein may be by binding the antigen-binding protein with a secondary antibody (an anti-immunoglobulin antibody) which is detectably labeled and, then, detecting the presence of the secondary antibody label.

[000157] The anti-CoV-S antigen-binding proteins (*e.g.*, antibodies and antigen-binding fragments (*e.g.*, of Table 1)) disclosed herein may also be used for immunohistochemistry. Such a method forms part of the present invention and comprises, *e.g.*,

(1) contacting tissue to be tested for the presence of CoV-S protein with an anti-CoV-S antigen-binding protein of the invention; and

(2) detecting the antigen-binding protein on or in the tissue.

[000158] If the antigen-binding protein itself is detectably labeled, it can be detected directly. Alternatively, the antigen-binding protein may be bound by a detectably labeled secondary antibody wherein the label is then detected.

EXAMPLES

[000159] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, room temperature is about 25°C, and pressure is at or near atmospheric.

Example 1: Generation of human antibodies to SARS-CoV-2 spike protein (SARS-CoV-2-S)

[000160] Human antibodies to SARS-CoV-2-Spike protein (SARS-CoV-2-S) were generated in a VELOCIMMUNE® mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions or human immunoglobulin heavy and lambda light chain variable regions. Each mouse was immunized with a vector expressing the SARS-CoV-2-S receptor binding domain (RBD) (amino acids 1-1273 of NCBI accession number (MN908947.3), SEQ ID NO: 832), followed by a booster with a SARS-CoV-2-S vector or a SARS-CoV-2-S protein. The antibody immune response was monitored by a SARS-CoV-2-S-specific immunoassay. When a desired immune response was achieved, lymphocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce SARS-CoV-2-S-specific antibodies. Anti-SARS-CoV-2-S antibodies were also isolated directly from antigen-positive mouse B cells without fusion to myeloma cells, as described in U.S. Patent 7582298, herein specifically incorporated by reference in its entirety. Using this method, fully human anti-SARS-CoV-2-S antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained.

[000161] Antibody variable regions were also isolated from human blood samples. Whole

blood was received from patients 3-4 weeks after a laboratory-confirmed PCR positive test for SARS-CoV- 2 and symptomatic COVID-19 disease. Red blood cells were lysed using an ammonium chloride based lysis buffer (Life Technologies) and B cells were enriched by negative selection. Single B cells that bound the SARS-CoV-2 spike protein were isolated by fluorescent-activated cell sorting (FACS). Isolated B cells were single-well plated and mixed with antibody light and heavy variable region-specific PCR primers. cDNAs for each single B cell were synthesized via a reverse transcriptase (RT) reaction. Each resulting RT product was then split and transferred into two corresponding wells for subsequent antibody heavy and light chain PCRs. One set of the resulting RT products was first amplified by PCR using a 5' degenerate primer specific for antibody heavy variable region leader sequence or a 5' degenerate primer specific for antibody light chain variable region leader sequence and a 3' primer specific for antibody constant region, to form an amplicon. The amplicons were then amplified again by PCR using a 5' degenerate primer specific for antibody heavy variable region framework 1 or a 5' degenerate primer specific for antibody light chain variable region framework 1 and a 3' primer specific for antibody constant region, to generate amplicons for cloning. The antibody heavy chain and light chain derived PCR products were cloned into expression vectors containing heavy constant region and light constant region, respectively, thereby producing expression vectors for hybrid antibodies. The expression vectors expressing full-length heavy and light chain pairs were transfected into CHO cells to produce antibody proteins for testing.

[000162] The biological properties of exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Heavy and light chain variable region amino acid and nucleotide sequences

[000163] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs, as well as the heavy chain and light chain sequences, of exemplary anti-SARS-CoV-2-S antibodies. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs									
Antibody Designation	**HCVR**	**HCDR 1**	**HCDR 2**	**HCDR 3**	**LCVR**	**LCDR 1**	**LCDR 2**	**LCDR 3**	**HC**	**LC**

mAb10913	2	4	6	8	10	12	14	16	18	20
mAb10915	22	24	26	28	30	32	34	36	38	40
mAb10916	2	4	6	8	10	12	14	16	42	20
mAb10917	44	46	26	49	51	53	55	57	59	61
mAb10918	22	24	26	28	30	32	34	36	63	40
mAb10920	65	67	69	71	73	75	55	77	79	81
mAb10921	83	85	26	87	89	91	55	93	95	97
mAb10922	99	101	103	105	107	109	111	113	115	117
mAb10923	119	121	123	125	127	129	55	131	133	135
mAb10924	137	139	141	143	145	147	149	151	153	155
mAb10925	65	67	69	71	73	75	55	77	157	81
mAb10926	83	85	26	87	89	91	55	93	159	97
mAb10927	99	101	103	105	107	109	111	113	161	117
mAb10928	119	121	123	125	127	129	55	131	163	135
mAb10929	137	139	141	143	145	147	149	151	165	155
mAb10930	167	169	171	173	175	129	55	177	179	181
mAb10931	167	169	171	173	175	129	55	177	183	181
mAb10932	185	187	26	189	191	75	194	196	198	200
mAb10933	202	204	206	208	210	212	55	214	216	218
mAb10934	220	222	224	226	228	230	194	232	234	236
mAb10935	238	24	26	240	242	244	194	246	248	250
mAb10936	252	254	256	258	260	129	55	262	264	266
mAb10937	268	270	272	274	276	129	55	278	280	282
mAb10940	284	169	286	288	290	292	294	296	298	300
mAb10938	302	24	26	304	306	308	194	310	312	314
mAb10939	316	187	319	321	323	325	55	327	329	331
mAb10941	333	85	26	336	338	340	294	296	342	344
mAb10942	185	187	26	189	191	75	194	196	346	200
mAb10943	202	204	206	208	210	212	55	214	348	218
mAb10944	220	222	224	226	228	230	194	232	350	236
mAb10945	238	24	26	240	242	244	194	246	352	250
mAb10946	252	254	256	258	260	129	55	262	354	266
mAb10947	268	270	272	274	276	129	55	278	356	282
mAb10948	302	24	26	304	306	308	194	310	358	314
mAb10949	316	187	319	321	323	325	55	327	360	331
mAb10951	333	85	26	336	338	340	294	296	362	344
mAb10950	284	169	286	288	290	292	294	296	364	300
mAb10954	366	85	26	370	372	244	194	375	377	379
mAb10955	381	383	26	385	387	389	194	310	392	394

mAb10956	396	187	26	399	401	389	194	403	405	407
mAb10957	409	411	26	414	416	53	55	418	420	422
mAb10958	366	85	26	370	372	244	194	375	424	379
mAb10959	381	383	26	385	387	389	194	310	426	394
mAb10960	396	187	26	399	401	389	194	403	428	407
mAb10961	409	411	26	414	416	53	55	418	430	422
mAb10964	432	434	436	438	440	442	55	445	447	449
mAb10965	451	453	26	455	457	459	34	462	464	466
mAb10966	468	187	26	470	472	389	194	474	476	478
mAb10967	480	24	483	485	487	389	194	489	491	493
mAb10969	495	497	499	501	503	389	194	214	506	508
mAb10970	510	24	26	512	514	516	194	518	520	522
mAb10971	524	411	26	528	530	532	55	534	536	538
mAb10973	432	434	436	438	440	442	55	445	540	449
mAb10974	451	453	26	455	457	459	34	462	542	466
mAb10975	468	187	26	470	472	389	194	474	544	478
mAb10976	480	24	483	485	487	389	194	489	546	493
mAb10977	548	550	552	554	556	558	294	560	562	564
mAb10978	495	497	499	501	503	389	194	214	566	508
mAb10979	510	24	26	512	514	516	194	518	568	522
mAb10980	524	411	26	528	530	532	55	534	570	538
mAb10981	548	550	552	554	556	558	294	560	572	564
mAb10982	574	187	576	578	580	582	584	586	588	590
mAb10983	574	187	576	578	580	582	584	586	592	590
mAb10984	594	596	26	598	600	12	14	602	604	606
mAb10985	608	169	610	612	614	616	584	618	620	622
mAb10986	624	626	26	628	630	582	632	634	636	638
mAb10987	640	642	499	644	646	648	650	652	654	656
mAb10988	658	660	662	664	666	668	670	672	674	676
mAb10989	678	680	682	684	686	688	650	690	692	694
mAb10990	594	596	26	598	600	12	14	602	696	606
mAb10991	608	169	610	612	614	616	584	618	698	622
mAb10992	624	626	26	628	630	582	632	634	700	638
mAb10993	640	642	499	644	646	648	650	652	702	656
mAb10994	658	660	662	664	666	668	670	672	704	676
mAb10995	678	680	682	684	686	688	650	690	706	694
mAb10996	708	24	26	711	713	129	55	715	717	719
mAb10997	708	24	26	711	713	129	55	715	721	719
mAb10998	723	187	26	725	727	129	55	729	731	733

mAb10999	723	187	26	725	727	129	55	729	735	733
mAb11000	737	24	26	739	741	743	55	745	747	749
mAb11001	737	24	26	739	741	743	55	745	751	749
mAb11002	753	24	26	755	713	129	55	715	757	719
mAb11003	753	24	26	755	713	129	55	715	759	719
mAb10914	44	46	26	49	51	53	55	57	762	61
mAb11004	764	766	499	768	770	91	55	772	774	776
mAb11005	764	766	499	768	770	91	55	772	778	776
mAb11006	780	782	26	784	786	53	55	788	790	792
mAb11007	780	782	26	784	786	53	55	788	794	792
mAb11008	796	24	26	798	800	53	55	802	804	806
mAb11009	796	24	26	798	800	53	55	802	808	806
mAb11010	810	812	814	816	818	129	820	822	824	826
mAb11011	810	812	814	816	818	129	820	822	828	826

Table 2: Nucleic Acid Sequence Identifiers

	SEQ ID NOs									
Antibody Designation	**HCVR**	**HCDR 1**	**HCDR 2**	**HCDR 3**	**LCVR**	**LCDR 1**	**LCDR 2**	**LCDR 3**	**HC**	**LC**
mAb10913	1	3	5	7	9	11	13	15	17	19
mAb10915	21	23	25	27	29	31	33	35	37	39
mAb10916	1	3	5	7	9	11	13	15	41	19
mAb10917	43	45	47	48	50	52	54	56	58	60
mAb10918	21	23	25	27	29	31	33	35	62	39
mAb10920	64	66	68	70	72	74	54	76	78	80
mAb10921	82	84	47	86	88	90	54	92	94	96
mAb10922	98	100	102	104	106	108	110	112	114	116
mAb10923	118	120	122	124	126	128	54	130	132	134
mAb10924	136	138	140	142	144	146	148	150	152	154
mAb10925	64	66	68	70	72	74	54	76	156	80
mAb10926	82	84	47	86	88	90	54	92	158	96
mAb10927	98	100	102	104	106	108	110	112	160	116
mAb10928	118	120	122	124	126	128	54	130	162	134
mAb10929	136	138	140	142	144	146	148	150	164	154
mAb10930	166	168	170	172	174	128	54	176	178	180
mAb10931	166	168	170	172	174	128	54	176	182	180
mAb10932	184	186	47	188	190	192	193	195	197	199
mAb10933	201	203	205	207	209	211	54	213	215	217

mAb10934	219	221	223	225	227	229	193	231	233	235
mAb10935	237	23	47	239	241	243	193	245	247	249
mAb10936	251	253	255	257	259	128	54	261	263	265
mAb10937	267	269	271	273	275	128	54	277	279	281
mAb10940	283	168	285	287	289	291	293	295	297	299
mAb10938	301	23	47	303	305	307	193	309	311	313
mAb10939	315	317	318	320	322	324	54	326	328	330
mAb10941	332	334	47	335	337	339	293	295	341	343
mAb10942	184	186	47	188	190	192	193	195	345	199
mAb10943	201	203	205	207	209	211	54	213	347	217
mAb10944	219	221	223	225	227	229	193	231	349	235
mAb10945	237	23	47	239	241	243	193	245	351	249
mAb10946	251	253	255	257	259	128	54	261	353	265
mAb10947	267	269	271	273	275	128	54	277	355	281
mAb10948	301	23	47	303	305	307	193	309	357	313
mAb10949	315	317	318	320	322	324	54	326	359	330
mAb10951	332	334	47	335	337	339	293	295	361	343
mAb10950	283	168	285	287	289	291	293	295	363	299
mAb10954	365	367	368	369	371	373	193	374	376	378
mAb10955	380	382	47	384	386	388	193	390	391	393
mAb10956	395	397	47	398	400	388	193	402	404	406
mAb10957	408	410	412	413	415	52	54	417	419	421
mAb10958	365	367	368	369	371	373	193	374	423	378
mAb10959	380	382	47	384	386	388	193	390	425	393
mAb10960	395	397	47	398	400	388	193	402	427	406
mAb10961	408	410	412	413	415	52	54	417	429	421
mAb10964	431	433	435	437	439	441	443	444	446	448
mAb10965	450	452	47	454	456	458	460	461	463	465
mAb10966	467	397	412	469	471	388	193	473	475	477
mAb10967	479	481	482	484	486	388	193	488	490	492
mAb10969	494	496	498	500	502	388	193	504	505	507
mAb10970	509	481	412	511	513	515	193	517	519	521
mAb10971	523	525	526	527	529	531	54	533	535	537
mAb10973	431	433	435	437	439	441	443	444	539	448
mAb10974	450	452	47	454	456	458	460	461	541	465
mAb10975	467	397	412	469	471	388	193	473	543	477
mAb10976	479	481	482	484	486	388	193	488	545	492
mAb10977	547	549	551	553	555	557	293	559	561	563
mAb10978	494	496	498	500	502	388	193	504	565	507

mAb10979	509	481	412	511	513	515	193	517	567	521
mAb10980	523	525	526	527	529	531	54	533	569	537
mAb10981	547	549	551	553	555	557	293	559	571	563
mAb10982	573	186	575	577	579	581	583	585	587	589
mAb10983	573	186	575	577	579	581	583	585	591	589
mAb10984	593	595	47	597	599	11	13	601	603	605
mAb10985	607	168	609	611	613	615	583	617	619	621
mAb10986	623	625	47	627	629	581	631	633	635	637
mAb10987	639	641	498	643	645	647	649	651	653	655
mAb10988	657	659	661	663	665	667	669	671	673	675
mAb10989	677	679	681	683	685	687	649	689	691	693
mAb10990	593	595	47	597	599	11	13	601	695	605
mAb10991	607	168	609	611	613	615	583	617	697	621
mAb10992	623	625	47	627	629	581	631	633	699	637
mAb10993	639	641	498	643	645	647	649	651	701	655
mAb10994	657	659	661	663	665	667	669	671	703	675
mAb10995	677	679	681	683	685	687	649	689	705	693
mAb10996	707	709	47	710	712	128	54	714	716	718
mAb10997	707	709	47	710	712	128	54	714	720	718
mAb10998	722	186	47	724	726	128	54	728	730	732
mAb10999	722	186	47	724	726	128	54	728	734	732
mAb11000	736	23	47	738	740	742	54	744	746	748
mAb11001	736	23	47	738	740	742	54	744	750	748
mAb11002	752	23	47	754	712	128	54	714	756	718
mAb11003	752	23	47	754	712	128	54	714	758	718
mAb10914	760	45	47	48	50	52	54	56	761	60
mAb11004	763	765	498	767	769	90	54	771	773	775
mAb11005	763	765	498	767	769	90	54	771	777	775
mAb11006	779	781	47	783	785	52	54	787	789	791
mAb11007	779	781	47	783	785	52	54	787	793	791
mAb11008	795	709	47	797	799	52	54	801	803	805
mAb11009	795	709	47	797	799	52	54	801	807	805
mAb11010	809	811	813	815	817	128	819	821	823	825
mAb11011	809	811	813	815	817	128	819	821	827	825

[000164] Antibodies disclosed herein have fully human variable regions but can have mouse constant regions (e.g., a mouse IgG1 Fc or a mouse IgG2 Fc (a or b isotype)) or human constant regions (e.g., a human IgG1 Fc or a human IgG4 Fc). As will be appreciated by a person of

ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 1 and 2 will remain the same, and the binding properties to antigen are expected to be identical or substantially similar regardless of the nature of the constant domain.

[000165] The variable regions of antibodies derived from VELOCIMMUNE® mice and from human samples were sequenced by Next Generation Sequencing and the repertoire for heavy and light chain pairs was identified (Figure 10A and Figure 10B). The predominant lineage of VI antibodies utilized VH3-53 paired with VK1-9, VK1-33, or VK1-39 while human-derived antibodies utilized VH3-66 paired with VK1-33 or VH2-70 paired with VK1-39. Further analysis of overlaid sequences showed strong overlap in the repertoire of isolated kappa chains between VI and human-derived antibodies. Although the repertoire of Lambda chains did not overlap well, that may be due to only two lambda mice being included in this trial. The average CDR length for heavy chain was similar between VI and human derived antibodies with an average length of 13 and 14.5 amino acids, respectively. Average kappa CDR length was the same for VI and human derived antibodies at 9 amino acids and was close for lambda chains with an average length of 11.1 and 10.6 amino acids, respectively. Availability of humanized mouse and human-derived antibodies allowed for more diversity of V genes and enabled the later identification of noncompeting antibodies.

[000166] As described above, the antibodies were obtained from hybridomas generated from VELOCIMMUNE® mice, by direct isolation from antigen-positive VELOCIMMUNE® mouse B cells, or derived from variable regions cloned from antigen-positive human B cells. A summary of these sources is shown in Table 3.

Table 3: Antibody/Variable Region sources

Antibody	**Source**
mAb10913	mouse B cells
mAb10915	mouse B cells
mAb10916	mouse B cells

mAb10917	mouse B cells
mAb10918	mouse B cells
mAb10920	mouse B cells
mAb10921	mouse B cells
mAb10922	mouse B cells
mAb10923	mouse B cells
mAb10924	mouse B cells
mAb10925	mouse B cells
mAb10926	mouse B cells
mAb10927	mouse B cells
mAb10928	mouse B cells
mAb10929	mouse B cells
mAb10930	mouse B cells
mAb10931	mouse B cells
mAb10932	mouse B cells
mAb10933	mouse B cells
mAb10934	mouse B cells
mAb10935	mouse B cells
mAb10936	mouse B cells
mAb10937	mouse B cells
mAb10940	mouse B cells
mAb10938	mouse B cells
mAb10939	mouse B cells
mAb10941	mouse B cells

mAb10942	mouse B cells
mAb10943	mouse B cells
mAb10944	mouse B cells
mAb10945	mouse B cells
mAb10946	mouse B cells
mAb10947	mouse B cells
mAb10948	mouse B cells
mAb10949	mouse B cells
mAb10951	mouse B cells
mAb10950	mouse B cells
mAb10954	human B cells
mAb10955	human B cells
mAb10956	human B cells
mAb10957	human B cells
mAb10958	human B cells
mAb10959	human B cells
mAb10960	human B cells
mAb10961	human B cells
mAb10964	human B cells
mAb10965	human B cells
mAb10966	human B cells
mAb10967	human B cells
mAb10969	human B cells
mAb10970	human B cells

mAb10971	human B cells
mAb10973	human B cells
mAb10974	human B cells
mAb10975	human B cells
mAb10976	human B cells
mAb10977	human B cells
mAb10978	human B cells
mAb10979	human B cells
mAb10980	human B cells
mAb10981	human B cells
mAb10982	mouse B cells
mAb10983	mouse B cells
mAb10984	human B cells
mAb10985	human B cells
mAb10986	human B cells
mAb10987	human B cells
mAb10988	human B cells
mAb10989	human B cells
mAb10990	human B cells
mAb10991	human B cells
mAb10992	human B cells
mAb10993	human B cells
mAb10994	human B cells
mAb10995	human B cells

mAb10996	hybridoma
mAb10997	hybridoma
mAb10998	hybridoma
mAb10999	hybridoma
mAb11000	hybridoma
mAb11001	hybridoma
mAb11002	hybridoma
mAb11003	hybridoma
mAb10914	mouse B cells
mAb11004	hybridoma
mAb11005	hybridoma
mAb11006	hybridoma
mAb11007	hybridoma
mAb11008	hybridoma
mAb11009	hybridoma
mAb11010	hybridoma
mAb11011	hybridoma

Example 3: Characterization of hybridoma supernatants by binding ELISA

[000167] An ELISA binding assay was performed to identify antibody supernatants that bound to the SARS-CoV-2-Spike protein receptor binding domain (RBD). A protein composed of the RBD of SARS-CoV-2 (amino acids 319-541) expressed with a 6X histidine tag and two myc epitope tags at the C-terminus (SARS-CoV-2-S-RBD-mmH; see also NCBI Accession Number MN908947.3) was coated at 1 μg/ml on a 96-well plate in PBS buffer overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. Antibody supernatants or media alone were diluted 1:40 or 1 :50 in the PSA+0.5% BSA

blocking buffer and transferred to the washed microtiter plates. After one hour of incubation at room temperature, the wells were washed, and plate-bound supernatant was detected with either goat-anti-human IgG antibody conjugated with horseradish peroxidase (HRP) (Jackson Immunoresearch), or anti-mouse IgG antibody conjugated with horseradish peroxidase (HRP) (Jackson Immunoresearch). The plates were then developed using TMB substrate solution (BD Biosciences) according to manufacturer's recommendation and absorbance at 450nm was measured on a Victor X5 plate reader.

[000168] The ability of anti-SARS-CoV-2-S antibodies to bind the receptor binding domain of SARS-CoV-2-S (SARS-CoV-2-S-RBD) was assessed, as described above, using a binding ELISA with the SARS-CoV-2-S-RBD-mmH protein coated on a microplate. Single point antibody supernatant binding to SARS-COV-2-S-RBD-mmH coated on 96-well microtiter plates was detected with an HRP conjugated anti-hFc or anti-mFc antibody.

[000169] The binding results of three trials are summarized in Table 4. The SARS-CoV-2 binding signals (absorbance 450nm) are indicated, with the media only background provided as a negative reference per experiment. A sample marked IC (Inconclusive) had an experimental anomaly to the plate and is therefore reported without a value. As shown in comparison to the media only control, the supernatants tested showed substantial binding to the SARS-CoV-2-S-RBD.

Table 4: Supernatant binding to SARS-CoV-2 spike protein receptor binding domain

Supernatant	Supernatant Dilution	Detection Antibody	Binding Signal (absorbance at 450nm)
mAb10913	1:50	a-hFc	2.752
mAb10914	1:50	a-hFc	2.857
mAb10915	1:50	a-hFc	2.76
mAb10932	1:50	a-hFc	2.718
mAb10933	1:50	a-hFc	2.762
mAb10934	1:50	a-hFc	2.688
mAb10935	1:50	a-hFc	2.676
mAb10936	1:50	a-hFc	2.644
mAb10937	1:50	a-hFc	2.664
mAb10920	1:50	a-hFc	2.683

mAb10921	1:50	a-hFc	2.633
mAb10922	1:50	a-hFc	2.595
mAb10923	1:50	a-hFc	2.353
mAb10924	1:50	a-hFc	2.269
mAb10930	1:50	a-hFc	2.451
mAb10938	1:50	a-hFc	2.536
mAb10939	1:50	a-hFc	2.516
mAb10940	1:50	a-hFc	2.77
mAb10941	1:50	a-hFc	IC
mAb10982	1:50	a-hFc	2.537
mAb10984	1:50	a-hFc	0.716
mAb10985	1:50	a-hFc	2.35
mAb10986	1:50	a-hFc	2.331
mAb10987	1:50	a-hFc	2.438
mAb10988	1:50	a-hFc	3.062
mAb10989	1:50	a-hFc	3.116
mAb10969	1:50	a-hFc	2.629
mAb10970	1:50	a-hFc	2.807
mAb10971	1:50	a-hFc	3.052
mAb10964	1:50	a-hFc	3.086
mAb10965	1:50	a-hFc	2.918
mAb10966	1:50	a-hFc	0.421
mAb10967	1:50	a-hFc	1.732
mAb10954	1:50	a-hFc	1.963
mAb10955	1:50	a-hFc	2.469
mAb10956	1:50	a-hFc	2.6
mAb10957	1:50	a-hFc	2.49
mAb10977	1:50	a-hFc	2.925
mAb11010	1:40	a-mFc	2.896
mAb11004	1:40	a-mFc	2.908
mAb11000	1:40	a-mFc	2.725
mAb11006	1:40	a-mFc	2.619
mAb11008	1:40	a-mFc	2.907
mAb10998	1:40	a-mFc	2.835
mAb10996	1:40	a-mFc	2.826
mAb11002	1:40	a-mFc	2.581
Media only	1:50	a-hFc	0.069
Media only	1:40	a-mFc	0.058

Media only	1:50	a-hFc	0.055

Example 4: Antibody binding to SARS-CoV-2-S-expressing virus-like particle

[000170] To investigate the ability of a panel of anti-SARS-CoV-2-S monoclonal antibodies to bind the SARS-CoV-2 spike glycoprotein, an in vitro binding assay utilizing SARS-CoV-2 spike protein-expressing viral-like particles (VLPs) in an electrochemiluminescence based detection platform (MSD) was developed.

