

NEUTRALIZING ANTIBODIES THAT BIND THE SARS-COV-2 S PROTEIN

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 63/020,952, filed May 6, 2020; U.S. Provisional Application No. 63/027,812, filed May 20, 2020; U.S. Provisional Application No. 63/063,017, filed August 7, 2020; U.S. Provisional Application No. 63/084,550, filed September 28, 2020, and U.S. Provisional Application No. 63/085,037, filed September 29, 2020, the contents of each of which are incorporated by reference herein in their entirety.

TECHNICAL FIELD

[0002] The present disclosure provides antigen binding proteins that specifically bind the spike protein (S protein) of SARS-CoV-2 and nucleic acids that encode the antigen binding proteins, vectors comprising the nucleic acids, host cells harboring the vectors, and methods of use thereof, including methods of treating SARS-CoV-2 infection and methods of preventing infection with SARS-CoV-2.

BACKGROUND

[0003] The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV), is the causative agent of the deadly Covid-19 pandemic. By the end of June 2020, over 10 million infections were reported worldwide, with over half a million deaths.

[0004] SARS-CoV-2 gains entry to human cells by using the angiotensin-converting enzyme 2 (ACE2) protein as a receptor. The spike (S) proteins of both SARS-CoV and SARS-CoV-2 are transmembrane glycoproteins that form homotrimers. Binding of ACE2 on host cells by the S protein leads to internalization of the virus.

[0005] The SARS-CoV-2 spike protein (S protein, NCBI Accession YP_009724390, isolate "Wuhan-Hu-1") includes two regions or domains known as S1 (the N-terminus to amino acid 685) and S2 (amino acids 686 to 1273) that are cleaved into subunits by a cellular protease during the infection process. The S1 subunit, which mediates the interaction between the Spike (S) protein and ACE2, includes the "N-terminal domain" (NTD) which is followed by the receptor binding domain (RBD) at amino acids 331 to 524. The S2 subunit, which includes an extracellular domain, a transmembrane domain, and a cytoplasmic tail, mediates virus-host membrane fusion that results in entry of the virus into the host cell.

[0006] There is an urgent need to find therapeutic and prophylactic agents that can be rapidly deployed to halt the destructive spread of the SARS-CoV-2 pandemic.

SUMMARY

[0007] To treat and prevent infection of individuals with SARS-CoV-2 and, potentially, other related coronaviruses, fully human neutralizing antibodies have been engineered that are able to inhibit binding of SARS-CoV-2 to target cells expressing the ACE2 protein. In some embodiments these antibodies are further engineered to include mutations of the Fc region, such as mutations to reduce antibody-dependent-enhancement (ADE) of infection.

[0008] Provided herein in a first aspect are antigen-binding proteins that specifically bind the spike (S) protein of the SARS-CoV-2 coronavirus (Genbank Accession QHD43416), where the antigen-binding proteins comprise a heavy chain variable domain sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6 and a light chain variable region having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7. In some examples an antigen-binding protein as provided herein that specifically binds the S protein of the SARS-CoV-2 coronavirus comprises a heavy chain variable domain having the sequence of SEQ ID NO:6 and a light chain variable domain having the sequence of SEQ ID NO:7.

[0009] In a related aspect are antigen-binding proteins are provided that specifically bind the S protein of SARS-CoV-2 where the antigen-binding proteins comprise a heavy chain variable region comprising a heavy chain complementarity-determining region (CDR) 1 sequence having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 sequence having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 sequence having the amino acid sequence of SEQ ID NO:10, and further comprise a light chain variable region comprising a light chain CDR1 sequence having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 sequence having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 sequence having the amino acid sequence of SEQ ID NO:13. In various embodiments the antigen-binding proteins having the heavy chain CDR1 sequence of SEQ ID NO:8, the heavy chain CDR2 sequence of SEQ ID NO:9, the heavy chain CDR3 sequence of SEQ ID NO:10, the light chain CDR1 sequence of SEQ ID NO:11, the light chain CDR2 sequence of SEQ ID NO:12, and the

light chain CDR3 sequence of SEQ ID NO:13 and have a heavy chain variable region comprising an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising an amino acid sequence 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7.

[0010] An antigen-binding protein as provided herein can be, comprise, or be derived from an antibody, such as an IgG, IgA, IgD, IgE, or IgM antibody that specifically binds the S protein of SARS-CoV-2. In various embodiments the anti-S protein antibody comprises or is derived from an IgG1, IgG2, IgG3, or IgG4 antibody. For example, the anti-S protein antibody or antigen-binding protein can comprise or be derived from an IgG1 or IgG4 antibody.

[0011] In further embodiments, an antigen-binding protein as provided herein comprising a heavy chain variable domain sequence having at least 95% amino acid sequence identity to SEQ ID NO:6 and a light chain variable region having at least 95% amino acid sequence identity to SEQ ID NO:7 and/or having the heavy chain CDR sequences of SEQ ID NOs:8, 9, and 10 and the light chain CDR sequences of SEQ ID NOs:11, 12, and 13 can be or comprise an antibody fragment, such as for example a Fab fragment, a Fab' fragment, or F(ab')₂ fragment. In additional embodiments an antigen-binding protein as provided herein having an amino acid sequence with at least 95% identity to SEQ ID NO:6 and an amino acid sequence with at least 95% identity to SEQ ID NO:7 and/or having the heavy chain CDR sequences of SEQ ID NOs:8, 9, and 10 and the light chain CDR sequences of SEQ ID NOs:11, 12, and 13 can be or comprise a single chain antibody (*e.g.*, an ScFv).

[0012] In some embodiments, the antigen-binding protein provided herein is or is derived from a fully human antibody or a fully human antibody fragment, for example, a fully human IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, or IgM, or a fully human single chain antibody, fully human Fab fragment, a single chain antibody, or is an antigen binding protein derived from or comprising any of these.

[0013] In some embodiments the antibody is an IgG antibody having one or more mutations in the Fc region, for example one or more mutations that decreases antibody dependent enhancement (ADE) and/or one or more mutations that increases antibody half-life. In some embodiments the antibody has one or more mutations in the Fc region that reduce ADE selected from L234A; L235A or L235E; N297A, N297Q, or N297D; and P329A or P329G. For example,

the anti-S antibody can include the mutations L234A and L235A (referred to as a LALA mutant). Alternatively or in addition, the antibody may have one or more mutations in the Fc region that increase the half-life of the antibody in serum, for example, the antibody may have one or more mutations selected from M252Y; S254T; T256D or T256E; T307Q or T307W; M428L; and N434S. For example, the anti-S antibody can include the mutations M252Y; S254T; and T256E (referred to as a YTE mutant).

[0014] In some embodiments, the antigen-binding protein that specifically binds the S protein of SARS-CoV-2, which can be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein comprising or derived from any thereof, specifically binds a coronavirus spike protein (e.g., a spike protein comprising SEQ ID NO:1 or SEQ ID NO:2, or a spike protein of a coronavirus comprising a sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:1 or SEQ ID NO:2) with a K_d of less than 200 nM, less than 100 nM, less than 50 nM, less than 10 nM, less than 1 nM, less than 0.1 nM, or less than 0.01 nM. For example, the antigen-binding protein can bind the S protein of a coronavirus (e.g., the S protein of SARS-CoV-2) with a K_d of between about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In some embodiments, the antibody is the S1D2 antibody having a heavy chain variable sequence of SEQ ID NO:6 and a light chain variable sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, having a K_d for binding the S protein of SARS-CoV-2 of between about 100 nM and about 1 nM or between about 60 nM and about 5 nM. In some embodiments, any K_d described herein for binding the S protein is determined by surface plasmon resonance, e.g., as described in Example 3.