[000171] To transiently express the SARS-CoV-2 spike protein (NCBI Accession number MN908947.3, amino acids 16-1211; SEQ ID NO: 833), Vesicular stomatitis virus (VSV) lacking glycoprotein G (VSV delta G) was pseudotyped with SARS-CoV-2 spike protein (VSV-SARS-CoV-2-S) and generated in HEK293T cells. As a negative binding control, VSV delta G was pseudotyped with VSV G protein (VSV-G).

[000172] Experiments were carried out according to following procedure. The two types of VLPs described above were diluted in PBS, seeded into 96-well carbon electrode plates (MULTI-ARRAY high bind plate, MSD), and incubated overnight at 4 °C to allow the VLPs to adhere. Nonspecific binding sites were blocked by 2% BSA (w/v) in PBS for 1 hour at room temperature. Supernatants containing antibodies produced from SARS CoV-2-immunized mice or infected human sera, along with media-only controls which were diluted 1:10 or 1:20 in 1x PBS + 0.5% BSA buffer, were added to the plate-bound particles. The plates were then incubated for 1 hour at room temperature with shaking, after which the plates were washed with 1x PBS to remove the unbound antibodies using an AquaMax2000 plate washer (MDS Analytical Technologies). The plate-bound antibodies were detected with a SULFO-TAGTM-conjugated anti-human IgG antibody (Jackson Immunoresearch) or a SULFO-TAGTM-conjugated anti-mouse IgG antibody (Jackson Immunoresearch) for 1 hour at room temperature. After washes, the plates were developed with the Read Buffer (MSD) according to manufacturer's recommended procedure and the luminescent signals were recorded with a SECTOR Imager 600 (Meso Scale Development) instrument. Direct binding signals (in RLU) were captured, and a ratio of SARS-CoV-2-S-expressing VLPs to the irrelevant VLP was calculated.

[000173] The ability of the anti-SARS-CoV-2-S monoclonal antibodies to bind to SARS-CoV-2-S-expressing VLPs compared with binding to irrelevant VSV-expressing VLPs was assessed

using an immunobinding assay, as described above. Single-point binding to the immobilized VLPs on 96-well High Bind plates (MSD) was performed with an antibody supernatant dilution of 1:10 or 1:20, bound for 1 hour, and detected using SULFO-TAGTM-conjugated anti-human IgG or anti-mouse IgG antibody. The binding signals from electrochemiluminescence were recorded on a Sector Imager 600 (MSD). RLU values were determined for the antibody binding to VLPs. Ratios were calculated comparing the SARS-CoV-2-S-expressing VLP binding signals to control VLPs.

[000174] The binding results from three experiments are summarized in Table 5. A signal observed from SARS-COV-2-S-expressing VLPs indicates binding, while comparison with negative VLPs provides a relative background. Media alone samples provide baseline signals of secondary antibody binding to samples with no supernatant. The 46 antibodies bound specifically at >4-fold higher than the media-only samples (20-35 RLU) on the SARS-CoV-2-S-expressing VLPs, with a range of binding signals from 85-13,600 RLU. The ratios of SARS-CoV-2-S-expressing VSV: VSV-VLPs (negative control) ranged from 1.1- 22.7, with many having high background on VSV-VLPs. The ratio of mAb11002 of 0.9 is likely due to a low concentration of monoclonal antibody in the supernatant sample.

Table 5: SARS-CoV-2-S VLP binding

Supernatant	Supernatant Dilution	Secondary Detection Antibody	VSV-VLP Binding Signal (RLU)	VSV-SARS-CoV-2-S VLP Binding Signal (RLU)	Ratio of Binding Signals: VSV-SARS-CoV-2-S/VSV-VLP
mAb10913	1:10	a-hFc	2155	3244	1.5
mAb10914	1:10	a-hFc	3885	5181	1.3
mAb10915	1:10	a-hFc	980	9022	9.2
mAb10932	1:10	a-hFc	989	10451	10.6
mAb10933	1:10	a-hFc	507	966	1.9

mAb10934	1:10	a-hFc	3876	5041	1.3
mAb10935	1:10	a-hFc	2087	3867	1.9
mAb10936	1:10	a-hFc	2325	8076	3.5
mAb10937	1:10	a-hFc	1404	1920	1.4
mAb10920	1:10	a-hFc	8366	10041	1.2
mAb10921	1:10	a-hFc	1194	5436	4.6
mAb10922	1:10	a-hFc	1473	2229	1.5
mAb10923	1:10	a-hFc	1224	1859	1.5
mAb10924	1:10	a-hFc	487	969	2
mAb10930	1:10	a-hFc	1769	3207	1.8
mAb10938	1:10	a-hFc	1232	6623	5.4
mAb10939	1:10	a-hFc	1777	5074	2.9
mAb10940	1:10	a-hFc	606	2072	3.4
mAb10941	1:10	a-hFc	673	4588	6.8
mAb10982	1:10	a-hFc	1178	2016	1.7
mAb10984	1:10	a-hFc	2486	8989	3.6
mAb10985	1:10	a-hFc	2049	3279	1.6
mAb10986	1:10	a-hFc	2044	10831	5.3
mAb10987	1:10	a-hFc	1839	2450	1.3
mAb10988	1:10	a-hFc	1832	2305	1.3

mAb10989	1:10	a-hFc	672	1999	3
mAb10969	1:10	a-hFc	3096	3313	1.1
mAb10970	1:10	a-hFc	1364	5712	4.2
mAb10971	1:10	a-hFc	1135	7266	6.4
mAb10964	1:10	a-hFc	1439	8601	6
mAb10965	1:10	a-hFc	743	1370	1.8
mAb10966	1:10	a-hFc	1428	6574	4.6
mAb10967	1:10	a-hFc	1446	9510	6.6
mAb10954	1:10	a-hFc	641	6308	9.8
mAb10955	1:10	a-hFc	932	1788	1.9
mAb10956	1:10	a-hFc	1030	1581	1.5
mAb10957	1:10	a-hFc	604	5544	9.2
mAb10977	1:10	a-hFc	4141	13600	3.3
mAb11010	1:20	a-mFc	96	363	3.8
mAb11004	1:20	a-mFc	110	406	3.7
mAb11000	1:20	a-mFc	333	592	1.8
mAb11006	1:20	a-mFc	165	3747	22.7
mAb11008	1:20	a-mFc	103	324	3.1
mAb10998	1:20	a-mFc	74	218	2.9
mAb10996	1:20	a-mFc	51	85	1.7

mAb11002	1:20	a-mFc	156	146	0.9
Media only	1:10	a-hFc	30	35	1.2
Media only	1:20	a-mFc	35	20	0.6
Media only	1:10	a-hFc	39	29	0.7

Example 5: Antibody neutralization of VSV-SARS-CoV-2-S pseudovirus infectivity

[000175] To investigate the ability of a panel of anti-SARS-CoV-2-S monoclonal antibodies to neutralize SARS-CoV-2, an in vitro neutralization assay utilizing VSV-SARS-CoV-2-S pseudovirus was developed.

[000176] As described above, VSV pseudotype viruses were generated by transiently transfecting 293T cells with a plasmid encoding for SARS-CoV-2 spike protein. Cells were seeded in 15 cm plates at $1.2x10^7$ cells per plate in DMEM complete media one day prior to transfection with 15 µg/plate spike protein DNA using 125 µL Lipofectamine LTX, 30 µL PLUS reagent, and up to 3 mL Opti-Mem. 24 hours post transfection, the cells were washed with 10 mL PBS, then infected with an MOI of 0.1 $VSV^{\Delta G:mNeon}$ virus in 10 mL Opti-Mem. Virus was incubated on cells for 1 hour, with gentle rocking every 10 minutes. Cells were washed 3 times with 10 mL PBS, then overlaid with 20 mL Infection media before incubation at 37 C, 5% CO_2 for 24 hours. Supernatant was collected into 250 mL centrifuge tubes on ice, then centrifuged at 3000 rpm for 5 minutes to pellet any cellular debris, aliquoted on ice, then frozen to -80 °C. Infectivity was tested on Vero cells prior to use in neutralization assays. This material will be referred to as VSV-SARS-CoV-2-S.

Neutralization assay with VSV-SARS-CoV-2-S

[000177] On day 1, Vero cells were seeded at 80% confluency in T225 flasks. To seed cells, media was removed from the cells, the cells were washed with 20mL PBS (Gibco: 20012-043), and 5mL TrypLE was added and incubated for ~5 minutes at 37 °C until the cells dislodged. 5 mL of complete DMEM was added to inactivate the trypsin, and pipetted up and down to distribute the cells. To count the resuspended cells, 20,000 Vero cells were plated in 100 µL prewarmed Complete DMEM per well in a 96 Well Black Polystyrene Microplate (Corning: 3904).

[000178] On day 2, VSV-SARS-CoV-2-S was thawed on ice and diluted 1:1 with infection media.

[000179] In a V-bottom 96 well plate, a dilution of each supernatant was generated in 60ul infection media. For media (negative) controls, 60 µl of diluted conditioned media was added to the wells. 60 µL of diluted VSV-SARS-CoV-2-S were added to every well except the media control wells. To those wells, 60 µL of infection media was added. Pseudoviruses were then incubated with supernatant dilutions for 30 minutes at room temperature. Media was removed from the Vero cell plates, 100 µL of supernatant/pseudovirus mixtures were transferred to the cells, and the plate was incubated at 37 °C, 5% CO_2 for 24 hours. The final supernatant dilutions of 1:4 and 1:20, and for some samples 1:100, were used to assess neutralization of VSV-SARS-CoV-2-S pseudoviruses.

[000180] On day 3, after the 24 hr incubation, supernatant was removed from the cell wells and replaced with 100 µL of PBS. The plates were then read on a SpectraMax i3 with MiniMax imaging cytometer.

[000181] The ability of the anti-SARS-CoV-2-S antibodies to neutralize VSV-based SARS-CoV-2-S-expressing pseudotyped virus was assessed using a neutralization fluorescence focus assay. The binding results of three assays are summarized below. The neutralization potency of antibody at each dilution is represented as a percentage compared to mock supernatant control. All antibodies demonstrated neutralization capacity, and particularly for the set of antibodies that were evaluated 1:100, those showing higher neutralization may represent more potent neutralization capacity.

Table 6: Neutralization of VLPs

Supernatant	**Neutralization (1:4 dilution)**	**Neutralization (1:20 dilution)**	**Neutralization (1:100 dilution)**
mAb10913	99.5	95.5	69.1
mAb10914	94.2	74.8	43.6
mAb10915	96.7	74.2	29.6

mAb10932	99.8	94.6	68
mAb10933	99.8	98.9	88.4
mAb10934	99.9	99.8	98.4
mAb10935	99.6	98.5	88.8
mAb10936	99.7	99.1	92.9
mAb10937	97.5	87.7	56.3
mAb10920	99.5	95.5	69.1
mAb10921	98.2	91.4	46.1
mAb10922	99.8	99.1	88.4
mAb10923	99.5	92.9	67.7
mAb10924	98.1	85.4	55.2
mAb10930	99.1	91.1	59
mAb10938	98.1	83	54.2
mAb10939	98.6	90.5	64
mAb10940	97	89.9	66.4
mAb10941	98.9	92.9	73.8
mAb10982	97.4	83.8	44.5
mAb10984	99.8	95.1	83.4
mAb10985	99.7	88.4	63.5
mAb10986	99.7	98	86

mAb10987	99.3	97.7	94.6
mAb10988	97.6	87.6	62.2
mAb10989	100	99.8	98.2
mAb10969	97.2	91	63.7
mAb10970	99.6	96.7	82.4
mAb10971	99.5	97	73.9
mAb10964	99.7	99.7	94.1
mAb10965	98.5	87.6	68.6
mAb10966	99.5	95.5	76.2
mAb10967	98.9	91.4	69.2
mAb10954	99.8	96	70.7
mAb10955	98.8	88.6	62.7
mAb10956	97.1	84.1	61.6
mAb10957	97.6	76.4	48
mAb10977	95.5	79	47.7
mAb11010	85	54	NT
mAb11004	77	40	NT
mAb11000	98	82	NT
mAb11006	91	54	NT
mAb11008	96	77	NT

mAb10998	88	59	NT
mAb10996	85	58	NT
mAb11002	35	-1	NT

*NT: not tested

Example 6: Characterization of antibodies in an antibody-dependent cell-mediated toxicity surrogate assay

[000182] The ability of antibodies targeting the spike protein of SARS-CoV-2 to interact with FcγR3a, an Fc-receptor prominently expressed on natural killer (NK) cells that induces antibody dependent cell-mediated cytotoxicity (ADCC), was measured in a surrogate bioassay using reporter cells and target cells bound to antibodies. This assay used Jurkat T cells that were engineered to express the reporter gene luciferase under the control of the transcription factor NFAT (NFAT-Luc) along with the high affinity human FcγR3a ^{176}Val allotype receptor (Jurkat/NFAT-Luc/hFcγR3a ^{176}Val). Target cells were engineered Jurkat T cells expressing human CD20 (used as a positive control with a CD20-targeting human IgG1 antibody) and the full-length SARS-CoV-2 spike protein controlled by a doxycycline-inducible promoter. Reporter cells were incubated with target cells and engagement of FcγR3a via the Fc domain of human IgG1 antibodies bound to target cells led to the activation of the transcription factor NFAT in the reporter cells and drove the expression of luciferase which was then measured via a luminescence readout.

[000183] Jurkat T cells were engineered to constitutively express full length human CD20 (amino acids M1-P297 of NCBI accession number NP_690605.1), Tet3G transactivator protein (cloned using a Takara pEF1α-Tet3G Vector, Catalog # 631167), as well as a doxycycline-inducible full-length SARS-CoV-2 spike protein (amino acids M1-T1273 of NCBI accession number YP_009724390.1). Engineered Jurkat/Tet3G/hCD20/SARS-CoV2 spike protein-expressing cells were sorted for high expression of the spike protein and subsequently maintained in RPMI + 10% Tet-free FBS + P/S/G + 500 µg/ml G418 + 1 µg/ml puromycin + 250 µg/ml hygromycin growth medium.

[000184] Jurkat T cells were engineered to stably express a Nuclear Factor of Activated T-cells (NFAT) luciferase reporter construct along with the high affinity human FcγR3a ^{176}Val

allotype receptor (amino acids M1-K254 of NCBI accession number P08637 VAR_003960). Engineered reporter cells were maintained in RPMI1640 + 10% FBS + P/S/G + 0.5 μg/ml puromycin + 500 μg/ml G418 growth media.

[000185] 36 hours prior to the start of the surrogate ADCC assay, 5 x 10^5 target cells/ml were induced in RPMI + 10% Tet-free FBS + P/S/G cell culture media containing 1 μg/ml doxycycline (Sigma). A day before the experiment, reporter cells were split to a density of 7.5 x 10^5 cells/ml in RPMI 1640 + 10% FBS + P/S/G + 0.5 μg/ml puromycin + 500 μg/ml G418 growth media.

[000186] Briefly, on the day of the experiment, the target and reporter cells were transferred into assay media (RPMI + 10% Tet-free FBS + P/S/G) and added at a 3:2 ratio (3 x 10^4/well target cells and 2 x 10^4/well reporter cells) to 384-well white microtiter plates, followed by the addition of anti-SARS-CoV-2-S antibody supernatant of varying concentrations. A positive control (CD20 antibody with human IgG1) sample and a negative control sample containing no antibody was included on each plate to normalize detected ADCC activities of anti-SARS-CoV-2-S antibody supernatants. Plates were incubated at 37 °C/5% CO_2 for 5 h followed by the addition of an equal volume of ONE-Glo™ (Promega) reagent to lyse cells and detect luciferase activity. The emitted light was captured in Relative Light Units (RLU) on a multi-label plate reader Envision (PerkinElmer), and data was analyzed and normalized using the following equation:

$$\text{ADCC activity (\%)} = 100 \times \frac{\text{Mean RLU (test samples) - Mean RLU (background signal))}}{\text{(Mean RLU (positive control) - Mean RLU (background signal))}}$$

[000187] The ability of anti-SARS-COV-2-S antibodies to activate FcγR3a receptors was evaluated in a surrogate ADCC assay using Jurkat/NFAT-Luc/FcγR3a ^{176}Val) as reporter cells and Jurkat/hCD20/SARS-CoV2 Spike as target cells. Each antibody tested contained an IgG1 domain.

[000188] Table 7 summarizes the results, showing the raw luciferase activity and the calculated % of positive control are indicated. A range of % ADCC activity was observed indicating FcγR3a activation by the antibody supernatants. All samples demonstrated some measure of surrogate ADCC activity, and 10 of the antibody supernatants demonstrated surrogate ADCC activity better than observed in positive controls.

Table 7: ADCC surrogate activity of anti-SARS-CoV-2-S antibody supernatants.

mAb	ADCC Mean RLU	ADCC (Activity (%)
mAb10913	11,480	111.9
mAb10914	21,960	265.8
mAb10915	14,280	153
mAb10932	13,020	108.8
mAb10933	9,740	68.5
mAb10934	11,680	92
mAb10935	11,540	90.4
mAb10936	15,160	133.8
mAb10937	12,340	100.1
mAb10920	15,480	137.8
mAb10921	10,080	67.7
mAb10922	9,140	56.3
mAb10923	13,340	107.1
mAb10924	7,220	33
mAb10930	8,900	53.4
mAb10938	12,960	102.5

mAb10939	9,440	59.7
mAb10940	12,520	106.2
mAb10941	10,340	77.2
mAb10982	7,900	59.4
mAb10984	6780	6.8
mAb10985	5840	2.8
mAb10986	6200	4.4
mAb10987	12020	29.4
mAb10988	7200	8.7
mAb10989	10200	21.5
mAb10969	10500	23.1
mAb10970	7640	10.6
mAb10971	7480	10
mAb10964	6380	5.1
mAb10965	6780	6.9
mAb10966	7080	10.4
mAb10967	6740	8.6
mAb10954	6940	9.8
mAb10955	6740	8.7
mAb10956	6760	8.8

mAb10957	7120	10.8
mAb10977	12980	33.8

Example 7: Anti-SARS-CoV-2-S antibody binding specificity assay

[000189] A Luminex binding assay was performed to determine the binding of anti-SARS-COV-2-S antibodies to a panel of antigens. For this assay, antigens were amine-coupled or captured by streptavidin to Luminex microspheres as follows: approximately 10 million MagPlex microspheres (Luminex Corp., MagPlex Microspheres, Cat. No. MC10000 and MC12000), were resuspended by vortexing in 500 µL 0.1M $NaPO_4$, pH 6.2 (activation buffer) and then centrifuged to remove the supernatant. Microspheres were protected from light, as they are light sensitive. The microspheres were resuspended in 160 µL of activation buffer and the carboxylate groups (-COOH) were activated by addition of 20 µL of 50 mg/mL of N-hydroxysuccinimide (NHS, Thermo Scientific, Cat. No. 24525) followed by addition of 20 µL of 50 mg/mL 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC, ThermoScientific, Cat. No. 22980) at 25 °C. After 10 minutes, the pH of the reaction was reduced to 5.0 with the addition of 600 µL 50 mM MES, pH 5 (coupling buffer), and the microspheres were vortexed and centrifuged to remove supernatant. The activated microspheres were immediately mixed with 500 µL of 25 µg/mL of the protein antigen or Streptavidin in coupling buffer and incubated for two hours at 25 °C. The coupling reaction was quenched by addition of 50 µL of 1M Tris-HCl, pH 8.0 and the microspheres were vortexed, centrifuged, and washed three times with 800 µL of PBS 0.005% (Tween20 0.05%), to remove uncoupled proteins and other reaction components. Microspheres were resuspended in 1 mL of PBS 2% BSA 0.05% Na Azide at 10 million microspheres/mL. For Streptavidin capture of antigens, 500 µL of 12.5 µg/mL of biotinylated protein in PBS was added to Streptavidin-coupled microspheres and incubated for one hour at 25 °C. Microspheres were vortexed, centrifuged, and washed three times with 800 µL of PBS, and then blocked using 500 µL 30mM Biotin (Millipore-Sigma, Cat. No. B4501) in 0.15M Tris pH 8.0. Microspheres were incubated for 30 minutes then vortexed, centrifuged, and washed three times with 800 µL of PBS. Microspheres were resuspended in 1 mL of PBS 2% BSA 0.05% Na Azide at 10 million microspheres/mL.

[000190] Microspheres for the different proteins and biotinylated proteins were mixed at 2700

beads/ml, and 75 µL of microspheres were plated per well on a 96 well ProcartaPlex flat bottom plate (ThermoFisher, Cat. No: EPX-44444-000) and mixed with 25 µL of individual anti-SARS-CoV-2 supernatant containing antibody. Samples and microspheres were incubated for two hours at 25°C and then washed twice with 200 µL of DPBS with 0.05% Tween 20. To detect bound antibody levels to individual microspheres, 100 µL of 2.5 µg/mL R-Phycoerythrin conjugated goat F(ab')2 anti-human kappa (Southern Biotech, Cat# 2063-09) in blocking buffer (for antibodies with murine Fc regions) or 100 µL of 1.25 µg/mL R-Phycoerythrin AffiniPure $F(ab')_2$ Fragment Goat Anti-Mouse IgG, $F(ab')_2$ Fragment Specific (Jackson Immunoresearch, Cat. No: 115-116-072) in blocking buffer (for antibodies with human Fc regions), was added and incubated for 30 minutes at 25 °C. After 30 minutes, the samples were washed twice with 200 µl of washing buffer and resuspended in 150 µL of wash buffer. The plates were read in a Luminex FlexMap 3D® (Luminex Corp.) and Luminex xPonent® software version 4.3 (Luminex Corp.). The SARS-CoV-2 proteins used in the assay are as follows:

RBD_ (R319-F541).mmh: SEQ ID NO: 829

RBD_ (R319-F541).mFc: SEQ ID NO: 830

RBD_ (R319-F541).hFc): SEQ ID NO: 831

[000191] The results of the Luminex binding are shown in Table 8 and Table 9 as median fluorescence intensity (MFI) signal intensities. The results show that the 46 anti-SARS-CoV-2-S antibody supernatants bound specifically to SARS-CoV-2-S RBD proteins. These results also show that five of these antibodies cross-react with SARS Coronavirus spike RBD proteins with binding signal greater than 1000 MFI.