[0015] In some embodiments, an antigen-binding protein provided herein, which can in various embodiments be derived from, comprise, or be an antibody, such as a fully human antibody, and may be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein derived from any thereof, specifically binds the S1 subunit of a coronavirus S protein (e.g., SEQ ID NO:4 or an S1 subunit of a spike protein of a coronavirus comprising a sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:4) with a K_d of less than 200 nM, less than 100 nM, less than 50 nM, less than 10 nM, less than 1 nM, less than 0.1 nM, or less than 0.01 nM. For example, the antigen-binding protein can bind the S1 subunit of a coronavirus (e.g., the S1 subunit of SARS-CoV-2) with a K_d

of between of between about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In one embodiment, the antibody is the fully human S1D2 antibody or antibody fragment, or an antigen-binding protein derived therefrom, having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the S1 subunit of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM or between about 60 nM and about 5 nM.

[0016] In some embodiments, an antigen-binding protein provided herein, which can be in various embodiments be an antibody, such as a fully human antibody, and may be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein derived from any thereof, specifically binds the receptor binding domain (RBD) of a coronavirus S protein (e.g., SEQ ID NO:5 or an RBD of a spike protein of a coronavirus comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:5) with a K_d of less than 100 nM, less than 50 nM, less than 10 nM, less than 1 nM, less than 0.1 nM, or less than 0.01 nM. For example, the antigen-binding protein can bind the RBD of the S protein of a coronavirus (e.g., the RBD the SARS-CoV-2 S protein) with a K_d of between of between about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In some embodiments, the antigen binding protein is the fully human S1D2 antibody having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the RBD of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM or between about 60 nM and about 2 nM. For example, the antigen binding protein may be the fully human S1D2 antibody disclosed herein having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally having L234A and L235A mutations in the Fc region, where the antibody binds the RBD of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM, or between about 60 nM and about 5 nM, or between 50 nM and 40 nM as measured by SPR.

[0017] In various embodiments, the antigen-binding proteins described herein block binding between the S protein of a coronavirus (such as HCoV-NL63, SARS-CoV, or SARS-CoV-2) and the ACE2 protein, for example, block binding of the ectodomain of the human ACE2 protein (hACE2) by the S protein of a coronavirus. In various embodiments, the antigen-binding proteins

described herein block binding between the S protein of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of between about 0.01 $\mu\text{g/ml}$ and about 100 $\mu\text{g/ml}$, between about 0.05 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, or between about 0.1 $\mu\text{g/ml}$ and about 5 $\mu\text{g/ml}$. In some embodiments, the antigen binding proteins described herein block binding between the S1 domain or subunit of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of between about 0.01 $\mu\text{g/ml}$ and about 100 $\mu\text{g/ml}$, between about 0.05 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, or between about 0.1 $\mu\text{g/ml}$ and about 5 $\mu\text{g/ml}$. For example, an antigen-binding protein as disclosed herein can block the binding of the S1 subunit of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of less than 100 nM, less than 50 nM, less than 10 nM, less than 5 nM, or less than 1 nM. In some embodiments, any IC_{50} described herein is determined according to a procedure described herein, e.g., as set forth in Example 7 (for blocking the binding of the S1 subunit of SARS-CoV-2 and the human ACE2 protein) or Example 8 (for neutralization of infection).