Table 8: Binding signal (MFI) of SARS-CoV-2 Spike RBD, SARS-CoV-2 Spike S1, SARS RBD, SARS Spike S1, MERS Spike and MERS RBD proteins to anti-SARS-CoV-2 monoclonal antibodies (with hFc)

	SARS-CoV-2								
Supernatant	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	Bt-SARS-CoV-2 Spike Protein (RBD)(R319-F541).mFc	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mFc	Bt-SARS-CoV-2 Spike Protein (RBD)(R319-F541).hFc	SARS-CoV-2 Spike Protein (RBD)(R319-F541).hFc	SARS-CoV-2 Spike (RBD, Fc Tag) (Sino 40592-V05H)	SARS-CoV-2 Spike Protein (S1 Subunit, Fc Tag) (Sino 40591-V02H)	SARS-CoV-2 Spike Protein (S1 Subunit, His Tag) (Sino 40591-V08H)
mAb10913	30709	29247	16645	33023	27452	31929	31561	18899	24931
mAb10914	31967	29650	15986	30740	25957	30464	30591	14914	21609
mAb10915	31795	30293	20062	31772	30625	32437	31267	17595	22917
mAb10932	29984	30133	17697	30640	26220	30559	29880	17627	22099
mAb10933	33356	32090	19383	34944	30110	35106	34484	21178	27509
mAb10934	33797	32649	21238	34325	33016	35841	33636	20643	27483
mAb10935	34853	32603	19328	35886	31444	35611	35037	19991	25554
mAb10936	33947	32305	21636	33740	32810	33912	33613	19487	25187
mAb10937	33866	32225	19689	34233	31501	34624	33878	19553	26404
mAb10920	34842	34440	20254	36415	31708	36828	36277	21085	28516
mAb10921	24977	23596	11307	19429	18186	22306	21766	8959	12212
mAb10922	31768	30755	18629	32355	27854	33609	31376	18287	24678
mAb10923	35208	34289	19593	37372	33555	37756	36324	22502	28855
mAb10924	29730	27987	17044	28308	26898	28744	28423	15672	20577
mAb10930	25119	25131	16563	28560	25922	28870	28744	16530	21151
mAb10938	29409	27069	17205	30533	24638	29593	29134	15431	21163
mAb10939	32196	30883	18746	33900	28857	32864	32472	18171	23928
mAb10940	35221	35290	21000	35978	30675	36507	34945	21350	25807
mAb10941	32392	31171	20428	34061	28431	33347	33232	19668	26738
mAb10982	24263	22180	12278	23296	19935	23020	23066	10847	14017
mAb10984	27854	26197	17054	28350	22479	28442	27808	15590	19245

mAb10985	30214	27854	15488	29443	24827	31054	28936	16219	20787
mAb10986	27187	25196	15921	28407	23388	27693	27693	16034	19061
mAb10987	32171	29074	16736	33115	26059	32757	31238	17465	23089
mAb10988	23858	22160	12659	26095	21793	24822	23949	12910	16208
mAb10989	17687	17286	11189	19568	16117	22435	19316	12263	14234
mAb10969	29550	27587	15391	31386	26565	31042	30950	18466	23959
mAb10970	33154	31662	20184	34739	29182	34991	34704	21047	24625
mAb10971	29355	28850	16660	28746	24602	30032	29848	16986	21579
mAb10964	31754	28907	19225	32420	27736	33074	32317	18650	24154
mAb10965	30812	26863	13707	27139	23351	29618	28034	14133	18864
mAb10966	30939	27440	17905	30363	25115	30778	29869	16403	23308
mAb10967	28453	26496	16771	29650	24263	28660	28061	15776	21869
mAb10954	30410	28281	18394	31284	24677	31768	29604	16626	21270
mAb10955	29627	28476	16785	30790	24689	31227	31054	17858	22675
mAb10956	27900	25690	12891	28349	24505	30225	28810	15013	19981
mAb10957	23411	20615	10566	18692	16725	22560	20258	8451	11989
mAb10977	16770	14605	8845	13827	12774	15216	16783	6476	9406

Table 8 (cont'd)

	SARS			**MERS**							
Supernatant	Human SARS Coronavirus Spike Protein (Receptor Binding Domain) rabbit Fc (Sino 40150-V31B2)	Human SARS Coronavirus Spike Protein (Receptor Binding Domain, His Tag) (Sino 40150-V08B2)	Human SARS Coronavirus Spike S1 Subunit Protein (His Tag) (Sino 40150-V08B1)	MERS-CoV (SARS-CoV-2) Spike Protein (ECD, aa 1-1297, His Tag) (Sino	MERS-CoV (SARS-CoV-2) Spike Protein S2 (aa 726-1296, His Tag) (Sino	MERS-CoV (SARS-CoV-2) Spike Protein S1 (aa 1-725, His Tag) (Sino 40069-V08H)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein fragment (RBD, aa 367-606, His Tag) (Sino 40071-V08B1)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein S1 Protein (aa 1-725, His Tag) (Sino 40069-V08B1)	MERS.mFc (mAb2663-L1)	MERS.hFc (mAb2664-L1)	Bt-MERS.hFc (mAb2664-L2)

				40069-V08B)	40070-V08B)						
mAb10913	35	39	21	20	26	14	34	26	29	28	29
mAb10914	47	39	22	19	28	15	31	23	86	71	49
mAb10915	42	40	21	18	23	15	31	24	86	91	56
mAb10932	34	26	19	14	19	12	26	19	60	49	40
mAb10933	39	31	18	14	19	14	24	17	22	21	26
mAb10934	38	27	18	15	18	10	24	20	77	68	47
mAb10935	37	25	21	15	18	14	25	17	74	67	42
mAb10936	46	36	20	19	21	13	29	20	32	26	32
mAb10937	44	50	21	19	26	14	27	22	21	23	29
mAb10920	59	68	26	24	30	13	39	27	38	35	44
mAb10921	35	31	19	19	19	12	23	18	55	44	39
mAb10922	36	41	18	19	18	9	29	22	20	21	24
mAb10923	53	66	29	23	36	14	37	25	24	29	39
mAb10924	41	30	18	17	19	12	29	22	19	22	28
mAb10930	42	49	19	16	20	14	27	22	29	24	29
mAb10938	38	36	19	16	19	13	25	20	86	65	46
mAb10939	38	50	19	16	18	14	27	19	41	27	30
mAb10940	32	28	20	15	18	11	22	19	18	21	25
mAb10941	45	37	22	19	22	15	30	24	82	69	47
mAb10982	30	54	24	17	21	13	29	20	64	60	42
mAb10984	33	31	22	21	25	13	29	20	237	341	172
mAb10985	31537	32343	22721	18	28	14	31	22	168	195	159
mAb10986	39	38	21	15	19	14	27	20	233	286	184
mAb10987	33	27	22	15	23	15	28	23	196	235	172
mAb10988	41	67	25	17	29	14	32	25	169	181	130
mAb10989	47	73	21	16	22	11	24	19	161	206	186

mAb10969	37	34	20	16	20	11	26	19	21	22	29
mAb10970	38	25	19	14	16	15	23	17	35	23	28
mAb10971	32	31	20	15	13	13	20	19	44	29	24
mAb10964	19999	23855	5186	15	17	13	20	19	19	22	26
mAb10965	30	23	16	19	20	12	26	23	58	53	43
mAb10966	35	21	20	16	16	12	24	16	56	53	42
mAb10967	35	30	21	17	19	13	23	21	61	71	45
mAb10954	30	26	15	17	16	10	18	21	57	61	41
mAb10955	36	21	14	15	18	16	20	19	57	48	42
mAb10956	32	24	16	15	16	13	22	24	58	49	41
mAb10957	32	22	16	15	18	11	22	19	40	29	28
mAb10977	36	28	23	17	19	13	24	21	17	20	25

Table 9: Binding signal (MFI) of SARS-CoV-2 RBD, SARS-CoV-2 Spike S1, SARS RBD, SARS Spike S1, MERS SPIKE and MERS RBD proteins to anti-SARS-CoV-2-S monoclonal antibodies (with mFc)

	SARS-CoV-2								
Supernatant	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH (mAb10620-L1)	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH (mAb10620-L2)	Bt-SARS-CoV-2 Spike Protein (RBD)(R319-F541).mFc (mAb10621-L2)	SARS-CoV-2 Spike Protein (RBD)(R319-F541).mFc (mAb10621-L1)	Bt-SARS-CoV-2 Spike Protein (RBD)(R319-F541).hFc (mAb10622-L2)	SARS-CoV-2 Spike Protein (RBD)(R319-F541).hFc (mAb10622-L1)	SARS-CoV-2 Spike (RBD, Fc Tag) (Sino 40592-V05H)	SARS-CoV-2 (2019-nCoV) Spike Protein (S1 Subunit, Fc Tag) (Sino 40591-V02H)	SARS-CoV-2 (2019-nCoV) Spike Protein (S1 Subunit, His Tag) (Sino 40591-V08H) t
mAb11010	11024	12885	9349	14432	15688	8880	9628	5136	10794
mAb11004	3350	11337	4299	4583	7625	4877	6905	4482	9526
mAb11000	17802	10971	11335	23007	11593	22316	5671	9356	5415
mAb11006	5134	4744	1396	2866	3812	3985	3749	2052	1037
mAb11008	4047	3178	3047	4260	4106	2570	2311	6880	1419
mAb10998	1847	3837	2228	2230	467	1740	2005	724	717
mAb10996	9142	2906	4319	8738	5398	2084	16101	1425	6232
mAb11002	11558	10181	2197	9530	5471	9382	8461	1107	2867

Table 9 (cont'd)

SARS	MERS

Supernatant	Human SARS Coronavirus Spike Protein (Receptor Binding Domain) rabbit Fc (Sino 40150-V31B2)	Human SARS Coronavirus Spike Protein (Receptor Binding Domain, His Tag) (Sino 40150-V08B2)	Human SARS Coronavirus Spike S1 Subunit Protein (His Tag) (Sino 40150-V08B1)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein (ECD, aa 1-1297, His Tag) (Sino 40069-V08B)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein S2 (aa 726-1296, His Tag) (Sino 40070-V08B)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein S1 (aa 1-725, His Tag) (Sino 40069-V08H)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein fragment (RBD, aa 367-606, His Tag) (Sino 40071-V08B1)	MERS-CoV (NCoV / Novel coronavirus) Spike Protein S1 Protein (aa 1-725, His Tag) (Sino 40069-V08B1)	MERS mFc (mAb 2663-L1)	MER .hFc (mAb 2664-L1)	Bt-MERS. hFc (mAb 2664-L2)
mAb11010	18276	16793	7421	7	14	14	19	18	134	28	28
mAb11004	5524	740	33	15	20	12	26	17	228	24	25
mAb11000	39	31	18	13	19	9	27	17	384	82	49
mAb11006	615	667	339	18	17	13	15	18	156	16	24
mAb11008	120	174	31	18	16	15	20	18	45	19	32
mAb10998	29	37	16	19	18	14	24	19	48	29	32
mAb10996	1355	1279	28	13	21	14	26	18	185	132	95
mAb11002	80	56	31	10	22	13	25	18	288	52	32

Example 8: Anti-SARS-CoV-2-S antibody diversity assay

[000192] A binding assay was performed to determine the binding profile of anti-SARS-COV-2-S antibodies. For this assay, antigens were amine coupled as described for the Luminex binding assay above. Briefly, approximately 9 million MagPlex microspheres for 16 different bead regions (Luminex Corp., MagPLex Microspheres, Cat. No. MagPLex MC10000 and MC12000), were resuspended by vortexing in 500 µL 0.1M $NaPO_4$, pH 6.2 and then centrifuged to remove the supernatant. The microspheres were resuspended in 160 µL of activation buffer and the carboxylate groups (-COOH) were activated by addition of 20 µL of 50 mg/mL of N-hydroxysuccinimide (NHS, Thermo Scientific, Cat# 24525) followed by addition of 20 µL of 50 mg/mL of 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC, ThermoScientific, Cat# 22980) at 25°C. After 10 minutes, the pH of the reaction was reduced to 5.0 with the addition of 600 µL of 50 mM MES, pH 5 (coupling buffer), and the microspheres were vortexed and centrifuged to remove supernatant. The activated microspheres were immediately mixed with 500 µL of 20 µg/mL of SARS-CoV-2 Spike Protein (RBD)(R319-F541)-mmH in coupling buffer and incubated for two hours at 25°C. The coupling reaction was quenched by addition of 50 µL of 1M Tris-HCl, pH 8.0 and the microspheres were vortexed, centrifuged, and washed three times with 1000 µL of PBS. Microspheres were resuspended in 250 µL of PBS at 9 million microspheres/mL.

[000193] 15 out of the 16 microsphere regions with amine-coupled protein were modified for the binning assay as follows: microspheres were washed twice with PBS 5% DMSO, and 500 µl of a chemical or enzyme were dissolved per manufacturing recommendations and added at 10 nM to the amine-coupled microspheres described above. This was subsequently vortexed and incubated for 2 hours at room temperature with rotation. Wash microspheres 3 times with PBS 2% BSA. Microspheres were resuspended in 1 mL of PBS at 9 million microspheres/mL.

[000194] Protein-modified and protein-unmodified (intact) microspheres were mixed at 2700 beads/ml, and 75 µL of microspheres were plated per well on a 96 well ProcartaPlex 96 well flat bottom plate (ThermoFisher, Cat. No: EPX-44444-000) and mixed with 25 µL of individual anti-SARS-CoV-2-S supernatant-containing antibody. Samples and microspheres were incubated for two hours at 25 °C and then washed twice with 200 µL of DPBS with 0.05% Tween 20. To detect bound antibody levels to individual microspheres, 100 µL of 2.5 µg/mL R-Phycoerythrin conjugated goat F(ab')2 anti-human kappa (Southern Biotech, Cat# 2063-09) in blocking buffer

(for antibodies with hFc), or 100 µL of 1.25 µg/mL R-Phycoerythrin AffiniPure $F(ab')_2$ Fragment Goat Anti-Mouse IgG, $F(ab')_2$ Fragment Specific (Jackson Immunoresearch, Cat. No: 115-116-072) in blocking buffer (for antibodies with mFc), or 100 µL of 1.25 µg/mL R-Phycoerythrin Anti-His (Biolegend, Cat. No: 362603) in blocking buffer (for ACE-2 control, R&D, Cat. No. 933-ZN), was added and incubated for 30 minutes at 25 °C. After 30 minutes, the samples were washed twice with 200 µl of washing buffer and resuspended in 150 µL of wash buffer. The plates were read in FlexMap 3D® (Luminex Corp.) and Luminex xPonent® software version 4.3 (Luminex Corp.).

[000195] The results of the Luminex binning results are shown in Table 10 as median fluorescence intensity (MFI) signal intensities. To determine clusters, data was normalized to the intact protein (unmodified microspheres) and clustered. The 46 anti-SARS-CoV-2 antibodies were classified in 9 clusters with 2 or more antibodies, and 11 antibodies were classified as single nodes. Clusters were assigned by based on these results of the hierarchical clustering and dendrogram. These results show that the 46 anti-SARS-CoV-2-S antibody supernatants had diverse binding characteristics and profiles, suggesting that the collection of antibodies bound to different epitopes on the SARS-CoV-2 spike protein.

Table 10: Binding signal (MFI) and cluster assignment of anti-SARS-CoV-2-S monoclonal antibodies to SARS-COV-2-S RBD.mmH (unmodified and chemically or enzymatically modified)

Sample	CLUSTER	UNMODIFIED - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD1 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD2 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD3 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD4 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD5 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD6 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH	MOD7 - SARS-CoV-2 Spike Protein (RBD)(R319 - F541).mmH
Human_ACE2 (10 nM)	1	5727	873	5119	1852	5106	202	5408	5013
Human_ACE2 (100 nM)	1	10681	1447	10320	2260	9661	559	9593	8624
Human_ACE2 (50 nM)	1	9269	991	8238	2185	7707	391	7859	7577
mAb10969	3	28551	54	24177	425	26049	3546	20577	23878
mAb10965	3	28080	38	21996	135	25727	3250	22419	24062
mAb10913	4	31694	102	28389	23270	29344	5018	28738	27854
mAb10920	4	35534	162	26783	28090	32185	7105	32942	30958
mAb10923	4	38711	153	32305	33866	36082	7540	35335	33924
mAb10930	4	29502	110	21579	21533	27843	6195	26600	25103
mAb10940	4	38871	94	34337	33453	36690	7817	36128	34544
mAb10989	4	19671	49	16697	18260	15785	3369	19568	15206
mAb11006	4	2044	30	705	3773	2553	517	2024	2503
mAb10934	5	33057	81	27716	25092	31664	6648	30801	29926
mAb10924	5	39205	118	32707	29366	36507	6378	35565	34210
mAb10939	5	33647	62	24895	26392	31390	6276	31275	29594
mAb10988	5	23009	68	15983	14842	20830	3536	20176	19499
mAb10957	5	20879	52	15728	19383	19993	3582	17727	17989
mAb10914	6	36047	143	32282	26967	34199	7162	32787	31823
mAb10915	6	36690	159	32489	26427	33545	9731	33568	31823
mAb10932	6	34024	191	28833	28557	31560	9946	31123	29765
mAb10938	6	34522	174	28465	19403	31252	8932	29225	30918

mAb10941	**6**	36369	140	31868	26129	33637	9455	33154	31478
mAb10984	**6**	25759	109	22445	20925	24747	6880	23630	23895
mAb10985	**6**	27394	99	24286	22986	26151	5519	25874	25023
mAb10986	**6**	25414	118	20868	20557	23619	6591	23066	22813
mAb10977	**6**	16980	54	14108	16590	15851	3505	14528	12779
mAb10933	**7**	35267	69	30617	5243	32665	6161	32930	31043
mAb10982	**7**	27505	80	20338	6650	25051	4585	24178	23770
mAb10987	**7**	29327	54	25311	2235	27981	4110	27095	25690
mAb10935	**8**	31883	81	28683	12724	30329	6457	27417	27785
mAb10970	**8**	32271	94	26863	22547	30537	7029	27679	28333
mAb10971	**8**	27415	106	23890	22184	27850	6869	25337	25164
mAb10964	**8**	29963	122	23580	23419	27896	7085	27483	25968
mAb10921	**9**	31657	91	28216	18123	30441	6821	28629	28756
mAb10966	**9**	29489	85	22836	19866	25736	5869	24217	26013
mAb10967	**9**	26784	107	20787	13760	25104	6192	21329	23434
mAb10954	**9**	28476	74	21915	19038	26186	5948	25299	24332
mAb10955	**9**	28637	39	24585	21155	27912	4141	23849	24862
mAb10996	**S1**	3403	20	5275	164	5562	488	3042	9125
mAb10937	**S2**	33561	94	24890	104	31164	5904	30327	28675
mAb10936	**S3**	32919	136	26818	312	31261	7856	31008	29293
mAb10922	**S4**	33183	102	25384	1107	31348	5822	31313	29386
mAb11002	**S5**	9881	16	3348	155	8615	153	9542	7562
mAb10956	**S6**	24562	29	21685	19337	23769	2275	19422	21961
mAb11010	**S7**	6388	18	4155	5441	8832	384	7444	5766
mAb11008	**S8**	7096	26	926	1525	2776	198	2750	1007
mAb10998	**S9**	2557	18	247	1336	1524	104	2937	723
mAb11004	**S10**	6514	18	2205	604	3566	1155	4522	2229
mAb11000	**S11**	16670	19	3416	12787	13493	2009	17756	12409

Table 10 (cont'd)

Sample	CLUSTER	MOD8 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD9 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD10 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD11 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD12 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD13 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD14 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH	MOD15 - SARS-CoV-2 Spike Protein (RBD)(R319-F541).mmH
Human_ACE2 (10 nM)	1	36	4500	4091	4618	4505	5094	4743	3173
Human_ACE2 (100 nM)	1	36	6212	7922	8440	8957	8948	7927	5370
Human_ACE2 (50 nM)	1	35	5518	6447	7064	7233	7600	7112	4407
mAb10969	3	154	18918	24407	22409	27036	24269	23672	14196
mAb10965	3	110	19061	22355	21414	25635	23144	23156	14072
mAb10913	4	15939	28645	27110	28878	31159	28971	27784	26272
mAb10920	4	17228	32758	31463	31910	35144	32185	32323	29949
mAb10923	4	20961	34187	33809	36323	38596	35381	33338	33131
mAb10930	4	10235	23744	24516	26738	27958	26968	25126	23951
mAb10940	4	14572	35967	34704	36070	39285	35462	34922	33614
mAb10989	4	6136	17756	15530	16838	15137	17411	18100	15946
mAb11006	4	299	2442	3749	1076	4622	2818	3344	3568
mAb10934	5	6410	31261	30364	30709	32873	30502	28591	27785
mAb10924	5	6594	32856	33797	35875	38424	34647	33476	31524
mAb10939	5	4808	28465	29444	30699	33475	30596	29721	27129
mAb10988	5	2980	18329	19660	20692	21770	20130	18948	16558
mAb10957	5	2171	17357	19487	18596	21247	18757	17810	16081
mAb10914	6	5475	31226	31467	33235	35175	32626	31100	29217
mAb10915	6	9277	33442	31984	32902	35462	31937	32397	30009
mAb10932	6	9711	30122	29074	30433	33379	30283	29880	26795
mAb10938	6	7536	28109	30308	31264	33394	30814	30538	27751
mAb10941	6	7518	29802	31421	33958	35290	32925	31777	29159
mAb10984	6	3527	20212	22065	22318	26163	23227	22283	19349
mAb10985	6	6821	23642	23572	24654	27394	24677	24493	20787

mAb10986	**6**	2838	20672	21766	21720	25207	23400	22214	19694
mAb10977	**6**	4005	14193	12616	13320	16332	13632	14312	13136
mAb10933	7	1556	27705	29926	30801	34427	30409	30525	24367
mAb10982	7	1065	20361	23131	23247	26412	24027	23549	16765
mAb10987	7	1444	25621	25345	26335	29995	27049	26082	22871
mAb10935	**8**	2534	26151	27958	28752	30847	28522	27452	24816
mAb10970	**8**	1968	25233	27793	27610	31869	29871	26909	23775
mAb10971	**8**	1598	22587	25646	24384	27391	25761	24774	19590
mAb10964	**8**	2414	24740	25658	26439	29113	27243	26783	22405
mAb10921	**9**	941	23674	27586	27367	30969	28480	28331	21220
mAb10966	**9**	833	21800	24332	24977	27440	26554	24585	18580
mAb10967	**9**	574	19521	22352	22997	25506	22641	22836	17387
mAb10954	**9**	929	22237	24516	23457	28200	24897	24539	19717
mAb10955	**9**	1141	22191	24805	23688	27210	25575	24677	18944
mAb10996	**S1**	28	8940	6336	6789	6229	5821	3484	1312
mAb10937	**S2**	1231	27597	27092	29937	32116	29661	29386	20543
mAb10936	**S3**	2916	29074	28775	30813	31711	29189	28522	21674
mAb10922	**S4**	2248	29845	28629	30373	32931	30625	28962	23399
mAb11002	**S5**	17	4144	6415	6790	8465	7688	6804	2016
mAb10956	**S6**	331	16954	21282	21524	26646	21547	22767	15077
mAb11010	**S7**	162	5567	6718	9557	12522	5287	5898	4915
mAb11008	**S8**	60	2350	2759	2824	3301	2745	2130	2831
mAb10998	**S9**	85	1611	2260	1206	2513	2186	727	1029
mAb11004	**S10**	71	1465	12665	10667	5925	5531	11578	1144
mAb11000	**S11**	56	14151	19230	17204	21718	17952	17117	5151

Example 9: Biacore binding kinetics of anti-SARS-CoV-2-S monoclonal antibodies

[000196] Equilibrium dissociation constants (K_D) for different SARS-CoV-2-S antibodies from primary supernatants from CHOt cells or from hybridomas were determined using a real-time surface plasmon resonance-based Biacore T200/Biacore 8K biosensor. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25 °C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with either mouse anti-human Fc specific mAb or rabbit anti-mouse Fcγ monoclonal antibody (GE, Catalog # BR-1008-38) to capture anti-SARS-CoV-2 antibodies. Binding studies were performed on a human SARS-CoV-2 RBD extracellular domain expressed with a C-terminal myc-myc-hexahistidine tag (SARS-COV-2 RBD-MMH), SARS-CoV-2 RBD extracellular domain expressed with a C-terminal mouse IgG2a (SARS-COV-2 RBD-mFc), or SARS-CoV-2 RBD extracellular domain expressed with a C-terminal human IgG1 (SARS-COV-2 RBD-hFc). Single concentrations of SARS-COV-2 RBD-MMH, (100nM); SARS-COV-2 RBD-mFc (50nM), or SARS-COV-2 RBD-hFc (50nM), prepared in HBS-ET running buffer, were injected for 1.5 minutes at a flow rate of 30μL/min while the dissociation of antibody-bound different SARS-CoV-2 RBD reagents was monitored for 2 minutes in HBS-ET running buffer. At the end of each cycle, the SARS-CoV-2 RBD antibody capture surface was regenerated using either a 10 sec injection of 20mM phosphoric acid for the mouse anti-human Fc specific monoclonal antibody surface or a 40 sec injection of 10mM Glycine, HCl, pH1.5 for the rabbit anti-mouse Fcγ specific polyclonal antibody. The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using BiaEvaluation software v3.1 or Biacore Insight Evaluation software v2.0. or curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life ($t_{1/2}$) were calculated from the kinetic rates as:

$$\mathbf{K_D\ (M)} = \frac{kd}{ka}, \quad \textbf{and} \quad \mathbf{t_{1/2}\ (min)} = \frac{\ln(2)}{60 * kd}$$

[000197] Binding kinetics parameters for different SARS-CoV-2 monoclonal antibodies binding to different anti-SARS-COV-2 RBD reagents of the invention at 25°C are shown in Tables 11 and 12.

Table 11: Binding kinetics of SARS-COV-2 RBD-MMH binding to anti-SARS-CoV-2 monoclonal antibodies at 25 °C

Supernatant	mAb Capture Level (RU)	50nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	KD (M)	t½ (min)
mAb10913	2010	381	4.91E+05	2.28E-02	4.64E-08	0.5
mAb10914	3169	174	3.49E+05	1.36E-02	3.89E-08	0.8
mAb10915	824	109	8.85E+04	3.18E-04	3.59E-09	36.3
mAb10932	2261	326	8.50E+04	1.26E-04	1.48E-09	92
mAb10933	1414	428	1.05E+06	4.08E-03	3.88E-09	2.8
mAb10934	2918	981	1.01E+06	4.35E-03	4.32E-09	2.7
mAb10935	3293	694	2.11E+05	3.99E-03	1.89E-08	2.9
mAb10936	2491	717	3.03E+05	8.81E-04	2.91E-09	13.1
mAb10937	1846	504	3.81E+05	5.73E-03	1.50E-08	2
mAb10920	1295	234	6.22E+05	2.20E-02	3.54E-08	0.5
mAb10921	1024	141	9.52E+04	4.99E-04	5.24E-09	23.1
mAb10922	2395	786	3.91E+05	2.00E-03	5.11E-09	5.8
mAb10923	1278	322	2.94E+05	6.04E-03	2.06E-08	1.9
mAb10924	766	166	1.97E+05	3.65E-03	1.85E-08	3.2

mAb10930	3137	328	8.90E+04	1.85E-03	2.08E-08	6.2
mAb10938	2167	180	6.60E+04	3.48E-04	5.28E-09	33.2
mAb10939	1505	241	1.69E+05	3.38E-03	2.00E-08	3.4
mAb10940	2149	698	3.34E+05	2.38E-03	7.15E-09	4.9
mAb10941	1811	288	9.85E+04	5.17E-04	5.25E-09	22.3
mAb10982	1096	188	1.32E+05	2.71E-03	2.06E-08	4.3
mAb10984	1654	387	1.55E+05	3.70E-04	2.39E-09	31.2
mAb10985	1974	749	9.41E+05	1.45E-03	1.54E-09	8
mAb10986	1560	524	3.21E+05	2.56E-04	7.97E-10	45.2
mAb10987	1242	356	4.50E+05	1.04E-02	2.32E-08	1.1
mAb10988	1227	291	1.27E+06	3.52E-02	2.77E-08	0.3
mAb10989	692	257	1.60E+06	3.14E-03	1.96E-09	3.7
mAb10969	2200	427	1.80E+05	4.71E-03	2.61E-08	2.5
mAb10970	1865	438	1.37E+05	7.99E-04	5.82E-09	14.4
mAb10971	1482	358	1.68E+05	4.49E-04	2.67E-09	25.8
mAb10964	1208	460	1.06E+06	7.56E-04	7.14E-10	15.3
mAb10965	1046	168	1.19E+05	2.73E-03	2.28E-08	4.2
mAb10966	1422	343	1.57E+05	4.40E-04	2.81E-09	26.3
mAb10967	1421	175	1.12E+05	1.08E-04	9.66E-10	106.9

mAb10954	1150	338	2.34E+05	4.05E-04	1.73E-09	28.5
mAb10955	1032	199	1.38E+05	2.69E-03	1.95E-08	4.3
mAb10956	1303	184	2.02E+05	5.31E-03	2.62E-08	2.2
mAb10957	736	163	1.34E+05	3.15E-04	2.35E-09	36.7
mAb10977	221	57	2.33E+05	7.17E-04	3.08E-09	16.1
mAb11010	1027	108	3.35E+05	1.48E-03	4.42E-09	7.8
mAb11004	1111	161	1.88E+05	3.12E-03	1.66E-08	3.7
mAb11000	381	16	1.40E+05	2.41E-02	1.72E-07	0.5
mAb11006	1118	49	8.97E+04	3.67E-04	4.10E-09	31.5
mAb11008	887	56	6.73E+04	4.00E-03	5.94E-08	2.9
mAb10998	1155	69	1.95E+05	2.28E-02	1.17E-07	0.5
mAb10996	616	28	1.53E+05	1.10E-02	7.18E-08	1.1
mAb11002	1070	8	3.21E+05	2.54E-02	7.93E-08	0.5

Table 12: Binding kinetics of SARS-COV-2 RBD-mFc or SARS-COV-2 RBD-hFc binding to anti-SARS-CoV-2-S monoclonal antibodies at 25 °C

Supernatant	**mAb Capture Level (RU)**	**50nM Ag Bound (RU)**	k_a **(1/Ms)**	k_d **(1/s)**	**KD (M)**	**t½ (min)**

mAb10913	961	575	6.23E+05	1.52E-04	2.44E-10	76.1
mAb10914	1467	313	1.83E+05	1.00E-05*	5.47E-11	1155*
mAb10915	392	141	2.81E+05	1.00E-05*	3.56E-11	1155*
mAb10932	1060	372	2.42E+05	1.00E-05*	4.13E-11	1155*
mAb10933	681	465	1.23E+06	2.12E-04	1.73E-10	54.4
mAb10934	1401	949	1.41E+06	1.17E-04	8.32E-11	98.3
mAb10935	1667	830	3.83E+05	1.00E-05*	2.61E-11	1155*
mAb10936	1171	699	6.52E+05	1.00E-05*	1.53E-11	1155*
mAb10937	904	575	6.39E+05	7.28E-05	1.14E-10	158.7
mAb10920	617	357	7.02E+05	2.92E-04	4.16E-10	39.5
mAb10921	489	170	2.66E+05	1.00E-05*	3.75E-11	1155*
mAb10922	1286	828	7.19E+05	2.42E-05	3.36E-11	478.2
mAb10923	613	362	6.51E+05	2.83E-05	4.35E-11	407.7
mAb10924	465	223	3.67E+05	8.13E-05	2.22E-10	142.1
mAb10930	2156	449	2.32E+05	1.00E-05*	4.31E-11	1155*
mAb10938	1363	333	3.11E+05	1.00E-05*	3.22E-11	1155*
mAb10939	904	324	2.99E+05	1.15E-05	3.87E-11	1004.3
mAb10940	1508	893	5.61E+05	2.86E-05	5.09E-11	403.8
mAb10941	1132	371	2.60E+05	1.00E-05*	2.15E-11	1155*
mAb10982	529	236	3.10E+05	1.69E-05	5.44E-11	683.6

mAb10984	1213	573	4.02E+05	1.00E-05*	2.49E-11	1155*
mAb10985	1463	1040	1.09E+06	1.27E-05	1.17E-11	910.9
mAb10986	1168	752	6.33E+05	1.00E-05*	1.58E-11	1155*
mAb10987	902	632	8.20E+05	1.70E-04	2.08E-10	67.8
mAb10988	892	628	1.24E+06	3.46E-04	2.79E-10	33.4
mAb10989	505	378	2.07E+06	9.30E-05	4.50E-11	124.2
mAb10969	1658	738	3.05E+05	1.51E-05	4.96E-11	764
mAb10970	1370	661	3.48E+05	1.00E-05*	2.88E-11	1155*
mAb10971	1081	556	3.95E+05	1.00E-05*	2.53E-11	1155*
mAb10964	875	651	1.43E+06	1.00E-05*	7.00E-12	1155*
mAb10965	762	322	2.97E+05	1.00E-05*	3.36E-11	1155*
mAb10966	921	430	4.02E+05	1.00E-05*	2.49E-11	1155*
mAb10967	945	355	3.99E+05	1.00E-05*	2.51E-11	1155*
mAb10954	734	414	5.77E+05	1.00E-05*	1.73E-11	1155*
mAb10955	634	292	3.96E+05	2.34E-05	5.92E-11	493.6
mAb10956	842	339	3.74E+05	1.48E-04	3.95E-10	78
mAb10957	449	209	3.58E+05	1.00E-05*	2.79E-11	1155*
mAb10977	161	102	5.56E+05	1.04E-04	1.87E-10	110.9
mAb11010	1014	163	4.24E+05	1.00E-05*	2.36E-11	1155*
mAb11004	1101	241	3.46E+05	6.63E-05	1.91E-10	174.2

mAb11000	380	61	4.38E+05	1.83E-03	4.17E-09	6.3
mAb11006	1112	75	1.88E+05	1.00E-05*	5.32E-11	1155*
mAb11008	872	110	1.61E+05	1.15E-04	7.15E-10	100.4
mAb10998	1140	227	3.30E+05	5.21E-04	1.58E-09	22.2
mAb10996	629	83	2.88E+05	9.32E-04	3.24E-09	12.4
mAb11002	1068	60	2.69E+05	4.49E-03	1.67E-08	2.6

***:** Estimated value based on the limit of measurement of the dissociative rate constant and dissociative half-life under the experimental conditions.

Example 10: Characterization of anti-SARS-CoV-2-S monoclonal antibodies by blocking ELISA

[000198] An ELISA-based blocking assay was developed to determine the ability of anti-SARS-CoV2-S antibodies to block the binding of the SARS-CoV-2 spike protein receptor binding domain (RBD) to human angiotensin converting enzyme 2 (hACE2).

[000199] The SARS-CoV-2 protein used in the experiments was comprised of the receptor binding domain (RBD) portion of the SARS-CoV-2 spike protein (amino acids Arg319 to Phe541) expressed with the Fc portion of the human IgG1 at the c-terminus (SARS-CoV-2 RBD-hFc; see NCBI accession number MN908947.3) The human ACE2 protein used in the experiments was purchased from R&D systems and is comprised of amino acids glutamine 18 to serine 740 with a c-terminal 10X-Histidine tag (hACE2-His; NCBI accession number Q9BYF1).

[000200] Experiments were carried out using the following procedure. A monoclonal anti-Penta-His antibody (Qiagen) was coated at 1 µg/ml in PBS on a 96-well microtiter plate overnight at 4 °C. The hACE2-His receptor was added at 0.2 µg/ml in PBS and bound for 2 hours at room temperature. Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. In other microtiter plates, a constant amount of 10 pM or 15 pM (as indicated in Table 13) of SARS-CoV-2 RBD-hFc protein was bound with antibodies diluted 1:10 or 1:20 in PBS + 0.5% BSA. These antibody-protein complexes, after a one-hour incubation, were transferred to the microtiter plate coated with hACE2-His. After 1.5 hours of

incubation at RT, the wells were washed, and plate-bound SARS-CoV-2 RBD-hFc protein was detected with goat-anti-human IgG antibody conjugated with horseradish peroxidase (HRP) (Jackson). The plates were then developed using TMB substrate solution (BD Biosciences, catalog #555214) according to manufacturer's recommendation and absorbance at 450nm was measured on a Victor X5 plate reader.

[000201] Data analysis was performed by calculating the % reduction of signal of the fixed SARS-CoV-2-S RBD-hFc concentration in the presence of the antibody vs in the absence of the antibody. In the calculation, binding signal of the sample of the constant SARS-CoV-2-S RBD-hFc without the presence of the antibody for each plate was referenced as 100% binding or 0% blocking; and the baseline signal of the sample of media only without the presence of SARS-CoV-2 RBD-hFc was referenced as 0% binding or 100% blocking.

[000202] The ability of anti-SARS-CoV-2-S antibodies to block SARS-CoV-2-S RBD from binding to human ACE2 was assessed using a blocking ELISA format. Single point test antibody supernatant blocking of either 10 pM or 15 pM SARS-CoV-2-S RBD-hFc binding to hACE2-His, which was presented on anti-His antibody coated on 96-well microtiter plates, was detected with an HRP conjugated anti-hFc antibody.

[000203] The blocking results of three assays are summarized in Table 13. The SARS-CoV-2-S binding signal (450 nm) and theG calculated % blocking are indicated. A range of blocking is observed for the test samples. For samples where an NA is indicated in columns 6 and 7, a plate-corrected value is included in columns 4 and 5, as data was consistent with a single plate switch occurring for those samples. 43 of 46 antibody supernatants blocked greater than 50% of the SARS-CoV-2-S RBD-hFc binding to plate-coated human ACE2, with 16 of them blocking >90% of the signal.

Table 13: Blocking ELISA Results

Supernatant	SARS-CoV-2 RBD Fixed Concentration	Supernatant dilution	Plate corrected SARS-CoV-2 RBD-hFc Binding to His presented ACE2	Plate corrected SARS-CoV-2 RBD-hFc Binding to His presented ACE2 % Blocking	SARS-CoV-2 RBD-hFc Binding to His presented ACE2 (Abs 450nm)	SARS-CoV-2 RBD-hFc Binding to His presented ACE2 % Blocking

			(Abs 450nm)			
mAb10913	15pM	1:10	0.206	80.5	0.206	80.5
mAb10914	15pM	1:10	0.326	59.1	0.326	59.1
mAb10915	15pM	1:10	0.171	89.7	0.171	89.7
mAb10932	15pM	1:10	0.254	57.3	0.254	57.3
mAb10933	15pM	1:10	0.158	96.3	0.158	96.3
mAb10934	15pM	1:10	0.209	78	0.209	78
mAb10935	15pM	1:10	0.238	69.4	0.238	69.4
mAb10936	15pM	1:10	0.234	70.6	0.234	70.6
mAb10937	15pM	1:10	0.176	88.1	0.176	88.1
mAb10920	15pM	1:10	0.601	-56.5	0.601	-56.5
mAb10921	15pM	1:10	0.192	82.7	0.192	82.7
mAb10922	15pM	1:10	0.181	86.4	0.181	86.4
mAb10923	15pM	1:10	0.237	43.6	0.237	43.6
mAb10924	15pM	1:10	0.175	78.2	0.175	78.2
mAb10930	15pM	1:10	0.241	42.5	0.241	42.5
mAb10938	15pM	1:10	0.169	87.5	0.169	87.5
mAb10939	15pM	1:10	0.204	65.6	0.204	65.6
mAb10940	15pM	1:10	0.152	95.2	0.152	95.2
mAb10941	15pM	1:10	0.174	97.2	0.174	97.2

mAb10982	15pM	1:10	0.195	83.5	0.195	83.5
mAb10984	15pM	1:10	0.166	96.3	NA	NA
mAb10985	15pM	1:10	0.162	97	NA	NA
mAb10986	15pM	1:10	0.158	97.8	NA	NA
mAb10987	15pM	1:10	0.243	81.8	NA	NA
mAb10988	15pM	1:10	0.244	84	0.244	84
mAb10989	15pM	1:10	0.155	101.8	0.155	101.8
mAb10969	15pM	1:10	0.221	87.8	0.221	87.8
mAb10970	15pM	1:10	0.164	97.7	0.164	97.7
mAb10971	15pM	1:10	0.17	96.7	0.17	96.7
mAb10964	15pM	1:10	0.169	96.9	0.169	96.9
mAb10965	15pM	1:10	0.158	98.8	0.158	98.8
mAb10966	15pM	1:10	0.157	94.2	0.157	94.2
mAb10967	15pM	1:10	0.145	97.9	0.145	97.9
mAb10954	15pM	1:10	0.147	97.3	0.147	97.3
mAb10955	15pM	1:10	0.162	92.7	0.162	92.7
mAb10956	15pM	1:10	0.189	84.5	0.189	84.5
mAb10957	15pM	1:10	0.154	95.1	0.154	95.1
mAb10977	15pM	1:10	0.315	71.5	0.315	71.5
mAb11010	10pM	1:20	0.186	82.1	0.186	82.1

mAb11004	10pM	1:20	0.211	70	0.211	70
mAb11000	10pM	1:20	0.173	72.7	0.173	72.7
mAb11006	10pM	1:20	0.236	58	0.236	58
mAb11008	10pM	1:20	0.213	69.1	0.213	69.1
mAb10998	10pM	1:20	0.185	61.6	0.185	61.6
mAb10996	10pM	1:20	0.295	-18.1	0.295	-18.1
mAb11002	10pM	1:20	0.177	79.2	0.177	79.2

Example 11: Epitope mapping of anti-SARS-CoV-2-S monoclonal antibodies to spike glycoprotein by Hydrogen-Deuterium Exchange Mass Spectrometry

[000204] Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) was performed to determine the amino acid residues of the SARS-CoV-2 Spike Protein Receptor Binding Domain (RBD (amino acids R319-F541)) that interact with mAb10989, mAb10987, mAb10934, mAb10933, mAb10920, mAb10922, mAb10936, mAb10954, mAb10964, mAb10977, mAb10984, and mAb10986. A general description of the HDX-MS method is set forth in e.g., Ehring (1999) *Analytical Biochemistry* 267(2):252-259; and Engen and Smith (2001) *Anal. Chem.* 73:256A-265A.

[000205] The HDX-MS experiments were performed on an integrated HDX-MS platform, consisting of a Leaptec HDX PAL system for the deuterium labeling and quenching, a Waters Acquity I-Class (Binary Solvent Manager) for the sample digestion and loading, a Waters Acquity I-Class (Binary Solvent Manager) for the analytical gradient, and a Thermo Q Exactive HF mass spectrometer for peptide mass measurement.

[000206] The labeling solution was prepared as PBS buffer in D_2O at pD 7.0 (10 mM phosphate buffer, 140 mM NaCl, and 3 mM KCl, equivalent to pH 7.4 at 25°C). For deuterium labeling, 10 μL of the RBD protein or RBD protein premixed with each one of the 12 antibodies listed above were incubated at 20 °C with 90 μL of D_2O labeling solution for various timepoints, in duplicate. For mAb10989, mAb10987, mAb10934, and mAb10933, the time points were 0

min (non-deuterated control), 5 min, and 10 min. For mAb10920, mAb10922, mAb10936, mAb10954, mAb10964, mAb10977, mAb10984, and mAb10986, the time points were 0 min (non-deuterated control) and 10 min. The deuteration reaction was quenched by adding 90 μL of pre-chilled quench buffer (0.5 M TCEP-HCl, 4 M urea and 0.5% formic acid) to each sample for a 90 second incubation at 20 °C. The quenched samples were then injected into the Leaptec HDX PAL system for online pepsin/protease XIII digestion. The digested peptides were trapped by a C18 column (2.1 mm x 5 mm, Waters) and separated by another C18 column (2.1 mm x 50 mm, Waters) at -5 °C with a 20 minute gradient (for mAb10989, mAb10987, mAb10934, and mAb10933) or a 10 minute gradient (for mAb10920, mAb10922, mAb10936, mAb10954, mAb10956, mAb10964, mAb10977, and mAb10984) from 0% to 90% of movbile phase B solution (mobile phase A solution: 0.5% formic acid and 4.5% acetonitrile in water, mobile phase B solution: 0.5% formic acid in acetonitrile). The eluted peptides were analyzed by a Thermo Q Exactive HF mass spectrometry in LC-MS/MS or LC-MS mode.

[000207] The LC-MS/MS data from the undeuterated RBD protein sample were searched against a database including amino acid sequences of the RBD protein, pepsin, protease XIII, and their reversed sequences using Byonic search engine (Protein Metrics). The search parameters were set as default using non-specific enzymatic digestion and human glycosylation as common variable modification. The list of identified peptides was then imported into HDExaminer software (version 3.1) to calculate the deuterium uptake (D-uptake) and differences in deuterium uptake percentage (Δ%D) for all deuterated samples. Difference in deuterium uptake percentage (Δ%D) was calculated as follows.

Difference in deuterium uptake (ΔD) = D-uptake (RBD-mAb) - D-uptake (RBD alone)

$$\text{Difference in deuterium uptake percentage } (\Delta\%D) = \frac{\Delta D}{\text{Theoretical maximum D uptake of the peptide}} \times 100$$

[000208] A total of 190 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10989 samples, representing 86.06% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 467-513 (DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL) (SEQ ID NO: 835) of the RBD were significantly protected by mAb10989.

[000209] A total of 187 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10987 samples, representing 86.06% sequence coverage of RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 432-452 (CVIAWNSNNLDSKVGGNYNYL) (SEQ ID NO: 836) of the RBD were significantly protected by mAb10987.

[000210] A total of 188 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10934 samples, representing 86.06% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 432-452 (CVIAWNSNNLDSKVGGNYNYL) (SEQ ID NO: 836), 467-474 (DISTEIYQ) (SEQ ID NO: 837), and 480-513 (CNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL) (SEQ ID NO: 838) of the RBD were significantly protected by mAb10934.

[000211] A total of 188 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10933 samples, representing 86.06% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 467-510 (DISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV) (SEQ ID NO: 839) of the RBD were significantly protected by mAb10933.

[000212] A total of 75 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10920 samples, representing 83.27% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 471-486 (EIYQAGSTPCNGVEGF) (SEQ ID NO: 840), and 491-515 (PLQSYGFQPTNGVGYQPYRVVVLSF) (SEQ ID NO: 841) of the RBD were significantly protected by mAb10920.

[000213] A total of 86 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10922 samples, representing 87.25% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less

than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 432-452 (CVIAWNSNNLDSKVGGNYNYL) (SEQ ID NO: 836) of the RBD were significantly protected by mAb10922.

[000214] A total of 81 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10936 samples, representing 82.07% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 351-360 (YAWNRKRISN) (SEQ ID NO: 842), 432-452 (CVIAWNSNNLDSKVGGNYNYL) (SEQ ID NO: 836), 467-486 (DISTEIYQAGSTPCNGVEGF) (SEQ ID NO: 843), and 491-513 (PLQSYGFQPTNGVGYQPYRVVVL) (SEQ ID NO: 844) of the RBD were significantly protected by mAb10936.

[000215] A total of 84 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10954 samples, representing 87.25% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 400-422 (FVIRGDEVRQIAPGQTGKIADYN) (SEQ ID NO: 845), 453-486 (YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGF) (SEQ ID NO: 846), and 490-515 (FPLQSYGFQPTNGVGYQPYRVVVLSF) (SEQ ID NO: 847) of the RBD were significantly protected by mAb10954.

[000216] A total of 109 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10964 samples, representing 83.67% sequence coverage of RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 401-424 (VIRGDEVRQIAPGQTGKIADYNYK) (SEQ ID NO: 848), and 471-513 (EIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL) (SEQ ID NO: 849) of the RBD were significantly protected by mAb10964.

[000217] A total of 78 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10977 samples, representing 87.25% sequence coverage of the RBD. Any

peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 351-364 (YAWNRKRISNCVAD) (SEQ ID NO: 850), and 471-486 (EIYQAGSTPCNGVEGF) (SEQ ID NO: 840) of the RBD were significantly protected by mAb10977.

[000218] A total of 88 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10984 samples, representing 87.25% sequence coverage of RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 400-422 (FVIRGDEVRQIAPGQTGKIADYN) (SEQ ID NO: 845), and 453-486 (YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGF) (SEQ ID NO: 846) of the RBD were significantly protected by mAb10984.

[000219] A total of 84 peptides from the RBD were identified from both RBD alone and RBD in complex with mAb10986 samples, representing 87.25% sequence coverage of the RBD. Any peptide that exhibited a reduction in deuterium uptake of 5% or greater (i.e., a Δ%D value of less than -5%, such as -6%, -10%, and so on) upon mAb binding was defined as significantly protected. Peptides corresponding to amino acids 400-422 (FVIRGDEVRQIAPGQTGKIADYN) (SEQ ID NO: 845), 453-486 (YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGF) (SEQ ID NO: 846), and 490-515 (FPLQSYGFQPTNGVGYQPYRVVVLSF) (SEQ ID NO: 847) of the RBD were significantly protected by mAb10986.