[0018] In some embodiments, an IC_{50} for blocking the binding of the S1 subunit of SARS-CoV-2 and the human ACE2 protein is determined using an ELISA wherein wells are coated with about 1 $\mu\text{g/mL}$ recombinant ACE2-Fc (e.g., the ACE2 polypeptide comprising the amino acid sequence of SEQ ID NO:23 fused to an Fc region (SEQ ID NO:25)), e.g., overnight at 4 °C. The plates may be washed three times with PBS-Tween PBS-T and blocked for 1 hour with 170 μL Blocker Casein in PBS at room temperature. The plates may then be washed three times with PBS-T. Two-fold serial dilutions of the antibody, e.g., starting from a concentration of 60 $\mu\text{g/mL}$ may be mixed 1:1 with about 3 $\mu\text{g/mL}$ recombinant SARS-CoV-2 S1 protein (e.g., SEQ ID NO:4), optionally including a his tag. Twenty-five μL of the S1D2 or test antibody dilutions /S1 protein mixtures may be added to the ELISA plate and incubated for 1 hour with shaking. The plate may be washed three times with PBS-T, then a secondary antibody such as rabbit anti-His polyclonal antibody-HRP (diluted at 1:5000 in casein) may be added and the plate may be incubated for 1 hour. Subsequently, TMB (3,3',5,5'-tetramethylbenzidine) may be added as substrate and developed for 30 minutes. The reaction may be stopped using 2 M H_2SO_4 and the OD may be read at 450 nm.

[0019] In some embodiments, an IC_{50} for neutralization of infection is determined using VeroE6 cells (e.g., in an amount of about 2×10^4 cells) and 100 x 50% tissue culture infective doses ($TCID_{50}$) of SARS-CoV-2, e.g., comprising a spike protein comprising the amino acid

sequence of SEQ ID NO: 2. The assay may be run in a volume of about 25 μ L (e.g., of DMEM+2% FBS) for about 1 hour and incubated at 37°C in a 5% CO₂ atmosphere. Virus supernatant may be removed and replaced with fresh medium, e.g., after about 1 hour of culture at 37 °C. Cytopathic effect (CPE), i.e., the appearance of plaques or discontinuity in the cell monolayer due to cell lysis, may be recorded on day 3 post-infection. At the end of the study, the media may be aspirated and the cells may be fixed with formalin and stained with 0.25% crystal violet. The concentration of antibody that completely prevents CPE in 50% of the wells (IC₅₀) may be calculated following the Reed & Muench method.

[0020] For example, an antigen-binding protein as disclosed herein that includes an amino acid sequence having at least 95% identity to SEQ ID NO:6 and an amino acid sequence having at least 95% identity to SEQ ID NO:7 can in some embodiments block the binding of the S1 subunit of SARS-CoV-2 (e.g., SEQ ID NO:4) to the ACE2 polypeptide (or the ACE2 ectodomain, e.g., SEQ ID NO:23 or SEQ ID NO:24) with an IC₅₀ of less than 100 nM, less than 50 nM, less than 10 nM, less than 5 nM, or less than 1 nM, for example, with an IC₅₀ of between about 100 nM and about 0.1 nM or between about 50 nM and about 0.5 nM, or between about 10 nM and about 1 nM, and in some embodiments between about 5 nM and about 1 nM. In some embodiments the antigen binding protein is the fully human S1D2 antibody having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the S1 subunit of the S protein of SARS-CoV-2 and can block binding of the SARS-CoV-2 S1 subunit to the ACE2 protein or the ectodomain thereof with an IC₅₀ of between about 10 nM and about 1 nM, for example between about 5 nM and about 1 nM.

[0021] In various embodiments provided herein, an antigen-binding protein as disclosed herein, which can be or comprise, as nonlimiting examples, an IgG1, IgG2, IgG3, or IgG4, a Fab fragment or a single chain antibody, or can be an antigen binding protein derived from or comprising any of these, is a neutralizing antigen binding protein that is able to inhibit binding to a target cell by a coronavirus such as HCoV-NL63, SARS-CoV, or SARS-CoV-2. For example, in various embodiments the antigen-binding protein, when included in a mixture that includes coronavirus and target cells expressing the ACE2 receptor, can reduce binding of the coronavirus to cells expressing the ACE2 receptor with an IC₅₀ of between about 0.001 μ g/ml and about 200 μ g/ml, or between about 0.01 μ g/ml and about 100 μ g/ml, or between about 0.01 μ g/ml and