[000220] In sum, the majority of the neutralizing antibodies tested contact the RBD in a manner that overlaps the RBD residues that comprise the ACE2 interface; furthermore, the antibodies can be grouped based on their pattern of contacting the RBD surface, as shown in Figure 15. The above data are also summarized in Tables 14-25.

Table 14: Spike protein receptor binding domain (RBD) peptides with significant protection upon formation of RBD-mAb compared to RBD alone

RBD	5 min incubation			10 min incubation			
	RBD-mAb10989	RBD		RBD-mAb10989	RBD		
Residues	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	Δ%D

467-474	2.67	3.16	-0.49	2.53	3.17	-0.64	**-10.5**
470-473	0.48	0.98	-0.50	0.47	0.98	-0.51	**-28.0**
470-474	0.99	1.46	-0.47	0.99	1.44	-0.45	**-16.9**
471-474	0.51	0.89	-0.38	0.51	0.89	-0.38	**-20.9**
475-486	2.20	2.93	-0.73	2.11	2.94	-0.83	**-9.7**
475-487	3.31	4.50	-1.19	3.61	4.48	-0.87	**-11.4**
475-489	2.77	4.48	-1.71	2.78	4.53	-1.75	**-16.0**
475-490	2.63	4.96	-2.33	2.67	4.97	-2.30	**-19.8**
480-489	1.82	3.67	-1.85	1.77	3.69	-1.92	**-26.2**
483-486	0.31	0.78	-0.47	0.30	0.78	-0.48	**-26.5**
487-489	0.05	0.40	-0.35	0.02	0.39	-0.37	**-40.4**
487-490	0.11	0.90	-0.79	0.11	0.84	-0.73	**-42.3**
487-491	0.10	1.05	-0.95	0.10	1.03	-0.93	**-52.0**
487-495	0.62	1.59	-0.97	0.67	1.57	-0.90	**-17.4**
487-509	5.63	6.99	-1.36	5.68	7.02	-1.34	**-8.3**
487-510	6.08	7.37	-1.29	6.08	7.44	-1.36	**-7.7**
487-512	5.72	6.48	-0.76	5.60	6.77	-1.17	**-5.1**
487-513	5.15	6.16	-1.01	5.07	6.14	-1.07	**-5.3**
488-490	0.03	0.22	-0.19	0.00	0.23	-0.23	**-23.2**
488-491	0.04	0.37	-0.33	0.04	0.36	-0.32	**-36.3**

Table 15: Spike protein RBD peptides with significant protection upon formation of RBD-mAb10987 complex comparing to RBD alone

RBD	5 min incubation			10 min incubation			
	RBD-mAb10987	RBD		RBD-mAb10987	RBD		
Residues	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	Δ%D
432-441	1.62	2.17	-0.55	1.64	2.18	-0.54	**-7.6**

432-449	5.60	6.59	-0.99	5.54	6.59	-1.05	**-7.1**
432-452	6.20	7.49	-1.29	6.20	7.46	-1.26	**-7.5**
433-441	1.50	2.00	-0.50	1.49	2.01	-0.52	**-8.1**
440-452	3.95	4.81	-0.86	4.03	4.80	-0.77	**-8.3**
442-449	2.49	2.98	-0.49	2.60	2.99	-0.39	**-8.2**

Table 16: RBD peptides with significant protection upon formation of RBD-mAb10934 complex comparing to RBD alone

RBD	5 min incubation			10 min incubation			
	RBD-mAb10934	RBD		RBD-mAb10934	RBD		
Residues	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	Δ%D
432-452	5.70	7.49	-1.79	5.62	7.46	-1.84	**-10.6**
433-441	1.60	2.00	-0.40	1.63	2.01	-0.38	**-6.2**
434-441	2.24	2.42	-0.18	2.13	2.52	-0.39	**-5.3**
440-452	3.12	4.81	-1.69	3.10	4.80	-1.70	**-17.1**
442-449	2.37	2.98	-0.61	2.37	2.99	-0.62	**-11.4**
442-452	2.67	4.21	-1.54	2.66	4.23	-1.57	**-19.1**
443-452	2.53	3.78	-1.25	2.52	3.78	-1.26	**-17.5**
444-451	1.79	2.73	-0.94	1.80	2.73	-0.93	**-17.2**
444-452	1.82	3.09	-1.27	1.75	3.09	-1.34	**-20.7**
445-452	1.24	2.42	-1.18	1.24	2.43	-1.19	**-22.0**
467-474	2.64	3.16	-0.52	2.58	3.17	-0.59	**-10.2**
470-473	0.51	0.98	-0.47	0.55	0.98	-0.43	**-25.0**
470-474	1.03	1.46	-0.43	1.01	1.44	-0.43	**-16.0**
471-474	0.56	0.89	-0.33	0.55	0.89	-0.34	**-18.6**
480-489	3.19	3.67	-0.48	3.19	3.69	-0.50	**-6.8**
487-489	0.04	0.40	-0.36	0.06	0.39	-0.33	**-38.6**

487-490	0.54	0.90	-0.36	0.53	0.84	-0.31	**-18.8**
487-491	0.63	1.05	-0.42	0.70	1.03	-0.33	**-20.5**
487-495	0.73	1.59	-0.86	0.71	1.57	-0.86	**-16.0**
487-509	5.55	6.99	-1.44	5.57	7.02	-1.45	**-8.9**
487-510	5.89	7.37	-1.48	6.00	7.44	-1.44	**-8.5**
487-513	4.37	6.16	-1.79	4.79	6.14	-1.35	**-7.9**
488-509	4.50	5.49	-0.99	4.60	5.52	-0.92	**-6.2**
488-510	5.84	6.58	-0.74	5.65	6.67	-1.02	**-5.4**
490-509	5.16	6.01	-0.85	5.30	6.12	-0.82	**-5.8**
490-512	5.15	6.37	-1.22	5.30	6.28	-0.98	**-6.4**
490-513	4.90	6.10	-1.20	5.05	6.05	-1.00	**-6.1**
503-509	1.19	1.39	-0.20	1.21	1.41	-0.20	**-5.5**

Table 17: RBD peptides with significant protection upon formation of RBD-mAb10933 complex comparing to RBD alone

RBD	5 min incubation			10 min incubation			
	RBD-mAb10933	RBD		RBD-mAb10933	RBD		
Residues	D-uptake	D-uptake	ΔD	D-uptake	D-uptake	ΔD	Δ%D
467-474	2.52	3.16	-0.64	2.55	3.17	-0.62	**-11.7**
470-474	1.03	1.46	-0.43	1.03	1.44	-0.41	**-15.6**
471-474	0.54	0.89	-0.35	0.54	0.89	-0.35	**-19.5**
475-487	3.62	4.50	-0.88	3.63	4.48	-0.85	**-9.6**
475-489	3.21	4.48	-1.27	3.26	4.53	-1.27	**-11.8**
480-486	1.79	2.06	-0.27	1.87	2.07	-0.20	**-5.1**
480-489	2.13	3.67	-1.54	2.18	3.69	-1.51	**-21.2**
483-486	0.61	0.78	-0.17	0.62	0.78	-0.16	**-9.3**
487-489	0.02	0.40	-0.38	0.02	0.39	-0.37	**-41.6**

487-490	0.42	0.90	-0.48	0.40	0.84	-0.44	**-25.6**
487-491	0.46	1.05	-0.59	0.46	1.03	-0.57	**-32.0**
487-495	0.74	1.59	-0.85	0.82	1.57	-0.75	**-14.8**
487-509	6.01	6.99	-0.98	6.14	7.02	-0.88	**-5.7**
487-510	6.29	7.37	-1.08	6.14	7.44	-1.30	**-7.0**
488-490	0.19	0.22	-0.03	0.13	0.23	-0.10	**-7.4**
488-491	0.26	0.37	-0.11	0.25	0.36	-0.11	**-12.3**

Table 18: RBD peptides with significant protection upon formation of RBD-mAb10920 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10920	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
471-486	4.63	5.40	-0.77	**-6.6**
475-486	2.74	3.27	-0.53	**-6.5**
491-513	5.45	6.57	-1.12	**-6.6**
495-510	4.51	5.43	-0.92	**-8.5**
495-513	4.41	5.13	-0.72	**-5.4**
496-515	3.58	4.35	-0.77	**-5.4**

Table 19: RBD peptides with significant protection upon formation of RBD-mAb10922 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10922	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
432-441	1.86	2.23	-0.37	**-5.3**

442-452	3.52	4.57	-1.05	**-13.0**

Table 20: RBD peptides with significant protection upon formation of RBD-mAb10936 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10936	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
351-360	2.68	3.10	-0.42	**-5.9**
432-441	1.85	2.23	-0.38	**-5.3**
442-452	2.55	4.57	-2.02	**-25.0**
443-452	2.98	4.01	-1.03	**-14.2**
467-470	0.69	0.84	-0.15	**-8.1**
471-486	4.73	5.40	-0.67	**-5.8**
491-513	5.48	6.57	-1.09	**-6.4**
495-510	4.38	5.43	-1.05	**-9.8**

Table 21: RBD peptides with significant protection upon formation of RBD-mAb10954 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10954	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
400-420	3.67	4.56	-0.89	**-5.5**
401-420	3.39	4.22	-0.83	**-5.5**
401-421	3.44	4.28	-0.84	**-5.2**
406-420	3.32	4.10	-0.78	**-7.2**
406-421	3.23	4.11	-0.88	**-7.6**

406-422	3.41	4.16	-0.75	**-5.9**
407-420	2.86	3.62	-0.76	**-7.7**
407-422	2.97	3.74	-0.77	**-6.6**
453-466	1.53	2.23	-0.70	**-7.1**
453-470	3.63	4.53	-0.90	**-6.7**
453-471	4.42	5.22	-0.80	**-5.6**
471-486	4.34	5.40	-1.06	**-9.1**
472-486	4.47	5.29	-0.82	**-7.6**
490-512	5.64	6.65	-1.01	**-5.9**
490-513	5.61	6.57	-0.96	**-5.3**
491-513	5.26	6.57	-1.31	**-7.7**
493-512	4.86	5.69	-0.83	**-5.7**
493-513	4.74	5.72	-0.98	**-6.4**
495-510	4.77	5.43	-0.66	**-6.2**
495-513	4.10	5.13	-1.03	**-7.6**
496-512	3.60	4.60	-1.00	**-8.6**
496-515	3.43	4.35	-0.92	**-6.4**

Table 22: RBD peptides with significant protection upon formation of RBD-mAb10964 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10964	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
401-421	3.87	4.84	-0.97	**-6.0**
406-419	3.34	3.91	-0.57	**-5.8**
406-420	3.47	4.15	-0.68	**-6.3**
406-421	3.53	4.22	-0.69	**-5.9**

406-422	3.66	4.37	-0.71	**-5.6**
406-424	3.31	4.24	-0.93	**-6.5**
410-422	3.04	3.56	-0.52	**-5.8**
471-486	4.65	5.41	-0.76	**-6.4**
475-489	3.34	4.56	-1.22	**-11.3**
480-489	2.32	3.19	-0.87	**-12.1**
487-509	6.38	7.58	-1.20	**-7.4**
495-513	4.50	5.20	-0.70	**-5.2**
496-512	4.17	4.80	-0.63	**-5.4**
496-513	3.90	4.85	-0.95	**-7.5**

Table 23: RBD peptides with significant protection upon formation of RBD-mAb10977 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10977	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
351-364	4.82	5.38	-0.56	**-5.2**
471-486	3.81	5.40	-1.59	**-13.6**
472-486	4.20	5.29	-1.09	**-10.1**

Table 24: RBD peptides with significant protection upon formation of RBD-mAb10984 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10984	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
400-420	3.73	4.56	-0.83	**-5.2**

401-421	3.47	4.28	-0.81	**-5.1**
406-420	3.35	4.10	-0.75	**-7.0**
406-421	3.31	4.11	-0.80	**-6.9**
406-422	3.47	4.16	-0.69	**-5.5**
407-420	2.88	3.62	-0.74	**-7.5**
407-422	2.94	3.74	-0.80	**-6.8**
453-466	1.51	2.23	-0.72	**-7.3**
453-470	3.70	4.53	-0.83	**-6.2**
453-471	4.49	5.22	-0.73	**-5.1**
471-486	4.45	5.40	-0.95	**-8.1**
472-486	4.63	5.29	-0.66	**-6.1**

Table 25: RBD peptides with significant protection upon formation of RBD-mAb10986 complex comparing to RBD alone

RBD	10 min incubation			
	RBD-mAb10986	RBD		
Residues	D-uptake	D-uptake	ΔD	Δ%D
400-420	3.58	4.56	-0.98	**-6.1**
400-421	3.60	4.61	-1.01	**-5.9**
401-420	3.30	4.22	-0.92	**-6.1**
401-421	3.29	4.28	-0.99	**-6.1**
401-422	3.44	4.43	-0.99	**-5.8**
406-420	3.28	4.10	-0.82	**-7.6**
406-421	3.24	4.11	-0.87	**-7.5**
406-422	3.35	4.16	-0.81	**-6.4**
407-420	2.81	3.62	-0.81	**-8.2**
407-422	2.91	3.74	-0.83	**-7.1**

453-466	1.53	2.23	-0.70	**-7.1**
453-470	3.55	4.53	-0.98	**-7.3**
453-471	4.41	5.22	-0.81	**-5.6**
471-486	4.13	5.40	-1.27	**-10.9**
490-510	5.13	6.44	-1.31	**-8.6**
490-512	5.33	6.65	-1.32	**-7.7**
490-513	5.25	6.57	-1.32	**-7.3**
491-513	4.29	6.57	-2.28	**-13.3**
493-512	4.46	5.69	-1.23	**-8.5**
493-513	4.62	5.72	-1.10	**-7.2**
495-513	3.89	5.13	-1.24	**-9.3**
496-513	3.36	4.53	-1.17	**-9.3**
496-515	3.05	4.35	-1.30	**-9.1**

Example 12: Neutralization of SARS-CoV-2 wild-type and variant spike proteins

[000221] To test whether anti-SARS-CoV-2 spike protein antibodies can neutralize SARS-CoV-2 variants, these antibodies were screened against a panel of VSV pseudotype viruses expressing wild-type and variant spike proteins. VSV pseudotype viruses were generated by transiently transfecting 293T cells with a plasmid encoding the SARS-CoV-2 spike protein or the same plasmid containing nucleotide variations that encode for known variants of the SARS-CoV-2 spike protein amino acid sequence. All plasmids were confirmed by Sanger sequencing. Cells were seeded in 15 cm plates at $1.2x10^7$ cells per plate in DMEM Complete Media (1000 mL DMEM, Gibco; 100 mL FBS, Gibco; 10 mL PSG, Gibco) one day prior to transfection with 15 µg/plate Spike DNA using 125 µL Lipofectamine LTX, 30 µL PLUS reagent, and up to 3 mL Opti-Mem. 24 hours post transfection, the cells were washed with 10 mL PBS, then infected with an MOI of 0.1 $VSV^{\Delta G:mNeon}$ virus in 10 mL of Opti-Mem. Virus was incubated on cells for 1 hour, with gentle rocking every 10 minutes. Cells were washed 3 times with 10 mL PBS, then overlaid with 20 mL Infection media (1000 mL DMEM, Gibco; 10 mL Sodium Pyruvate, Gibco; 7 mL BSA, Sigma; 5 mL Gentamicin, Gibco) before incubation at 37 °C, 5% CO_2 for 24 hours.

Pseudovirus supernatant was collected into 250 mL centrifuge tubes on ice, then centrifuged at 3000 rpm for 5 minutes to pellet any cellular debris, aliquoted on ice, then frozen to -80 °C. Infectivity was tested on Vero cells prior to use in neutralization assays. This material will be referred to as $VSV^{\Delta G:mNeon}$/Spike pseudovirus, or $VSV^{\Delta G:mNeon}$/Spike_(variant amino acid mutation) (for example, $VSV^{\Delta G:mNeon}$/Spike_H49Y).

[000222] On Day 1, Vero cells were seeded to 80% confluency in T225 flasks, the cells were washed with PBS (Gibco: 20012-043), TrypLE was added to detach cells from the flask, and Complete DMEM was added to inactivate trypsin. 20,000 Vero cells were plated in in 100 µL of prewarmed Complete DMEM per well in 96 Well Black Polystyrene Microplate (Corning: 3904). On Day 2, $VSV^{\Delta G:mNeon}$/Spike pseudovirus was thawed on ice and diluted with Infection media. Antibodies were diluted in a U-bottom 96 well plate, generating a dilution of each antibody in 210 µl Infection media at 2X assay concentration. 120 µL of diluted antibodies were transferred to a fresh U-bottom plate, and media and an IgG1 control antibody were added to each plate. 120 µl of diluted pseudovirus was added to every well except the media control wells. To those wells, 120 µL of Infection media was added. Pseudovirus with antibodies were incubated for 30 minutes at room temperature, then media was removed from Vero cells. 100 µL of antibody/pseudovirus mixture were added to the cells, and then incubated at 37 °C, 5% CO_2 for 24 hours. On day 3, supernatant was removed from cell wells and replaced with 100 µL of PBS. Plates were read on a SpectraMax i3 with MiniMax imaging cytometer.

[000223] In addition to testing neutralization capacity with non-replicating VSV-SARS-CoV-2-S virus, antibodies also were tested with SARS-CoV-2 virus. Monoclonal antibodies and antibody combinations were serially diluted in DMEM (Quality Biological), supplemented with 10% (v/v) heat inactivated fetal bovine serum (Sigma), 1% (v/v) penicillin/streptomycin (Gemini Bio-products) and 1% (v/v) L-glutamine (2 mM final concentration, Gibco) (VeroE6 media) to a final volume of 250 µL. Next, 250 µL of VeroE6 media containing SARS-CoV-2 (WA-1) (1000 PFU/mL) was added to each serum dilution and to 250 µL media as an untreated control. The virus-antibody mixtures were incubated for 60 min at 37 °C. Following incubation, virus titers of the mixtures were determined by plaque assay. Finally, 50% plaque reduction neutralization titer (PRNT50) values (the serum dilutions at which plaque formation was reduced by 50% relative to that of the untreated control) were calculated using a 4-parameter logistic curve fit to the percent neutralization data (GraphPad Software, La Jolla, CA).

[000224] Individual monoclonal antibody half maximal inhibitory concentration (IC50) against VSV-SARS-CoV-2 spike protein (S)-expressing pseudovirus encoding the Wuhan-Hu-1 (NCBI Accession Number MN908947.3) sequence of spike protein (S-wt) were determined in Vero cells (Table 26). The majority of antibodies displayed neutralization potency in the picomolar range (pM), with some exhibiting neutralization potency in nanomolar (nM) range.

[000225] While recombinant ACE2 was able to mediate neutralization of the VSV-spike pseudoparticles, as previously reported, its potency was far inferior to that of the monoclonal antibodies, with more than 1000-fold decrease in potency seen relative to the best neutralizing mAbs (Figure 10A). In addition, the potent neutralizing activity of mAb10987, mAb10989, mAb10933, and mAb10934 was confirmed in neutralization assays, including neutralization of SARS-CoV-2 in VeroE6 cells (Figure 10B). All neutralization assays generated similar potency across the four mAbs (mAb10987, mAb10989, mAb10933, and mAb10934) and no combinations demonstrated synergistic neutralization activity (Figure 10B).

Table 26: mAb neutralization potency (IC50 (M)) against wild-type strain of VSV-SARS-CoV-2-S pseudoparticles in Vero cells

Antibody	IC50 (M)
mAb10934	5.44E-11
mAb10936	1.11E-10
mAb10987	4.06E-11
mAb10924	1.36E-10
mAb10935	2.21E-10
mAb10913	2.31E-10
mAb10939	2.36E-10
mAb10937	2.62E-10
mAb10920	2.64E-10
mAb10941	2.78E-10

mAb10923	3.29E-10
mAb10915	3.40E-10
mAb10932	3.58E-10
mAb10921	3.74E-10
mAb10914	3.94E-10
mAb10940	5.43E-10
mAb10989	7.23E-12
mAb10938	6.65E-10
mAb10922	1.21E-10
mAb10930	1.07E-09
mAb10954	9.22E-11
mAb10955	1.19E-10
mAb10933	4.28E-11
mAb10956	1.28E-10
mAb10957	1.76E-10
mAb10964	5.70E-11
mAb10965	1.42E-10
mAb10966	1.00E-10
mAb10967	2.43E-10
mAb10970	1.26E-10
mAb10971	1.55E-10
mAb10977	5.15E-11
mAb10982	3.69E-10
mAb10984	9.73E-11

mAb10985	2.57E-10
mAb10986	9.91E-11
mAb10988	2.98E-10
mAb10969	2.27E-09
mAb10996	1.13E-08
mAb10998	9.51E-09
mAb11002	non-neutralizing
mAb11000	2.79E-08
mAb11004	6.00E-09
mAb11006	1.40E-09
mAb11008	2.05E-08
mAb11010	non-neutralizing

[000226] Amino acid variants in spike (S) protein were identified from over 7000 publicly available SARS-CoV-2 sequences, representing globally circulating isolates, and cloned into VSV pseudoparticles. Neutralization assays with variant-encoding pseudoparticles were performed to assess the impact of each variant on neutralization potency of the monoclonal antibodies. Table 27 illustrates the relative neutralization potency of monoclonal antibodies against variant encoding pseudoparticles relative to SARS-CoV-2 spike (S-wt) at a single concentration of 5 μg/ml. Percent of neutralization relative to S-wt was captured for each individual antibody and variant. None of the antibodies demonstrated loss of neutralization potency at the 5 μg/ml concentration with the exception of mAb10985 and the R408I variant. These data demonstrate broad functional neutralization coverage of monoclonal antibodies against globally circulating SARS-CoV-2 spike variants.

[000227] To further interrogate the impact of the S protein variants on neutralization potency of the monoclonal antibodies, full neutralization curves were run to determine the IC50 value of the most potent neutralizing antibodies against a subset of variants localized within the receptor binding domain (RBD) of the S protein. Table 28 shows the IC50 neutralization values for each

variant psuedoparticle. Intrinsic variability of up to 3-fold can be observed between pseudoparticle neutralization assays and does not indicate a change in neutralization potency. These data demonstrate that the antibodies retained their neutralization potency against a diverse panel of S protein RBD variants.