about 50 µg/ml, or between about 0.01 µg/ml and about 10 µg/ml, or between about 0.01 µg/ml and about 5 µg/ml, or between about 0.01 µg/ml and about 1 µg/ml, or between about 0.1 µg/ml and about 100 µg/ml, or between about 0.1 µg/ml and about 50 µg/ml. In some embodiments an antigen-binding protein can reduce binding of the coronavirus to a cell expressing the ACE2 receptor with an IC₅₀ of between about 1 µg/ml and about 50 µg/ml, or between about 1 µg/ml and about 10 µg/ml, or between about 1 µg/ml and about 5 µg/ml. In additional embodiments an antigen-binding protein can reduce binding of the coronavirus to a cell expressing the ACE2 receptor with an IC₅₀ of between about 0.1 µg/ml and about 10 µg/ml, or between about 0.1 µg/ml and about 5 µg/ml, or between about 0.1 µg/ml and about 1 µg/ml.

[0022] In various embodiments, the anti-S antigen binding proteins disclosed herein are neutralizing antigen-binding proteins, e.g., neutralizing antibodies, that can block infection of target cells, for example, can inhibit a cytopathic effect (CPE) resulting from infection by a coronavirus such as SARS-CoV or SARS-CoV-2 of susceptible cells with an IC₅₀ of between about 0.01 µg/ml and about 100 µg/ml, or between about 0.1 µg/ml and about 50 µg/ml, or between about 0.1 µg/ml and about 25 µg/ml, or between about 0.1 µg/ml and about 10 µg/ml, or between about 0.5 nM and about 500 nM, or between about 1 nM and about 200 nM, or between about 1 nM and about 100 nM, or between about 1 nM and about 50 nM. For example, in some embodiments the antigen binding protein is the fully human S1D2 antibody having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the S1 subunit of the S protein of SARS-CoV-2 and can inhibit CPE with an IC₅₀ of between about 5 nM and about 50 nM.

[0023] In a further aspect provided herein are pharmaceutical compositions that include an antigen-binding protein as disclosed herein that specifically binds the S protein of a coronavirus, such as the S protein of SARS-CoV-2, and a pharmaceutically carrier. The pharmaceutical can be formulated for intramuscular or subcutaneous injection, or for intravenous, oral, intranasal, or pulmonary delivery, as nonlimiting examples. The pharmaceutical composition can optionally be formulated as a liquid, solid, or gel, depending on the mode of delivery and/or storage and packaging considerations, and can optionally be formulated and packaged in single doses.

[0024] Also provided herein in another aspect is a method of treating a subject infected or suspected of being infected with a coronavirus such as SARS-CoV or SARS-Cov-2. The method

includes administering an effective amount of the antibody disclosed herein, for example in a pharmaceutical formulation as disclosed herein, to the subject. Administration can be, as nonlimiting examples, by intramuscular or subcutaneous injection, by intravenous delivery (e.g., by bolus injection or infusion), by oral delivery, by intranasal delivery, or by pulmonary delivery, for example by inhalation.

[0025] Further included is a method of preventing infection with a coronavirus such as SARS-CoV or SARS-Cov-2. The method includes administering an effective amount of the antibody disclosed herein, for example in a pharmaceutical formulation as disclosed herein, to the subject. Administration can be, as nonlimiting examples, by intramuscular or subcutaneous injection, or by intravenous, oral, intranasal, or pulmonary delivery, such as by inhalation.