Table 27. Relative neutralization of VSV-SARS-CoV-2 variants encoding S protein at 5 µg/ml antibody concentration in Vero cells

mAb	wt	H49Y	S50L	V341I	N354D	S359N	V367F	K378R
mAb10989	100%	100%	88%	100%	100%	99%	100%	100%
mAb10987	100%	100%	96%	99%	100%	99%	100%	100%
mAb10933	100%	100%	96%	99%	100%	99%	100%	99%
mAb10977	100%	100%	98%	100%	99%	100%	100%	100%
mAb10934	100%	100%	95%	100%	100%	99%	100%	99%
mAb10964	100%	100%	90%	100%	99%	99%	100%	100%
mAb10954	100%	100%	92%	100%	100%	99%	100%	100%
mAb10984	100%	100%	95%	100%	99%	99%	100%	99%
mAb10986	100%	100%	98%	100%	99%	99%	100%	100%
mAb10966	100%	100%	90%	100%	99%	99%	100%	100%
mAb10936	100%	100%	96%	100%	99%	99%	100%	100%
mAb10955	100%	100%	95%	99%	99%	99%	100%	100%
mAb10922	100%	100%	98%	99%	99%	99%	100%	99%
mAb10970	100%	100%	99%	100%	100%	100%	100%	99%
mAb10956	100%	100%	96%	99%	99%	99%	100%	100%
mAb10924	100%	100%	96%	100%	99%	99%	100%	99%
mAb10965	100%	100%	96%	100%	99%	100%	100%	100%
mAb10971	100%	100%	90%	99%	99%	99%	100%	99%
mAb10957	100%	100%	91%	99%	99%	98%	100%	99%
mAb10935	100%	NR	NR	NR	NR	99%	NR	99%

mAb10913	100%	100%	93%	100%	99%	98%	100%	99%
mAb10939	100%	100%	93%	98%	99%	100%	100%	99%
mAb10967	100%	100%	90%	99%	99%	98%	100%	100%
mAb10985	100%	100%	96%	99%	99%	98%	100%	99%
mAb10937	100%	100%	92%	99%	100%	98%	100%	99%
mAb10920	100%	100%	92%	99%	99%	99%	100%	99%
mAb10941	100%	99%	97%	99%	100%	99%	100%	100%
mAb10988	100%	100%	99%	100%	99%	98%	100%	100%
mAb10923	100%	101%	102%	97%	103%	105%	104%	103%
mAb10915	100%	100%	95%	100%	99%	99%	100%	99%
mAb10932	100%	100%	93%	100%	99%	99%	100%	99%
mAb10982	100%	100%	94%	99%	99%	99%	100%	100%

Table 27 (cont'd)

mAb	R408I	Q409E	A435S	K458R	G476S	Y483A	Y508H	H519P	D614G
mAb10989	100%	101%	100%	99%	99%	100%	100%	97%	100%
mAb10987	99%	100%	100%	99%	99%	99%	100%	97%	100%
mAb10933	100%	99%	100%	99%	99%	100%	100%	98%	100%
mAb10977	100%	100%	99%	99%	99%	99%	100%	97%	100%
mAb10934	100%	100%	100%	98%	98%	99%	100%	97%	100%
mAb10964	99%	100%	99%	98%	100%	99%	100%	96%	100%
mAb10954	100%	100%	100%	100%	100%	100%	100%	97%	100%
mAb10984	99%	100%	100%	99%	99%	100%	100%	96%	100%
mAb10986	100%	100%	100%	98%	99%	100%	100%	99%	100%
mAb10966	99%	100%	100%	99%	100%	99%	100%	96%	100%
mAb10936	99%	100%	100%	99%	99%	99%	100%	97%	100%
mAb10955	100%	100%	99%	99%	99%	99%	100%	97%	100%

mAb10922	99%	100%	100%	98%	99%	99%	100%	97%	99%
mAb10970	100%	101%	100%	100%	99%	99%	100%	99%	100%
mAb10956	100%	100%	99%	99%	100%	99%	100%	97%	100%
mAb10924	99%	100%	100%	99%	99%	99%	99%	98%	100%
mAb10965	99%	100%	100%	99%	100%	99%	100%	98%	100%
mAb10971	99%	100%	100%	99%	99%	99%	100%	98%	100%
mAb10957	99%	100%	99%	98%	99%	99%	100%	98%	100%
mAb10935	NR	NR	NR	NR	98%	NR	99%	NR	NR
mAb10913	99%	100%	100%	99%	98%	99%	99%	97%	100%
mAb10939	99%	100%	99%	98%	97%	98%	100%	96%	100%
mAb10967	99%	99%	99%	98%	99%	98%	100%	97%	100%
mAb10985	26%	100%	100%	99%	99%	100%	99%	97%	99%
mAb10937	100%	99%	99%	99%	99%	100%	99%	98%	100%
mAb10920	99%	100%	100%	99%	98%	100%	99%	98%	100%
mAb10941	99%	100%	100%	98%	98%	98%	100%	96%	100%
mAb10988	100%	101%	99%	99%	99%	100%	99%	98%	100%
mAb10923	103%	104%	100%	100%	96%	98%	101%	97%	101%
mAb10915	98%	100%	100%	98%	97%	100%	99%	97%	100%
mAb10932	99%	100%	99%	99%	98%	100%	99%	98%	100%
mAb10982	99%	100%	99%	98%	99%	99%	100%	98%	100%

Table 28. Neutralization IC50 (M) of VSV-SARS-CoV-2-S RBD variants in Vero cells

	Q321S	**V341I**	**A348T**	**N354D**	**S359N**	**V376F**	**K378S**	**R408I**
mAb10933	6.85E-11	3.37E-11	4.13E-11	5.89E-11	2.12E-11	2.40E-11	3.52E-11	1.98E-11
mAb10934	6.84E-11	7.42E-11	1.42E-10	9.76E-11	3.04E-11	3.20E-11	4.65E-11	2.75E-11
mAb10984	2.75E-10	2.49E-10	2.01E-10	2.64E-10	1.23E-10	1.53E-10	1.88E-10	1.35E-10
mAb10986	2.06E-10	1.92E-10	1.03E-10	2.49E-10	8.91E-11	1.49E-10	1.54E-10	6.14E-11

mAb10987	5.02E-11	3.38E-11	2.98E-11	2.68E-11	2.41E-11	1.78E-11	2.40E-11	1.71E-11
mAb10989	1.46E-11	1.61E-11	7.33E-12	1.14E-11	4.30E-12	1.33E-11	1.21E-11	1.09E-11
mAb10964	5.65E-11	1.13E-10	3.52E-11	1.93E-10	6.83E-11	8.92E-11	6.19E-11	4.96E-11
mAb10954	2.32E-10	2.52E-10	1.84E-10	2.84E-10	1.09E-10	1.29E-10	1.65E-10	9.88E-11
IgG1 Isotype Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 28 (cont'd)

	Q409E	**A435S**	**K458R**	**I472V**	**G476S**	**V483A**	**Y508H**	**H519P**
mAb10933	5.65E-11	4.71E-11	3.43E-11	9.17E-11	1.41E-10	1.54E-11	4.77E-11	3.03E-11
mAb10934	5.94E-11	8.07E-11	3.46E-11	9.40E-11	3.51E-11	4.43E-11	6.73E-11	3.56E-11
mAb10984	1.52E-10	2.18E-10	1.59E-10	2.61E-10	2.10E-10	1.71E-10	2.83E-10	1.08E-10
mAb10986	1.95E-10	1.51E-10	1.00E-10	2.24E-10	1.13E-10	9.70E-11	2.01E-10	6.14E-11
mAb10987	4.06E-11	3.88E-11	1.68E-11	4.18E-11	1.86E-11	2.60E-11	2.75E-11	2.20E-11
mAb10989	2.12E-11	1.10E-11	7.51E-12	2.27E-11	6.80E-12	8.78E-12	1.71E-11	4.51E-12
mAb10964	6.61E-11	7.90E-11	5.46E-11	1.01E-10	3.42E-11	4.50E-11	1.02E-10	4.45E-11
mAb10954	2.64E-10	2.11E-10	1.45E-10	3.44E-10	1.83E-10	1.12E-10	2.05E-10	1.40E-10
IgG1 Isotype Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Example 13: Biacore binding kinetics of purified anti-SARS-CoV-2-S monoclonal antibodies

[000228] Equilibrium dissociation constant (K_D) for different SARS-COV-2 RBD reagents binding to purified CHOt anti-SARS-COV-2 monoclonal antibodies (mAbs) were determined using a real-time surface plasmon resonance based Biacore T200/Biacore 8K biosensor. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-ET) running buffer at 25 °C and 37 °C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with either mouse anti-human Fc specific mAb (Regeneron, mAb2567) to capture anti-SARS-COV-2bmAbs. Binding studies were performed on human SARS-COV-2 RBD extracellular domain expressed with a C-terminal

myc-myc-hexahistidine (SARS-COV-2 RBD-MMH) and SARS-COV-2 RBD extracellular domain expressed with a C-terminal mouse IgG2a (SARS-COV-2 RBD-mFc). Use of these reagents allowed for the testing of the antibodies' ability to bind monomeric and dimeric RBD peptides, respectively.

[000229] Different concentrations of hSARS-COV-2 RBD-MMH, (90nM-3.33nM, 3-fold dilution) and SARS-COV-2 RBD-mFc (30nM-1.11nM 3-fold dilution) prepared in HBS-ET running buffer, were injected for 3 minutes at a flow rate of 50µL/min while the dissociation of mAb bound different SARS-COV-2 RBD reagents was monitored for 6-10 minutes in HBS-ET running buffer. At the end of each cycle, the SARS-COV-2 RBD mAb capture surface was regenerated using either 12sec injection of 20mM phosphoric acid for mouse anti-human Fc specific mAb surface. The association rate (k_a) and dissociation rate (k_d) were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using BiaEvaluation software v3.1 or Biacore Insight Evaluation software v2.0. or curve-fitting software. Binding dissociation equilibrium constant (K_D) and dissociative half-life ($t_{1/2}$) were calculated from the kinetic rates as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t_{1/2}\ (min) = \frac{\ln(2)}{60 * kd}$$

[000230] Binding kinetics parameters for different SARS-COV-2 mAbs binding to different anti-SARS-COV-2 RBD reagents of the invention at 25 °C and 37 °C are shown in Tables 29 through 32, respectively.

Table 29: Binding kinetics parameters of SARS-COV-2 RBD-MMH binding to anti-SARS-COV-2-S monoclonal antibodies at 25 °C.

mAb Captured (mAb#)	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	KD (M)	$t_{1/2}$ (min)
mAb10913	287 ± 3	55.9	4.04E+05	2.12E-02	5.26E-08	0.5
mAb10914	310± 2	51.1	8.81E+04	3.76E-03	4.26E-08	3.1
mAb10915	310± 2	63.2	9.61E+04	1.08E-04	1.13E-09	106.9
mAb10920	307 ± 3	73.9	4.52E+05	1.30E-02	2.87E-08	0.9
mAb10921	307 ± 3	61.4	1.01E+05	4.75E-04	4.71E-09	24.3
mAb10922	312.2±1.7	120.2	6.14E+05	1.48E-03	2.41E-09	7.8
mAb10923	283 ± 2	80.4	4.66E+05	6.17E-03	1.32E-08	1.9
mAb10924	319 ± 2	94.6	2.07E+05	1.74E-03	8.40E-09	6.6

mAb10930	284.7±0.7	59.6	1.24E+05	3.34E-03	2.70E-08	3.5
mAb10932	315 ± 3	79.4	8.99E+04	1.21E-04	1.35E-09	95.5
mAb10933	280 ± 1	99.8	1.52E+06	2.78E-03	1.83E-09	4.2
mAb10934	280 ± 1	103.4	4.82E+06	5.77E-03	1.20E-09	2.0
mAb10935	337 ± 2	107.8	3.93E+05	4.19E-03	1.07E-08	2.8
mAb10936	311 ± 2	107.3	5.45E+05	1.07E-03	1.97E-09	10.8
mAb10937	311 ± 2	102.2	5.72E+05	4.76E-03	8.34E-09	2.4
mAb10938	338 ± 3	61.5	7.27E+04	1.75E-04	2.41E-09	66.0
mAb10939	343 ± 2	82.3	1.63E+05	2.84E-03	1.74E-08	4.1
mAb10940	338 ± 3	103.5	8.01E+05	2.51E-03	3.13E-09	4.6
mAb10941	327 ± 1	92.1	1.20E+05	4.12E-04	3.43E-09	28.0
mAb10954	286.9±3	110.5	4.04E+05	3.64E-04	8.99E-10	31.7
mAb10955	298.3±2.5	88.8	1.61E+05	2.12E-03	1.32E-08	5.4
mAb10956	293.7±0.6	86.6	2.22E+05	4.06E-03	1.82E-08	2.8
mAb10957	286.7±2	93.0	1.38E+05	2.53E-04	1.84E-09	45.7
mAb10964	259.6±1.2	99.9	1.65E+06	3.90E-04	2.36E-10	29.6
mAb10965	253.1±1.9	63.6	1.24E+05	2.92E-03	2.35E-08	4.0
mAb10966	266.6±3	97.4	2.37E+05	3.65E-04	1.54E-09	31.6
mAb10967	260.2±0.9	70.7	1.24E+05	6.28E-05	5.08E-10	183.9
mAb10969	272.2±1.3	87.1	2.45E+05	3.80E-03	1.55E-08	3.0
mAb10970	307.3±1.3	102.8	2.27E+05	1.10E-03	4.85E-09	10.5
mAb10971	263.1±1.1	89.3	2.15E+05	3.75E-04	1.74E-09	30.8
mAb10977	305±3	98.5	2.43E+05	2.57E-04	1.06E-09	44.9
mAb10982	267.8±0.5	69.3	1.23E+05	2.06E-03	1.68E-08	5.6
mAb10984	334±2.1	117.9	2.04E+05	4.26E-04	2.09E-09	27.1
mAb10985	306.9±2.1	113.4	1.44E+06	1.55E-03	1.08E-09	7.5
mAb10986	268.8±0.9	104.3	4.64E+05	1.49E-04	3.21E-10	77.5
mAb10987	270.8±1.3	78.0	5.60E+05	1.20E-02	2.14E-08	1.0
mAb10988	279.2±2.3	63.6	8.29E+05	2.71E-02	3.27E-08	0.4
mAb10989	316.7±1.6	114.3	1.86E+06	2.78E-03	1.50E-09	4.2
mAb10996	414.2±2.8	37.5	1.41E+05	2.28E-02	1.61E-07	0.5
mAb10998	212.3±1	17.7	3.54E+05	1.84E-02	5.21E-08	0.6
mAb11000	322.6±3.5	73.6	1.09E+06	1.14E-03	1.04E-09	10.1
mAb11002	291.7±2.7	13.8	1.65E+05	6.73E-03	4.07E-08	1.7
mAb11004	232.9±0.6	76.4	3.79E+05	3.24E-03	8.54E-09	3.6
mAb11006	277.2±1.1	66.9	9.67E+04	4.40E-04	4.55E-09	26.3
mAb11008	214.9±1.5	40.8	9.30E+04	3.27E-03	3.52E-08	3.5
mAb11010	221.8±1.3	76.8	1.11E+06	2.74E-03	2.47E-09	4.2
mAb1932	205±0.8	5.3	NB	NB	NB	NB

Table 30: Binding kinetics parameters of SARS-COV-2 RBD-MMH binding to anti-SARS-COV-2-S monoclonal antibodies at 37 °C.

mAb Captured (mAb#)	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	KD (M)	t½ (min)
mAb10913	366 ± 6	49	5.29E+05	5.56E-02	1.05E-07	0.2
mAb10914	401 ± 3	63	2.51E+05	1.58E-02	6.27E-08	0.7
mAb10915	401 ± 3	93	1.57E+05	7.57E-04	4.84E-09	15.3
mAb10920	394 ± 3	73	6.10E+05	3.41E-02	5.60E-08	0.3
mAb10921	394 ± 3	87	1.60E+05	2.07E-03	1.29E-08	5.6
mAb10922	405.6±1.7	130	1.04E+06	9.27E-03	8.89E-09	1.2
mAb10923	355 ± 3	84	6.15E+05	2.76E-02	4.48E-08	0.4
mAb10924	406 ± 5	110	2.99E+05	6.18E-03	2.07E-08	1.9
mAb10930	373.9±3.5	42	2.30E+05	1.87E-02	8.14E-08	0.6
mAb10932	406 ± 4	119	1.43E+05	6.55E-04	4.57E-09	17.6
mAb10933	368 ± 3	124	2.37E+06	8.28E-03	3.49E-09	1.4
mAb10934	368 ± 3	117	4.62E+06	2.32E-02	5.02E-09	0.5
mAb10935	430 ± 5	75	4.37E+05	3.74E-02	8.56E-08	0.3
mAb10936	402 ± 3	126	9.75E+05	5.51E-03	5.65E-09	2.1
mAb10937	402 ± 3	107	9.68E+05	2.43E-02	2.51E-08	0.5
mAb10938	434 ± 3	100	1.06E+05	1.12E-03	1.05E-08	10.3
mAb10939	439 ± 5	90	2.40E+05	9.46E-03	3.95E-08	1.2
mAb10940	434 ± 3	124	1.42E+06	1.23E-02	8.70E-09	0.9
mAb10941	418± 3	134	1.97E+05	1.75E-03	8.87E-09	6.6
mAb10954	371.8±2	131	5.68E+05	1.35E-03	2.38E-09	8.6
mAb10955	384.1±6.3	81	2.85E+05	1.26E-02	4.43E-08	0.9
mAb10956	383±2.3	89	3.56E+05	1.30E-02	3.65E-08	0.9
mAb10957	322±2.1	124	2.44E+05	6.19E-04	2.54E-09	18.7
mAb10964	333.3±4.6	121	3.68E+06	2.08E-03	5.64E-10	5.6
mAb10965	326.8±1.2	67	2.23E+05	9.19E-03	4.12E-08	1.3
mAb10966	350.2±2.9	118	4.40E+05	1.67E-03	3.79E-09	6.9
mAb10967	336±2.2	108	1.91E+05	2.62E-04	1.38E-09	44.1
mAb10969	349.5±3	86	4.07E+05	1.59E-02	3.92E-08	0.7
mAb10970	393.8±3.4	104	3.33E+05	7.58E-03	2.28E-08	1.5
mAb10971	347±1.9	116	3.92E+05	9.79E-04	2.50E-09	11.8
mAb10977	341±1.4	122	4.35E+05	1.31E-03	3.01E-09	8.8
mAb10982	347.5±1.3	67	1.94E+05	9.42E-03	4.85E-08	1.2
mAb10984	422.5±0.7	144	3.28E+05	1.82E-03	5.55E-09	6.3
mAb10985	395.5±2.5	134	2.57E+06	4.23E-03	1.65E-09	2.7
mAb10986	349.3±1.5	129	8.24E+05	5.83E-04	7.07E-10	19.8
mAb10987	354±5.3	82	8.38E+05	2.51E-02	3.00E-08	0.5
mAb10988	364.4±2.6	52	9.19E+05	5.78E-02	6.29E-08	0.2
mAb10989	405.6±1.9	128	2.97E+06	1.16E-02	3.90E-09	1.0
mAb10996	524.3±2.8	43	1.06E+05	1.25E-02	1.19E-07	0.9
mAb10998	271.1±0.6	15	2.81E+05	7.54E-03	2.68E-08	1.5

mAb11000	418.2±1	87	2.89E+05	9.10E-03	3.14E-08	1.3
mAb11002	370.1±2.5	12	2.81E+05	7.54E-03	2.68E-08	1.5
mAb11004	297.8±0.4	79	1.75E+06	1.48E-03	8.48E-10	7.8
mAb11006	350.2±1.2	92	6.28E+05	1.48E-02	2.35E-08	0.8
mAb11008	289.4±2.7	38	1.42E+05	1.51E-03	1.06E-08	7.6
mAb11010	286.3±0.5	96	1.67E+05	1.45E-02	8.71E-08	0.8
mAb1932	265.3±1.4	5	NB	NB	NB	NB

Table 31: Binding kinetics parameters of SARS-COV-2 RBD-mFc binding to anti-SARS-COV-2-S monoclonal antibodies at 25 °C.

mAb Captured (mAb#)	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	KD (M)	t½ (min)
mAb10913	107 + 0.4	65	5.00E+06	2.77E-04	5.53E-11	41.7
mAb10914	116 + 0.8	44	2.59E+05	1.40E-04	5.40E-10	82.5
mAb10915	103 + 0.2	41	2.83E+05	9.13E-06	3.23E-11	1265.1
mAb10920	116 + 0.9	69	5.08E+06	2.55E-04	5.02E-11	45.3
mAb10921	104 + 0.2	39	2.66E+05	3.34E-05	1.25E-10	345.8
mAb10922	111.4 ± 0.8	80	3.20E+06	5.64E-05	1.76E-11	204.8
mAb10923	110 + 1.0	71	3.69E+06	1.35E-04	3.67E-11	85.6
mAb10924	121 + 0.5	74	8.09E+05	7.63E-05	9.43E-11	151.4
mAb10930	104.2 ± 0.9	61	9.43E+05	1.71E-04	1.81E-10	67.5
mAb10932	121 + 0.8	60	2.95E+05	2.85E-05	9.67E-11	405.3
mAb10933	108 + 0.5	72	6.16E+06	6.10E-05	9.89E-12	189.3
mAb10934	113 + 0.5	70	1.12E+07	1.56E-04	1.39E-11	74.0
mAb10935	128 + 0.8	88	1.35E+06	1.07E-04	7.94E-11	107.9
mAb10936	117 + 0.4	74	1.78E+06	5.04E-05	2.83E-11	229.2
mAb10937	106 + 0.3	67	1.78E+06	5.40E-05	3.04E-11	213.9
mAb10938	128 + 1.5	47	2.42E+05	1.69E-05	7.02E-11	683.4
mAb10939	127 + 0.8	67	7.22E+05	8.74E-05	1.21E-10	132.2
mAb10940	102 + 0.4	67	3.72E+06	4.66E-05	1.25E-11	247.9
mAb10941	125 + 0.2	68	3.70E+05	3.48E-05	9.43E-11	331.9
mAb10954	108.8 ± 1	86	2.35E+06	4.78E-05	2.03E-11	241.6
mAb10955	109.8 ± 0.8	76	1.20E+06	9.22E-05	7.71E-11	125.3
mAb10956	104.1 ± 0.5	74	1.46E+06	1.30E-04	8.87E-11	88.8
mAb10957	104.7 ± 0.5	77	1.02E+06	3.35E-05	3.27E-11	344.8
mAb10964	93.3 ± 0.3	70	9.30E+06	3.69E-05	3.97E-12	313.0
mAb10965	94.2 ± 0.8	63	6.94E+05	1.56E-04	2.25E-10	74.0
mAb10966	100.2 ± 0.4	73	1.50E+06	3.37E-05	2.24E-11	342.7
mAb10967	93.3 ± 0.2	60	6.64E+05	1.35E-05	2.03E-11	855.6
mAb10969	111.4 ± 0.8	80	4.64E+05	1.00E-04	2.16E-10	115.5
mAb10970	113.4 ± 0.7	85	2.19E+06	4.05E-04	1.85E-10	28.5

mAb10971	99 ± 0.5	72	1.40E+06	4.09E-05	2.92E-11	282.4
mAb10977	109.1 ± 0.4	73	1.82E+06	2.29E-05	1.26E-11	504.4
mAb10982	94.8 ± 0.1	59	9.10E+05	8.06E-05	8.86E-11	143.3
mAb10984	121 ± 0.6	89	1.39E+06	3.97E-05	2.86E-11	290.9
mAb10985	112.7 ± 0.3	77	8.09E+06	8.51E-05	1.05E-11	135.7
mAb10986	94.2 ± 0.5	66	2.70E+06	2.40E-05	8.88E-12	481.3
mAb10987	98 ± 0.7	73	3.19E+06	4.24E-04	1.33E-10	27.2
mAb10988	101.6 ± 0.6	69	4.96E+06	5.08E-04	1.02E-10	22.7
mAb10989	112.1 ± 0.4	77	1.08E+07	9.63E-05	8.95E-12	119.9
mAb10996	104.2 ± 0.9	61	5.62E+05	8.02E-04	1.43E-09	14.4
mAb10998	94.8 ± 0.1	59	1.47E+06	3.58E-03	2.44E-09	3.2
mAb11000	112.7 ± 0.3	77	1.11E+06	1.27E-04	1.15E-10	90.9
mAb11002	121 ± 0.6	89	5.54E+05	2.47E-03	4.46E-09	4.7
mAb11004	94.2 ± 0.5	66	6.95E+05	6.40E-05	9.21E-11	180.5
mAb11006	98 ± 0.7	73	3.30E+05	5.21E-05	1.58E-10	221.7
mAb11008	101.6 ± 0.6	69	3.90E+05	1.92E-04	4.92E-10	60.2
mAb11010	112.1 ± 0.4	77	1.14E+06	8.99E-05	7.89E-11	128.5
mAb1932	97.8 ± 0.2	3	NB	NB	NB	NB