[0026] Yet another aspect are methods of detecting a coronavirus using an antigen-binding protein that specifically binds the S protein of a coronavirus as disclosed herein. The methods include detecting the presence of a coronavirus, or a protein of a coronavirus, e.g., an S protein or S1 subunit of a coronavirus, in a sample, comprising: (a) contacting the sample with an antigen-binding protein as disclosed herein under conditions suitable to form an antibody-antigen complex; and (b) detecting the presence of the antibody-antigen complex to detect the presence of a coronavirus or protein thereof. In some embodiments, this method can be used to detect the presence of a coronavirus in a sample from a subject and thereby diagnose a subject suspected of having a coronavirus infection. In various embodiments the sample from the subject comprises phlegm, mucous, saliva, blood, pleural fluid, cheek scraping, tissue biopsy, or semen. In some embodiments, the antigen-binding protein that specifically binds the S protein, which can be an antibody or antibody fragment that specifically binds the S protein, can be labeled for direct or indirect detection of an antigen-antibody complex, where the label can comprise a radionuclide, fluorophore, enzyme, enzyme substrate, enzyme cofactor, enzyme inhibitor, or ligand (e.g., biotin, a hapten). In various embodiments, the presence of the antibody-antigen complex can be detected using any detection mode including detection of radioactivity, detection of fluorescence, detection of luminescence, or colorimetric, antigenic, or enzymatic detection, or detection of a magnetic or electrodense (e.g., gold) bead, and may optionally use binding moieties such as but not limited to biotin, streptavidin, or protein A.

[0027] Also included herein are nucleic acid molecules encoding an antigen-binding protein as provided herein comprising a heavy chain variable domain sequence having at least 95% amino

acid sequence identity to SEQ ID NO:6 and a light chain variable region having at least 95% amino acid sequence identity to SEQ ID NO:7, and/or having the heavy chain CDR sequences having the amino acid sequences of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, and the light chain CDR sequences of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13. A nucleic acid molecule that encodes one or both of a heavy chain variable domain sequence having at least 95% amino acid sequence identity to SEQ ID NO:6 and a light chain variable region having at least 95% amino acid sequence identity to SEQ ID NO:7 or includes the heavy chain CDRs 1, 2, and 3 of SEQ ID NOs:8, 9, and 10, respectively and the light chain CDRs 1, 2, and 3 of SEQ ID NOs:11, 12, and 13, respectively can be an expression vector that includes a promoter operably linked to the antigen-binding protein encoding sequence. For example, the expression vector can be a viral or plasmid vector and the promoter in some examples is a eukaryotic promoter and can be, as nonlimiting examples, an EF1a promoter, a CMV promoter, a JeT promoter, an RSV promoter, an SV40 promoter, a CAG promoter, a beta-actin promoter, an HTLV promoter, or an EF1a/HTLV hybrid promoter. The expression vector can be, for example, a viral or plasmid vector, and in some examples can be a nanoplasmid vector having fewer than 500 bp of a bacterial plasmid and having fewer than 10 CpG sequences.

[0028] Also provided herein is a pharmaceutical formulation comprising one or more nucleic acid molecules that encode an antigen-binding protein as provided herein. The pharmaceutical formulation can include the nucleic acid molecule(s) and one or more excipients or carriers. In some examples the pharmaceutical composition can include one or more compounds that enhance delivery of nucleic acid molecules into cells, such as for example a cationic lipid or amphiphilic block copolymers, for example, one or more poloxamers or poloxamines.

[0029] Also provided herein are methods for treating or preventing a coronavirus infection by administering an effective amount of a pharmaceutical composition as provided herein that includes at least one nucleic acid molecule encoding an antigen-binding protein as disclosed herein. The administering can be, for example, by injection, such as intradermal or intramuscular injection. Single or multiple doses, including multiple doses over weeks or months, can be administered. The amount of DNA (e.g., plasmid or plasmids encoding a neutralizing antibody) to be delivered can be determined for example, at least in part by experiments on non-human animals.

[0030] Further included are transgenic cells engineered to express an antibody comprising a heavy chain variable domain sequence having at least 95% amino acid sequence identity to SEQ ID NO:6 and a light chain variable region having at least 95% amino acid sequence identity to SEQ ID NO:7. The cells can be prokaryotic or eukaryotic. In some embodiments the transgenic cells are mammalian cells, such as cells of a mammalian cell line.

[0031] The embodiments described herein include, without limitation, the following. Embodiment 1 is an isolated antigen-binding protein that specifically binds the spike (S) protein of SARS-CoV-2, wherein the antigen-binding protein comprises a heavy chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:6 and a light chain variable domain having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7.

[0032] Embodiment 2 is an isolated antigen-binding protein that specifically binds the S protein of SARS-CoV-2, comprising a heavy chain variable region comprising a heavy chain complementarity determining region (CDR) 1 having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO:10, and a light chain variable region comprising a light chain CDR1 having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 having the amino acid sequence of SEQ ID NO:13.

[0033] Embodiment 3 is the isolated antigen-binding protein according to embodiment 2, wherein the heavy chain variable domain has at least 95% sequence identity to the amino acid sequence of SEQ ID NO:6 and the light chain variable domain has at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7.

[0034] Embodiment 4 is the isolated antigen-binding protein according to embodiment 2, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:6 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:7.

[0035] Embodiment 5 is the isolated antigen-binding protein of any one of embodiments 1-4, wherein the antigen-binding protein binds the S protein of SARS-CoV-2 with a dissociation constant (K_d) of 10^{-7} M or less.

[0036] Embodiment 6 is the isolated antigen-binding protein of embodiment 5, wherein the antigen-binding protein binds the S protein of SARS-CoV-2 with a K_d of 10^{-8} M or less.

[0037] Embodiment 7 is the isolated antigen-binding protein of any of embodiments 1-6, wherein the antigen-binding protein is an antibody or antibody fragment.

[0038] Embodiment 8 is the isolated antigen-binding protein of any of embodiments 1-6, wherein the antigen-binding protein is a fully human antibody, comprises a heavy chain variable region and a light chain variable region of a fully human antibody, or comprises an antibody fragment derived from a fully human antibody.

[0039] Embodiment 9 is the isolated antigen-binding protein of any of embodiments 1-8, comprising an IgG antibody, which is optionally an IgG1, IgG2, IgG3, or IgG4 antibody.

[0040] Embodiment 10 is the isolated antigen-binding protein of embodiment 9, comprising an IgG1 or IgG4 antibody.

[0041] Embodiment 11 is the isolated antigen-binding protein of embodiment 10, wherein the IgG1 or IgG4 antibody comprises a mutant Fc region.

[0042] Embodiment 12 is the isolated antigen-binding protein of embodiment 11, wherein the Fc region comprises at least one mutation that decreases antibody-dependent-enhancement (ADE) of infection.

[0043] Embodiment 13 is the isolated antigen-binding protein of embodiment 11 or 12, wherein Fc region comprises one or more mutations selected from N297A, N297Q, N297D, L234A, L235A, L235E, P329A, and P329G.

[0044] Embodiment 14 is the isolated antigen-binding protein of embodiment 13, wherein Fc region comprises the mutations L234A and L235A (LALA), optionally wherein the Fc region comprises the sequence of SEQ ID NO: 14 or 16.

[0045] Embodiment 15 is the isolated antigen-binding protein of any one of embodiments 11-14, wherein the Fc region comprises at least one mutation that increases antibody half-life.

[0046] Embodiment 16 is the isolated antigen-binding protein of any one of embodiments 11-15, wherein Fc region comprises one or more mutations selected from M252Y, T256D, T307Q, T307W, M252Y, S254T, T256E, M428L, and N434S.

[0047] Embodiment 17 is the isolated antigen-binding protein of embodiment 16, wherein Fc region comprises the mutations M252Y, S254T, and T256E (YTE), optionally wherein the Fc region comprises the sequence of SEQ ID NO: 15 or 16.