Table 32: Binding kinetics parameters of SARS-COV-2 RBD-mFc binding to anti-SARS-COV-2-S monoclonal antibodies at 37 °C

mAb Captured (mAb#)	mAb Capture Level (RU)	30nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	KD (M)	t½ (min)
mAb10913	147 + 0.8	75	6.32E+06	1.73E-03	2.74E-10	6.7
mAb10914	163 + 1.2	70	6.91E+05	2.20E-04	3.18E-10	52.5
mAb10915	141 + 0.6	63	4.41E+05	6.89E-05	1.56E-10	167.6
mAb10920	155 + 1.1	83	6.31E+06	7.53E-04	1.19E-10	15.3
mAb10921	135 + 0.3	62	4.58E+05	1.25E-04	2.73E-10	92.4
mAb10922	149.1 ± 1	97	4.60E+06	1.60E-04	3.49E-11	72.2
mAb10923	144 + 0.8	88	5.53E+06	1.85E-04	3.36E-11	62.4
mAb10924	160 + 1.1	98	1.17E+06	1.31E-04	1.12E-10	88.2
mAb10930	142.9 ± 0.4	72	1.49E+06	5.97E-04	3.99E-10	19.3
mAb10932	164+ 1.5	89	4.48E+05	6.86E-05	1.53E-10	168.4
mAb10933	152+ 0.9	89	7.30E+06	7.94E-05	1.09E-11	145.5
mAb10934	151 + 0.7	87	1.36E+07	2.93E-04	2.16E-11	39.4
mAb10935	171 + 0.8	101	5.68E+06	4.94E-04	8.69E-11	23.4
mAb10936	161+ 1.0	94	3.81E+06	6.75E-05	1.77E-11	171.1
mAb10937	141 + 0.6	85	4.47E+06	5.74E-05	1.29E-11	201.2
mAb10938	172 + 1.2	76	3.78E+05	6.56E-05	1.73E-10	176.1
mAb10939	169 + 0.6	92	1.06E+06	1.65E-04	1.55E-10	70.0
mAb10940	136 + 0.6	85	5.54E+06	5.04E-05	9.10E-12	229.2

mAb10941	164 + 0.8	100	8.02E+05	8.01E-05	1.00E-10	144.2
mAb10954	142.4 ± 0.8	105	3.02E+06	1.12E-04	3.69E-11	103.1
mAb10955	146.8 ± 0.7	91	1.92E+06	3.88E-04	2.02E-10	29.8
mAb10956	136.6 ± 0.4	91	2.17E+06	3.42E-04	1.58E-10	33.8
mAb10957	137.7 ± 1.2	100	1.55E+06	7.19E-05	4.63E-11	160.6
mAb10964	122.5 ± 0.3	84	1.05E+07	1.26E-04	1.20E-11	91.7
mAb10965	125.7 ± 1	81	1.42E+06	3.38E-04	2.37E-10	34.2
mAb10966	137.3 ± 1.1	92	2.45E+06	9.93E-05	4.05E-11	116.3
mAb10967	123.3 ± 0.9	81	1.45E+06	3.33E-05	2.29E-11	346.8
mAb10969	149.1 ± 1	97	8.11E+05	1.41E-04	1.74E-10	81.9
mAb10970	149.9 ± 0.6	102	2.18E+06	4.20E-04	1.92E-10	27.5
mAb10971	136.1 ± 0.8	90	2.37E+06	9.41E-05	3.97E-11	122.7
mAb10977	145.8 ± 0.7	93	2.50E+06	1.07E-04	4.28E-11	107.9
mAb10982	125.5 ± 0.8	74	1.23E+06	2.58E-04	2.10E-10	44.8
mAb10984	158.4 ± 0.7	110	2.07E+06	8.36E-05	4.04E-11	138.2
mAb10985	151.8 ± 0.7	87	9.36E+06	3.75E-04	4.01E-11	30.8
mAb10986	125 ± 0.7	83	4.59E+06	5.79E-05	1.26E-11	199.5
mAb10987	131.5 ± 0.7	87	5.04E+06	3.90E-04	7.75E-11	29.6
mAb10988	138.6 ± 0.5	82	8.34E+06	7.90E-04	9.47E-11	14.6
mAb10989	146.1 ± 0.6	92	1.38E+07	3.65E-04	2.65E-11	31.6
mAb10996	142.9 ± 0.4	72	9.35E+05	2.47E-03	2.64E-09	4.7
mAb10998	125.5 ± 0.8	74	8.79E+05	1.97E-02	2.24E-08	0.6
mAb11000	151.8 ± 0.7	87	1.63E+06	2.71E-04	1.66E-10	42.6
mAb11002	158.4 ± 0.7	110	5.06E+05	1.65E-02	3.26E-08	0.7
mAb11004	125 ± 0.7	83	1.01E+06	1.18E-04	1.17E-10	97.9
mAb11006	131.5 ± 0.7	87	3.88E+05	7.65E-05	1.97E-10	151.0
mAb11008	138.6 ± 0.5	82	4.64E+05	4.05E-04	8.72E-10	28.5
mAb11010	146.1 ± 0.6	92	1.59E+06	8.02E-05	5.05E-11	144.0
mAb1932	128 ± 0.3	5	NB	NB	NB	NB

Example 14: Anti-SARS-CoV-2 Antibodies block RBD binding to hACE2 as determined by ELISA

[000231] An ELISA-based blocking assay was used to determine the ability of anti-SARS-CoV-2 antibodies to block the binding of the SARS-COV-2 Spike protein receptor binding domain (RBD) to its receptor, human angiotensin converting enzyme 2 (hACE2).

[000232] The SARS-CoV-2 protein used in this assay was comprised of the receptor binding domain (RBD) portion of the SARS-CoV-2 Spike protein (amino acids Arg319-Phe541) expressed with the Fc portion of the human IgG1 at the c-terminus (SARS-CoV-2 RBD-hFc) The human ACE2 protein used in the experiments was purchased from R&D Systems and was

comprised of amino acids Gln18-Ser740 with a C-terminal 10X-Histidine tag (hACE2-His; NCBI Accession No. Q9BYF1).

[000233] Experiments were carried out using the following procedure. A monoclonal anti-Penta-His antibody (Qiagen) was coated at 1μg/ml in PBS on a 96-well microtiter plate overnight at 4 °C. The hACE2-His receptor was added at 0.2ug/ml in PBS and bound for two hours at room temperature (RT). Nonspecific binding sites were subsequently blocked using a 0.5% (w/v) solution of BSA in PBS. In other microtiter plates, a constant amount of 100pM of SARS-CoV-2 RBD-hFc protein was bound with anti-SARS-COV-2 antibodies and an isotype IgG1 antibody control at dilutions from 0.0008nM to 50nM in PBS +0.5% BSA. After a one-hour incubation, the mixture solutions were transferred to the microtiter plate coated hACE2-His. After 1.5 hours of incubation at RT, the wells were washed, and plate-bound SARS-COV2 was detected with goat-anti-human IgG antibody conjugated with horseradish peroxidase (HRP) (Jackson). The plates were then developed using TMB substrate solution (BD Biosciences, #555214) according to manufacturer's recommendation and absorbance at 450nm was measured on a Victor X5 plate reader.

[000234] Binding data were analyzed using a sigmoidal dose-response model within Prism™ software (GraphPad). The calculated IC50 value, defined as the concentration of antibody required to block 50% of SARS-CoV-2 RBD-hFc binding to plate-coated hACE2-His, was used as an indicator of blocking potency. Percent blocking was defined based on the background-corrected binding signal observed at the highest antibody concentration tested using this formula and reported for all tested antibodies:

$$\%\ \text{Blocking} = 100 - \left(\frac{[\text{Experimental Signal}_{(\text{highest Ab conc})} - \text{Background Signal}_{(\text{buffer})}]}{[\text{Maximum Signal}_{(\text{hEGF.mFc alone})} - \text{Background Signal}_{(\text{buffer})}]} \times 100 \right)$$

[000235] Antibodies that blocked binding less than or equal to 50% at the highest concentration tested were classified as non-blockers and IC50 values were not reported for those antibodies.

[000236] The ability of anti-SARS-CoV-2 antibodies to block SARS-CoV-2 RBD binding to human ACE2 was assessed using a blocking ELISA. In this assay 100pM SARS-COV-2 RBD-hFc was titrated with a wide range of the concentrations of the anti-SARS-CoV-2-S antibody and

the inhibition of the presence of the antibody on RBD binding to hACE2-His was evaluated. The plate-bound RBD-hFc was detected with an HRP conjugated anti-hFc antibody.

[000237] The blocking IC50s and maximum blocking at the highest tested concentrations of the anti-SARS-CoV-2-S antibodies are summarized in Table 33, and the blocking curves shown in Figures 1-8. Of the 46 antibodies tested, 44 displayed antibody concentration-dependent blocking of RBD.hFc binding to hACE-2. IC50 values ranged from 41pM to 4.5nM and maximum blocking ranging from 55% to about 100% at the highest antibody concentration tested. Two antibodies out of 46 tested showed no blocking activities under the assay conditions. The irrelevant isotype control antibody showed no blocking activity, as expected.

Table 33: Blocking potency of Anti-SAR-COV-2 Antibodies on Spike RBD-hFc Binding to Immobilized Human ACE-2

mAb	Assay Run #	Blocking 100pM (RBD).hFc to ACE2 IC_{50},M	Blocking 100pM (RBD).hFc to ACE2 % Blocking
mAb10913	1	2.17E-10	80
mAb10914	1	9.80E-10	93
mAb10915	1	3.21E-10	99
mAb10920	1	3.38E-10	95
mAb10920	3	1.39E-10	87
mAb10921	1	4.33E-10	99
mAb10921	3	5.07E-10	94
mAb10922	2	6.65E-11	97
mAb10923	1	1.49E-10	94
mAb10923	3	1.84E-10	85
mAb10924	1	1.63E-10	98

mAb10924	2	1.27E-10	98
mAb10930	2	2.82E-10	86
mAb10932	1	3.73E-10	99
mAb10933	1	7.07E-11	99
mAb10933	3	6.53E-11	95
mAb10933	2	5.22E-11	101
mAb10934	1	6.60E-11	96
mAb10934	3	5.97E-11	98
mAb10934	2	4.80E-11	96
mAb10935	1	1.02E-10	99
mAb10935	2	6.94E-11	98
mAb10936	1	8.75E-11	95
mAb10936	2	7.10E-11	97
mAb10937	1	6.49E-11	99
mAb10938	1	2.75E-10	99
mAb10939	1	1.75E-10	97
mAb10939	3	2.63E-10	93
mAb10940	1	6.52E-11	92
mAb10941	1	2.27E-10	100
mAb10941	2	2.06E-10	100
mAb10954	2	7.11E-11	95
mAb10955	2	1.41E-10	97
mAb10956	2	1.85E-10	99
mAb10957	2	1.69E-10	99

mAb10964	3	6.83E-11	93
mAb10964	2	6.25E-11	95
mAb10965	2	2.13E-10	97
mAb10966	2	1.60E-10	99
mAb10967	2	2.80E-10	98
mAb10969	3	2.15E-10	95
mAb10970	2	1.07E-10	97
mAb10971	2	1.49E-10	98
mAb10977	3	8.71E-11	77
mAb10977	2	7.11E-11	65
mAb10982	2	1.16E-10	93
mAb10984	2	7.75E-11	90
mAb10985	3	6.96E-11	97
mAb10985	2	4.11E-11	99
mAb10986	2	7.54E-11	98
mAb10987	3	2.85E-10	93
mAb10987	2	1.81E-10	95
mAb10988	2	8.64E-11	95
mAb10989	3	5.91E-11	96
mAb10989	2	4.28E-11	98
mAb10996	3	6.10E-09	71
mAb10998	3	4.30E-09	55
mAb11000	3	4.50E-09	75
mAb11002	3	NBD	7

mAb11004	3	NBD	9
mAb11006	3	2.20E-10	85
mAb11008	3	1.49E-09	93
mAb11010	3	1.47E-10	83
mAb193250 IgG1 Control	1	--	-8
mAb193250 IgG1 Control	3	--	-19
mAb193250 IgG1 Control	2	--	-15

Note: RBD-hFc at 100 pM was titrated with anti-SARS-COV-2-S antibodies in serial dilutions from 50nM and bound RBD-hFc on immobilized hACE2 with a 10x histidine tag, and detected with HRP-conjugated anti-hFc antibody. NBD; no blocking detected.

Example 15: Cross-competition between mAb10987, mAb10989, mAb10933, and mAb10934

[000238] mAb10987, mAb10989, mAb10933, and mAb10934 were examined in cross-competition binding assays (Figure 11), identifying several pairs of non-competing mAbs with picomolar neutralization potency that could potentially be combined to form antibody cocktails, e.g., mAb10987 and mAb0933.

[000239] Epitope binning of the anti-SARS-CoV-2-S mAbs was conducted in a pre-mix sandwich format involving competing mAbs against one another in a pairwise combinatorial manner for binding to SARS-CoV-2 RBD-MMH protein using a ForteBio Octet HTX biolayer interferometry instrument (Molecular Devices ForteBio LLC, Fremont, CA) with running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4, 1 mg/mL BSA. Assays were performed at 30 ºC with continuous agitation at 1000 rpm. After obtaining an initial baseline in running buffer 20 μg/mL of anti-COVID19 mAbs was captured onto anti-human Fc (AHC) biosensor tips for 300 s. To block remaining free unsaturated binding sites on AHC biosensor tips, all sensors were exposed for 240 s to blocking solution well containing 100 μg/mL irrelevant IgG1. Following this process, biosensors were immersed into wells containing pre-mix

solution of 100nM SARS CoV-2 RBD-MMH protein and 600 nM of anti-COVID19 mAb binding site of a second mAbs for 300 s. Binding response at each step was recorded and specific signal was normalized by subtracting self-blocking mAb competing control from dataset. Data analysis was performed with Octet Data Analysis HT 10.0 software using the Epitope Binning.

[000240] Comparing the cross-competition binding assays with the HDX-MS results described above provides structural insights into the mechanism by which non-competing pairs of antibodies can simultaneously bind the RBD, and can thus be ideal partners for a therapeutic antibody cocktail. mAb10987 and mAb10933 represent such a pair of antibodies. mAb10933 targets the spike-like loop region on one edge of the ACE2 interface. Within that region, the residues that show the most significant HDX protection by mAb10933 face upward, suggesting that the Fab region of mAb10933 binds the RBD from the top direction, where mAb10933 will have significant collisions with ACE2. In order to avoid competition with mAb10933, mAb10987 only binds to the HDX-defined protected regions from the front or the lower left side (in the front view of mAb10987 in Figure 12). This is consistent with the neutralization data described above, as mAb10987 would orient it in a position that has high probability to interfere with ACE2.

Example 16: Structure determination of antibody-bound spike protein

[000241] To better understand the binding of mAb10933 and mAb10987 to the spike protein RBD, structural analysis was performed via cryo-electron microscopy (cryoEM). Fab fragments of mAb10933 and mAb10987 were isolated using FabALACTICA kit (Genovis). 600 µg of the mAb10933 Fab and 600 µg of mAb10987 Fab were mixed with 300 µg of SARS-CoV-2-S RBD and incubated on ice for ~1 hour then injected into a Superdex 200 increase gel filtration column equilibrated to 50 mM Tris pH 7.5, 150 mM NaCl. Peak fractions containing the mAb10933 Fab - mAb10987 Fab - RBD complex were collected and concentrated using a 10 kDa MWCO centrifugal filter. For cryoEM grid preparation, the protein sample was diluted to 1.5 mg/mL and 0.15% PMAL-C8 amphipol was added. 3.5 µL of protein was deposited onto a freshly plasma cleaned UltrAufoil grid (1.2/1.3, 300 mesh). Excess solution was blotted away using filter paper and plunge frozen into liquid ethane using a Vitrobot Mark IV. The cryoEM grid was transferred to a Titan Krios (Thermo Fisher) equipped with a K3 detector (Gatan). Movies were collected using EPU (Thermo Fisher) at 105,000x magnification, corresponding to a pixel size of 0.85 Å.

A dose rate of 15 electrons per pixel per second was used and each movie was 2 seconds, corresponding to a total dose of ~40 electrons per Å2.

[000242] All cryoEM data processing was carried out using cryoSPARC v2.14.2. 2,821 movies were aligned using patch motion correction and patch CTF estimation. 2,197 aligned micrographs were selected for further processing on the basis of estimated defocus values and CTF fit resolutions. An initial set of particles picked using blob picker were subjected to 2D classification to generate templates for template picking. 989,553 particles picked by template picking were subjected to multiple rounds of 2D classification to remove unbound fabs and particles containing an incomplete complex. Ab initio reconstruction with three classes generated a single class containing 61,707 particles that corresponded to the mAb10933 Fab - mAb10987 Fab - RBD complex. Heterogenous refinement of the particles in this class followed by non-uniform refinement resulted in a 3.9 Å resolution (FSC=0.143) map containing 48,140 particles that was used for model building. Into this map, models of the RBD (taken from PDB code 6M17) and the two Fabs (taken from prior antibody structures, except for the lambda light chain of mAb10987 which came from PDB code 5U15), were manually placed. These models were then manually rebuilt using Coot and real-space refined against the map using Phenix.

[000243] Confirming the above-described data, single-particle cryoEM of the complex of SARS-CoV-2 spike RBD bound to Fab fragments of mAb10933 and mAb10987 shows that the two antibodies in this cocktail can simultaneously bind to distinct regions of the RBD (Figure 13A, Figure 13B, and Figure 14). A 3D reconstructed map of the complex with nominal resolution of 3.9Å shows that the both Fab fragments bind at different epitopes on the RBD, confirming that they are non-competing antibodies. mAb10933 binds at the top of the RBD, extensively overlapping the binding site for ACE2. On the other hand, the epitope for mAb10987 is located on the side of the RBD, well away from the mAb10933 epitope, and has little to no overlap with the ACE2 binding site.

Example 17: Cross-competition between anti-SARS-CoV-2-S mAbs

[000244] Binding competition between anti-SARS-CoV-2-S monoclonal antibodies (mAbs) was determined using a real time, label-free bio-layer interferometry (BLI) assay on the Octet HTX biosensor platform (Pall ForteBio Corp.). The entire experiment was performed at 25 °C in 10mM HEPES, 150mM NaCl, 3mM EDTA, and 0.05% v/v Surfactant Tween-20, 1mg/mL BSA,

pH 7.4 (HBS-EBT) buffer with the plate shaking at a speed of 1000rpm. To assess whether two mAbs were able to compete with one another for binding to their respective epitopes on the SARS-COV-2-S RBD extracellular domain expressed with a C-terminal myc-myc-hexahistidine (SARS-COV-2 RBD-MMH), ~0.51nm of SARS-COV-2-S RBD-MMH was first captured onto anti-Penta-His antibody coated Octet biosensor tips (Fortebio Inc, # 18-5122) by submerging the biosensor tips for 1 minute in wells containing a 10 µg/mL solution of SARS-COV-2-S RBD-MMH. The SARS-COV-2-S RBD-MMH captured biosensor tips were then saturated with a first anti-SARS-CoV-2-S monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 5 minutes. The biosensor tips were then subsequently dipped into wells containing 50µg/mL solution of a second anti-SARS-CoV-2 monoclonal antibody (subsequently referred to as mAb-2) for 5 minutes. The biosensor tips were washed in HBS-ETB buffer in between every step of the experiment. The real-time binding response was monitored during the entire course of the experiment and the binding response at the end of every step was recorded. The response of mAb-2 binding to SARS-COV-2 RBD-MMH pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different anti-SARS-CoV-2 monoclonal antibodies was determined as shown in Table 34.

Table 34: Cross-competition between anti-SARS-CoV-2-S antibodies

mAb-1	mAb-2 Competing with mAb-1
mAb10977	mAb10924
	mAb10989
	mAb10920
	mAb10913
	mAb10923
	mAb10930
	mAb10969
	mAb10988

	mAb10964
	mAb10996
	mAb10966
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	mAb10970
	mAb10957
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	mAb10941
	mAb10939
	mAb10935
	mAb10914
	mAb10982
	mAb11008
	mAb11002
	mAb10933
	mAb10940
	mAb10922
	mAb11004
	mAb10937
	mAb10936

	mAb10934
mAb10924	**mAb10977**
	mAb10989
	mAb10920
	mAb10913
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mAb10989	**mAb10977**
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Example 18: pH sensitivity of anti-SARS-CoV-2-S monoclonal antibodies binding to monomeric SARS-CoV-2-S RBD reagents measured at 37 °C

[000245] The dissociation rate constants (k_d) for different anti-SARS-CoV-2-S monoclonal antibodies in pH 7.4, pH 6.0, and pH5.0 buffers were determined using a real-time surface plasmon resonance (SPR)-based Biacore T200 biosensor. All binding studies were performed at 37 °C using three running buffers, (i) PBS, 0.05% v/v Surfactant Tween-20, pH7.4 (PBS-T-pH7.4) (ii) PBS, 0.05% v/v Surfactant Tween-20, pH6.0 (PBS-T-pH6.0), and (iii) PBS, 0.05%

v/v Surfactant Tween-20, pH5.0 (PBS-T-pH5.0). The Biacore CM5 sensor chip surface was first derivatized by amine coupling with a mouse anti-human Fc specific mAb (Regeneron) to capture anti-SARS-CoV-2-S monoclonal antibodies. Binding studies were performed on human SARS-COV-2-S RBD extracellular domain expressed with a C-terminal myc-myc-hexahistidine (SARS-COV-2 RBD-MMH), Single concentrations of SARS-COV-2-S RBD-MMH (90nM) prepared in PBS-T-pH 7.4 buffer were injected at a flow rate of 25μL/min for 3 minutes followed by the dissociation of bound SARS-COV-2-S RBD-MMH in PBS-T-pH 7.4, PBS-T-pH 6.0 or PBS-T PBS-T-pH 5.0 running buffers for 5 minutes.

[000246] The dissociation rate constants (k_d) in four pH running buffers were determined by fitting the real-time binding sensorgrams to a 1:1 binding model using Scrubber 2.0c curve-fitting software. The dissociative half-life (t½) was calculated from the k_d values as:

$$\textbf{t½ (min)} = \frac{\ln(2)}{60 * kd}$$

[000247] The k_d and t½ values for SARS-COV-2-S RBD-MMH binding to different anti-SARS-CoV-2-S monoclonal antibodies in PBS-T-pH 7.4 followed by dissociation in PBS-T-pH 7.4 and PBS-T-pH 6.0 at 37 °C are shown in Table 35. The k_d and t½ values for SARS-COV-2-S RBD-MMH binding to different anti-SARS-CoV-2-S monoclonal antibodies in PBS-T-pH 7.4 followed by dissociation in PBS-T-pH 7.4 and PBS-T-pH 5.0 at 37 °C are shown in Table 36. The comparison of the dissociative half-life (t½) of SARS-COV-2 RBD-MMH in pH 7.4, pH 6.0 and pH 5.0 buffers.

Table 35: Binding of SARS-COV-2-S RBD-MMH to anti-SARS-CoV-2-S monoclonal antibodies in PBS-T-pH 7.4 buffer and dissociation in PBS-T-pH 7.4 & pH 6.0 buffer at 37°C.

	Running Buffer: PBS-T, pH7.4 @ 37C°				Running Buffer: PBS-T, Chase in pH6.0 @ 37C°				t½ Ratio
mAb Captured	mAb Capture Level (RU)	90nM RBD.mmh Bound (RU)	kd (1/s)	t½ (min)	mAb Capture Level (RU)	90nM RBD.mmh Bound (RU)	kd (1/s)	t½ (min)	pH7.4 / pH6.0
mAb10913	427	69	4.30E-02	0.3	421	67	4.38E-02	0.3	1
mAb10914	388	69	9.41E-03	1.2	386	62	1.16E-02	1	1.2
mAb10915	319	84	7.43E-04	15.5	312	88	1.51E-03	7.7	2
mAb10932	432	133	6.60E-04	17.5	438	131	1.28E-03	9	1.9
mAb10933	360	124	7.85E-03	1.5	353	119	8.53E-03	1.4	1.1
mAb10934	341	107	1.74E-02	0.7	334	108	2.11E-02	0.5	1.2
mAb10935	407	76	2.78E-02	0.4	404	72	1.71E-02	0.7	0.6
mAb10936	381	124	5.29E-03	2.2	375	120	8.69E-03	1.3	1.6
mAb10937	330	94	2.09E-02	0.6	323	98	2.09E-02	0.6	1
mAb10924	385	111	5.69E-03	2	379	110	1.20E-02	1	2.1
mAb10938	407	95	1.05E-03	11	407	90	2.99E-03	3.9	2.8
mAb10940	343	119	1.08E-02	1.1	339	127	1.04E-02	1.1	1

mAb10941	398	129	1.65E-03	7	396	127	2.04E-03	5.7	1.2
mAb10920	383	79	2.47E-02	0.5	380	73	5.39E-02	0.2	2.2
mAb10921	345	89	1.79E-03	6.5	339	92	2.01E-02	0.6	11.3
mAb10923	355	87	2.35E-02	0.5	349	88	2.43E-02	0.5	1
mAb10939	410	90	9.48E-03	1.2	412	83	1.18E-02	1	1.2
mAb10922	251	85	9.07E-03	1.3	240	92	9.61E-03	1.2	1.1
mAb10930	377	50	1.92E-02	0.6	372	46	1.67E-02	0.7	0.9
mAb10982	389	79	9.90E-03	1.2	387	74	7.72E-03	1.5	0.8
mAb10984	378	133	1.71E-03	6.8	370	135	1.94E-03	5.9	1.1
mAb10985	457	172	3.63E-03	3.2	464	172	3.19E-03	3.6	0.9
mAb10986	413	155	6.29E-04	18.4	411	152	1.24E-03	9.3	2
mAb10987	379	105	2.37E-02	0.5	372	109	1.83E-02	0.6	0.8
mAb10988	467	109	4.35E-02	0.3	469	103	5.37E-02	0.2	1.2
mAb10989	382	126	9.32E-03	1.2	375	119	7.36E-03	1.6	0.8
mAb10970	340	93	7.65E-03	1.5	334	96	6.37E-03	1.8	0.8
mAb10971	350	125	9.44E-04	12.2	342	125	1.27E-03	9.1	1.3
mAb10964	380	140	1.94E-03	6	379	137	2.51E-03	4.6	1.3

mAb10965	290	65	8.66E-03	1.3	281	70	9.47E-03	1.2	1.1
mAb10966	417	152	1.60E-03	7.2	409	149	1.41E-03	8.2	0.9
mAb10967	372	118	2.98E-04	38.8	367	115	3.45E-04	33.5	1.2
mAb10954	336	118	1.74E-03	6.6	331	124	2.70E-03	4.3	1.6
mAb10955	404	100	1.22E-02	0.9	403	97	1.46E-02	0.8	1.2
mAb10956	452	114	1.25E-02	0.9	446	106	1.50E-02	0.8	1.2
mAb10957	388	136	5.80E-04	19.9	382	140	7.67E-04	15.1	1.3
mAb10977	293	44	1.59E-02	0.7	285	44	3.39E-02	0.3	2.1
mAb10969	340	72	1.86E-02	0.6	336	71	1.01E-02	1.1	0.5
mAb10996	408	35	4.69E-02	0.2	405	37	4.37E-02	0.3	0.9
mAb10998	308	20	2.86E-02	0.4	307	19	2.84E-02	0.4	1
mAb11002	373	10	2.60E-02	0.4	368	4	5.91E-03	2	0.2
mAb11000	404	88	1.48E-03	7.8	403	90	2.85E-03	4.1	1.9
mAb11004	356	97	1.47E-02	0.8	353	96	2.09E-02	0.6	1.4
mAb11006	398	105	1.46E-03	7.9	398	98	1.98E-03	5.8	1.4
mAb11008	341	112	1.33E-03	8.7	338	118	1.28E-03	9	1
mAb11010	432	157	3.90E-03	3	431	156	7.51E-03	1.5	1.9

Isotype Control	430	4	NB	NB	427	9	NB	NB	NB

Table 36: Binding of SARS-COV-2-S RBD-MMH to anti-SARS-CoV-2 monoclonal antibodies in PBS-T-pH7.4 buffer and the dissociation in PBS-T-pH 7.4 & pH 5.0 buffer at 37°C.

	Running Buffer: PBS-T, pH7.4 @ 37C°				Running Buffer: PBS-T, Chase in pH5.0 @ 37C°				t½ Ratio
mAb Captured	mAb Capture Level (RU)	90nM RBD.mmh Bound (RU)	kd (1/s)	t½ (min)	mAb Capture Level (RU)	90nM RBD.mmh Bound (RU)	kd (1/s)	t½ (min)	pH7.4 / pH5.0
mAb10913	427	69	4.30E-02	0.3	430	65	3.53E-02	0.3	0.8
mAb10914	388	69	9.41E-03	1.2	391	57	1.00E-02	1.2	1.1
mAb10915	319	84	7.43E-04	15.5	316	94	2.05E-03	5.6	2.8
mAb10932	432	133	6.60E-04	17.5	452	131	2.11E-03	5.5	3.2
mAb10933	360	124	7.85E-03	1.5	353	114	1.14E-02	1	1.5
mAb10934	341	107	1.74E-02	0.7	338	109	1.71E-02	0.7	1
mAb10935	407	76	2.78E-02	0.4	413	70	1.28E-02	0.9	0.5
mAb10936	381	124	5.29E-03	2.2	379	116	1.60E-02	0.7	3
mAb10937	330	94	2.09E-02	0.6	326	104	1.55E-02	0.7	0.7
mAb10924	385	111	5.69E-03	2	390	113	1.48E-02	0.8	2.6

mAb10938	407	95	1.05E-03	11	417	82	7.61E-03	1.5	7.2
mAb10940	343	119	1.08E-02	1.1	341	135	8.23E-03	1.4	0.8
mAb10941	398	129	1.65E-03	7	407	128	2.21E-03	5.2	1.3
mAb10920	383	79	2.47E-02	0.5	382	68	2.93E-02	0.4	1.2
mAb10921	345	89	1.79E-03	6.5	345	100	5.46E-02	0.2	30.6
mAb10923	355	87	2.35E-02	0.5	357	90	2.13E-02	0.5	0.9
mAb10939	410	90	9.48E-03	1.2	419	78	1.14E-02	1	1.2
mAb10922	251	85	9.07E-03	1.3	240	102	8.08E-03	1.4	0.9
mAb10930	377	50	1.92E-02	0.6	383	44	1.20E-02	1	0.6
mAb10982	389	79	9.90E-03	1.2	391	66	6.27E-03	1.8	0.6
mAb10984	378	133	1.71E-03	6.8	378	140	2.33E-03	5	1.4
mAb10985	457	172	3.63E-03	3.2	471	170	3.36E-03	3.4	0.9
mAb10986	413	155	6.29E-04	18.4	417	148	3.18E-03	3.6	5.1
mAb10987	379	105	2.37E-02	0.5	377	115	8.80E-03	1.3	0.4
mAb10988	467	109	4.35E-02	0.3	492	103	6.98E-02	0.2	1.6
mAb10989	382	126	9.32E-03	1.2	379	105	6.13E-03	1.9	0.7
mAb10970	340	93	7.65E-03	1.5	341	102	6.02E-03	1.9	0.8

mAb10971	350	125	9.44E-04	12.2	352	129	1.70E-03	6.8	1.8
mAb10964	380	140	1.94E-03	6	379	132	3.02E-03	3.8	1.6
mAb10965	290	65	8.66E-03	1.3	284	77	7.40E-03	1.6	0.9
mAb10966	417	152	1.60E-03	7.2	422	151	1.25E-03	9.2	0.8
mAb10967	372	118	2.98E-04	38.8	377	114	4.05E-04	28.5	1.4
mAb10954	336	118	1.74E-03	6.6	335	132	5.33E-03	2.2	3.1
mAb10955	404	100	1.22E-02	0.9	416	96	1.85E-02	0.6	1.5
mAb10956	452	114	1.25E-02	0.9	462	101	2.18E-02	0.5	1.7
mAb10957	388	136	5.80E-04	19.9	390	146	7.93E-04	14.6	1.4
mAb10977	293	44	1.59E-02	0.7	287	46	4.81E-02	0.2	3
mAb10969	340	72	1.86E-02	0.6	344	69	1.33E-02	0.9	0.7
mAb10996	408	35	4.69E-02	0.2	415	42	9.02E-02	0.1	1.9
mAb10998	308	20	2.86E-02	0.4	311	21	2.32E-02	0.5	0.8
mAb11002	373	10	2.60E-02	0.4	371	1	7.15E-04	16.2	0
mAb11000	404	88	1.48E-03	7.8	411	96	2.46E-03	4.7	1.7
mAb11004	356	97	1.47E-02	0.8	362	98	2.70E-02	0.4	1.8
mAb11006	398	105	1.46E-03	7.9	411	93	2.10E-03	5.5	1.4

mAb11008	341	112	1.33E-03	8.7	340	127	1.10E-03	10.5	0.8
mAb11010	432	157	3.90E-03	3	440	156	7.15E-03	1.6	1.8
Isotype Control	430	4	NB	NB	435	15	NB	NB	NB

Example 19: Anti-SARS-CoV-2-S antibodies binding to virus-like particles

[000248] To investigate the ability of a panel of anti-SARS-CoV-2 monoclonal antibodies to bind SARS-CoV-2 Spike glycoprotein, an in vitro binding assay utilizing vesicular stomatitis virus (VSV) pseudotyped with SARS-CoV-2 Spike protein in an electrochemiluminescence based detection platform (MSD) was developed.

[000249] Pseudotyped vesicular stomatitis virus (VSV) viral like particles (VLPs) were generated from HEK293T cells to transiently express the SARS-CoV-2 Spike Protein (Accession number MN908947.3, amino acids 16-1211). VLPs expressing VSV only were also generated as a negative binding control.

[000250] Experiments were carried out according to following procedure. VLPs from the two sources described above were diluted in PBS, seeded into the 96-well carbon electrode plates (MULTI-ARRAY high bind plate, MSD) and incubated overnight at 4 °C to allow the VLPs to adhere. Nonspecific binding sites were blocked by 2% BSA (w/v) in PBS for 1 hour at room temperature. To the plate-bound particles, anti-SARS-CoV-2 antibodies and a non-binding human IgG1 control, diluted in PBS + 0.5% BSA at a range of concentrations from 0.0008nM to 50nM, and buffer with no antibody were added in duplicate and the plates incubated for 1 hour at room temperature with shaking. The plates were then washed with 1X PBS to remove the unbound antibodies using an AquaMax2000 plate washer (MDS Analytical Technologies). The plate-bound antibodies were detected with a SULFO-TAGTM-conjugated anti-human IgG antibody (Jackson Immunoresearch) for 1 hour at room temperature. After washes, the plates were developed with the Read Buffer (MSD) according to manufacturer's recommended procedure and the luminescent signals were recorded with a SECTOR Imager 600 (Meso Scale Development) instrument. The direct binding signals (in RLU) were captured for SARS-CoV-2-expressing VLPs and VSV only VLPs.

[000251] The ability of the anti-SARS-CoV-2-S monoclonal antibodies to bind to SARS-CoV-2-S expressing VLPs compared with binding to irrelevant VSV expressing VLPs was assessed using an immunobinding assay. Binding to the immobilized VLPs on 96-well High Bind plates (MSD) was performed with a series of antibody dilutions and the bound antibodies were detected using SULFO-TAGTM-conjugated anti-human IgG. The binding signals from electrochemiluminescence were recorded on a Sector Imager 600 (MSD). RLU values were determined for the antibody binding to VLPs. All antibodies displayed a concentration-

dependent binding and the ratios of binding on the SARS-COV-2-S expressing VLPs to VSV only were analyzed at 5.5nM and 0.20nM.

[000252] The binding results of anti-SARS-CoV-2-S mAbs at the two concentrations to VSV/spike and VSV-only VLPs are summarized in Table 37. Of 46 antibodies tested, 44 antibodies bound specifically to VSV/spike with a ratio to VSV of 3 or higher at either concentration. At 0.2nM antibody, the ratio of VSV/spike to VSV ranged from 3 to 56, and at 5nM the ratio ranged from 3 to 303. Although two antibodies (mAb10998 and mAb11002) displayed weak binding to the VSV/Spike VLPs, with ratios of less than 3 to the VSV VLPs, the signals at 5nM were higher on the VSV/spike than the VSV. An irrelevant IgG1 isotype antibody showed minimal binding, as expected.

Table 37: Specificity of anti-SARS-COV-2-S antibodies binding to spike protein-expressing VSV VLPs vs VSV by Electrochemiluminescence

	Antibody Binding Signal (RLU)				**Ratio**		
Antibody Concentration	**5.5nM**		**0.20nM**		**5.5nM**	**0.20nM**	
mAb#	**VSV/Spike**	**VSV**	**VSV/Spike**	**VSV**	**VSV/ Spike :VSV**	**VSV/ Spike :VSV**	**Expt#**
mAb10913	1140	302	434	51	4	9	1
mAb10914	6139	1823	911	85	3	11	1
mAb10915	16763	702	2868	77	24	37	1
mAb10920	7757	2536	1332	102	3	13	3
mAb10921	8174	705	938	89	12	11	3
mAb10922	1458	129	562	39	11	6	2
mAb10923	1444	132	446	33	11	14	3
mAb10924	1922	353	375	57	5	7	1
mAb10930	1488	291	429	38	5	4	2
mAb10932	11774	105	1282	35	113	37	1
mAb10933	631	82	446	29	8	16	1

mAb10934	1099	124	648	29	9	22	1
mAb10935	2526	387	611	47	7	13	1
mAb10936	5087	228	1702	41	22	42	1
mAb10937	1056	204	374	43	5	9	1
mAb10938	11418	395	1223	37	29	33	1
mAb10939	4656	637	948	99	7	10	3
mAb10940	947	58	384	34	16	11	1
mAb10941	7297	69	958	17	106	56	1
mAb10954	9727	205	2114	48	47	8	2
mAb10955	2189	270	397	55	8	6	2
mAb10956	1006	373	263	71	3	6	2
mAb10957	10624	127	1606	68	84	11	2
mAb10964	14252	47	9486	26	303	24	2
mAb10965	1039	87	279	58	12	14	2
mAb10966	9176	97	1406	88	95	15	2
mAb10967	10744	122	1090	32	88	8	2
mAb10969	1163	334	262	42	3	6	3
mAb10970	5640	76	1061	50	74	13	2
mAb10971	7995	60	1372	27	134	20	2
mAb10977	26895	4283	9330	165	6	2	2
mAb10982	1875	220	427	36	9	6	2
mAb10984	9142	195	2270	33	47	9	2
mAb10985	1497	90	529	65	17	8	2
mAb10986	11155	177	2315	65	63	11	2
mAb10987	1146	168	699	53	7	8	2
mAb10988	967	163	438	39	6	4	2
mAb10989	2195	128	1533	66	17	13	2

mAb10996	812	309	82	65	3	1	3
mAb10998	2253	1590	122	104	1	1	3
mAb11000	580	139	94	47	4	2	3
mAb11002	419	283	47	50	1	1	3
mAb11004	1061	56	386	28	19	14	3
mAb11006	26528	6299	7159	247	4	29	3
mAb11008	508	48	80	28	11	3	3
mAb11010	349	64	96	30	5	3	3
IgG1 Isotype Control	113	84	32	21	1	2	1
IgG1 Isotype Control	167	127	75	35	1	2	3
IgG1 Isotype Control	94	99	99	31	1	1	2

Example 20: Anti-SARS-CoV-2-S antibodies binding to spike protein-expressing cells

[000253] To investigate the ability of a panel of anti-SARS-CoV-2-S monoclonal antibodies to bind to SARS-CoV-2-S expressing cells, an in vitro binding assay utilizing SARS-CoV-2-S expressing cells in an electrochemiluminescence based detection platform (MSD) was developed.

[000254] Jurkat/Tet3G/hCD20/Tet-3G inducible cells were engineered to transiently express the SARS-CoV-2 Spike Protein (Accession number MN908947.3, amino acids 16-1211, Jurkat/Tet3G/hCD20/Tet-On 3G Inducible COVID-19 Spike Protein High Sorted), and flow cytometry sorted for selection of high expression of the SARS-CoV-2 protein. Parental Jurkat/Tet3G/hCD20/Tet-3G were also included in the experiments as a negative binding control.

[000255] Experiments were carried out according to following procedure. Cells from the two lines described above were induced with 1 μg/ml doxycycline at 37 °C for 36 hours prior to harvest, spun down, washed with PBS, then diluted in PBS, seeded into the 96-well carbon electrode plates (MULTI-ARRAY high bind plate, MSD), and incubated overnight at 4 °C to

allow the cells to adhere. Nonspecific binding sites were blocked by 2% BSA (w/v) in PBS for one hour at room temperature. To the plate-bound cells, anti-SARS-CoV-2 antibodies and a non-binding human IgG1 control, diluted in PBS + 0.5% BSA at a range of concentrations from 0.0008nM to 50nM, and buffer with no antibody were added in duplicate and the plates incubated for one hour at room temperature with shaking. The plates were then washed with 1X PBS to remove the unbound antibodies using an AquaMax2000 plate washer (MDS Analytical Technologies). The plate-bound antibodies were detected with a SULFO-TAGTM-conjugated anti-human IgG antibody (Jackson Immunoresearch) for one hour at room temperature. After washes, the plates were developed with the Read Buffer (MSD) according to manufacturer's recommended procedure and the luminescent signals were recorded with a SECTOR Imager 600 (Meso Scale Development) instrument. The direct binding signals (in RLU) were captured for SARS-CoV-2-S expressing cells and a negative control cell line.

[000256] The ability of the anti-SARS-CoV-2 monoclonal antibodies to bind to SARS-CoV-2 Spike Protein expressing cells compared with binding to parental cells was assessed using an immunobinding assay. Binding to the immobilized cells on 96-well high bind plates (MSD) was performed with a series of antibody dilutions and the bound antibodies were detected using SULFO-TAGTM-conjugated anti-human IgG. The binding signals from electrochemiluminescence were recorded on a Sector Imager 600 (MSD). All antibodies displayed a concentration-dependent binding and the ratio of the binding on spike expressing cells to the parental cells were analyzed at the concentration of 5.5nM and 0.20nM.

[000257] The binding results of the anti-SARS-COV-2-S mAbs at the two concentrations to Spike protein expressing and parental Jurkat cells are summarized in Table 38. Of the 46 antibodies tested, 44 antibodies bound specifically to Jurkat/spike cells (Jurkat/Tet3G/hCD20/Tet-On 3G Inducible SARS-CoV-2 Spike Protein High Sorted cells) with a ratio to the parental cells of 4 or higher at either concentration. At 0.2nM, the ratios of the binding signals on Jurkat/spike cells to the parental cells ranged from 4 to 36, and at 5 nM the ratio ranged from 4 to 63. Although the two antibodies (mAb10998 and mAb11002) displayed weak binding to Jurkat/spike cells with binding ratio to the parental cells less than 4, at 5 nM the binding signals were higher on Jurket/spike than on the parental cells. An irrelevant IgG1 isotype antibody showed minimal binding, as expected.

Table 38: Specificity of anti-SARS-CoV-2-S antibodies binding to spike protein-expressing Jurkat cells vs parental cells by electrochemiluminescence

	Antibody Binding Signal (RLU)				Ratio	
Antibody Concentration	**5.5nM**		**0.2nM**		**5.5nM**	**0.2nM**
mAb#	**Jurkat/ Spike**	**Parental**	**Jurkat/ Spike**	**Parental**	**Jurkat/Spike: Parental**	**Jurkat/Spike:Parental**
mAb10913	907	174	576	36	5	16
mAb10914	1624	569	262	64	3	4
mAb10915	1814	217	269	42	8	6
mAb10920	3501	597	1970	80	6	25
mAb10921	3746	272	436	60	14	7
mAb10922	399	63	225	22	6	10
mAb10923	2561	103	1137	46	25	25
mAb10924	1418	121	336	24	12	14
mAb10930	673	151	175	25	4	7
mAb10932	1525	65	206	29	23	7
mAb10933	898	171	671	73	5	9
mAb10934	762	146	697	46	5	15
mAb10935	1572	209	513	28	8	19
mAb10936	995	116	567	28	9	21
mAb10937	867	95	388	30	9	13
mAb10938	1678	165	195	30	10	7
mAb10939	3195	292	901	119	11	8
mAb10940	657	51	291	19	13	16
mAb10941	1196	37	192	33	33	6
mAb10954	929	110	327	46	8	7

mAb10955	750	134	274	28	6	10
mAb10956	801	136	214	42	6	5
mAb10957	846	76	211	48	11	4
mAb10964	896	37	724	20	24	36
mAb10965	681	49	135	69	14	2
mAb10966	969	65	245	53	15	5
mAb10967	928	121	168	26	8	6
mAb10969	2793	124	774	35	23	22
mAb10970	743	59	246	57	13	4
mAb10971	839	42	263	23	20	12
mAb10977	2031	975	604	76	2	8
mAb10982	737	117	211	25	6	8
mAb10984	889	95	282	26	9	11
mAb10985	527	63	179	65	8	3
mAb10986	1050	92	341	33	11	10
mAb10987	632	83	471	31	8	15
mAb10988	367	83	272	41	4	7
mAb10989	778	62	778	38	13	20
mAb10996	1399	172	185	27	8	7
mAb10998	1277	393	128	65	3	2
mAb11000	1745	70	261	22	25	12
mAb11002	241	160	30	36	2	1
mAb11004	2031	48	748	34	43	22
mAb11006	5052	1055	1044	70	5	15
mAb11008	2382	38	237	50	63	5
mAb11010	387	52	140	33	8	4

IgG1 isotype control	95	34	62	22	3	3
IgG1 isotype control	58	65	21	48	1	0
IgG1 isotype control	64	73	118	62	1	2

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[000258] All references cited herein are incorporated by reference to the same extent as if each individual publication, database entry (*e.g.*, Genbank sequences or GeneID entries), patent application, or patent, was specifically and individually indicated to be incorporated by reference. This statement of incorporation by reference is intended by Applicants to relate to each and every individual publication, database entry (*e.g.*, Genbank sequences or GeneID entries), patent application, or patent identified even if such citation is not immediately adjacent to a dedicated statement of incorporation by reference. The inclusion of dedicated statements of incorporation by reference, if any, within the specification does not in any way weaken this general statement of incorporation by reference. Citation of the references herein is not intended as an admission that the reference is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

CLAIMS

1. An isolated antibody or antigen-binding fragment thereof that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein said isolated antibody or antigen-binding fragment comprises a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID NO: 202, and a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 210.

2. The isolated antibody or antigen-binding fragment thereof of claim 1, which comprises:

(a) an immunoglobulin constant region;

(b) an IgG1 constant region; or

(c) a human IgG1 constant region.

3. The isolated antibody or antigen-binding fragment thereof of claim 1 or claim 2, which comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 216.

4. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-3, which comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 218.

5. The isolated antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein said antibody or antigen-binding fragment:

(a) is multispecific; and/or

(b) is a recombinant antibody or antigen-binding fragment.

6. A pharmaceutical composition, comprising:

i) the isolated antibody or antigen-binding fragment thereof of any one of claims 1-5; and

ii) a pharmaceutically acceptable carrier or diluent.

7. An isolated antibody or antigen-binding fragment thereof that binds a SARS-CoV-2 spike protein comprising the amino acid sequence set forth in SEQ ID NO: 832, wherein

said isolated antibody or antigen-binding fragment comprises a heavy chain variable region (HCVR) comprising the amino acid sequence set forth in SEQ ID NO: 640, and a light chain variable region (LCVR) comprising the amino acid sequence set forth in SEQ ID NO: 646.

8. The isolated antibody or antigen-binding fragment of claim 7, which comprises:

(a) an immunoglobulin constant region;

(b) an IgG1 constant region; or

(c) a human IgG1 constant region.

9. The isolated antibody or antigen-binding fragment of claim 7 or claim 8, which comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 654.

10. The isolated antibody or antigen-binding fragment of any one of claims 7-9, which comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 656.

11. The isolated antibody or antigen-binding fragment thereof of any one of claims 7-10, wherein said antibody or antigen-binding fragment:

(a) is multispecific; and/or

(b) is a recombinant antibody or antigen-binding fragment.

12. A pharmaceutical composition, comprising:

i) the isolated antibody or antigen-binding fragment thereof of any one of claims 7-11; and

ii) a pharmaceutically acceptable carrier or diluent.