[0048] Embodiment 18 is the isolated antigen-binding protein of any of embodiments 1-17, wherein the antigen-binding protein is a Fab fragment, a Fab' fragment, or a F(ab')₂ fragment.

[0049] Embodiment 19 is The isolated antigen-binding protein of any of embodiments 1-17, wherein the antigen-binding protein is a single chain antibody, optionally wherein the single chain antibody is an ScFv.

[0050] Embodiment 20 is the isolated antigen-binding protein of any one of the preceding embodiments, wherein the antigen-binding protein is a neutralizing antibody or antigen-binding fragment thereof that blocks binding of the SARS-CoV-2 S protein to the ACE2 protein.

[0051] Embodiment 21 is the isolated antigen-binding protein of embodiment 20, wherein the IC₅₀ for blocking of binding of S to the ACE2 protein is 10 nM or less.

[0052] Embodiment 22 is The isolated antigen-binding protein of embodiment 21, wherein the IC₅₀ for blocking of binding of S to the ACE2 protein is 5 nM or less.

[0053] Embodiment 23 is the isolated antigen-binding protein of any one of the preceding embodiments, wherein the antigen-binding protein is a neutralizing antibody or antigen-binding fragment thereof that blocks SARS-CoV-2 infection of target cells.

[0054] Embodiment 24 is the isolated antigen-binding protein of embodiment 23, wherein the IC₅₀ for blocking infection of target cells by SARS-CoV-2 is 10 nM or less.

[0055] Embodiment 25 is The isolated antigen-binding protein of embodiment 24, wherein the IC₅₀ for blocking infection of target cells by SARS-CoV-2 is 5 nM or less.

[0056] Embodiment 26 is a pharmaceutical composition, comprising the antigen-binding protein of any one of the preceding embodiments and a pharmaceutically-acceptable excipient.

[0057] Embodiment 27 is the pharmaceutical composition of embodiment 26, wherein the antigen-binding protein is an IgG antibody, a Fab, Fab' or F(ab')₂ fragment, or a single chain antibody.

[0058] Embodiment 28 is the pharmaceutical composition of embodiment 27, wherein the antigen-binding protein is an IgG antibody.

[0059] Embodiment 29 is the pharmaceutical composition of embodiment 28, wherein the antigen-binding protein is an IgG antibody having an Fc region comprising L234A and L235A mutations.

[0060] Embodiment 30 is a nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide comprising a heavy chain variable region having at least 95% identity to SEQ ID NO:6.

[0061] Embodiment 31 is the nucleic acid molecule of embodiment 30, wherein the nucleic acid molecule comprises a vector, wherein the vector comprises a promoter operably linked to the nucleic acid sequence encoding a polypeptide comprising the heavy chain variable region.

[0062] Embodiment 32 is a nucleic acid molecule that comprises a nucleic acid sequence encoding a polypeptide comprising a light chain variable region having at least 95% identity to SEQ ID NO:7.

[0063] Embodiment 33 is the nucleic acid molecule of embodiment 32, wherein the nucleic acid molecule comprises a vector, wherein the vector comprises a promoter operably linked to the nucleic acid sequence encoding a polypeptide comprising the light chain variable region.

[0064] Embodiment 34 is a vector comprising a nucleic acid sequence encoding a heavy chain variable region having at least 95% identity to SEQ ID NO:6 and a nucleic acid sequence encoding a light chain variable region having at least 95% identity to SEQ ID NO:7.

[0065] Embodiment 35 is One or more nucleic acid molecules encoding the antigen-binding protein of any one of embodiments 1-25.

[0066] Embodiment 36 is One or more vectors comprising the one or more nucleic acid molecules of embodiment 35.

[0067] Embodiment 37 is a host cell harboring the nucleic acid molecule or vector of any one of embodiments 30-36.

[0068] Embodiment 38 is a pharmaceutical composition comprising the one or more nucleic acid molecules of embodiment 35.

[0069] Embodiment 39 is a method for preparing a neutralizing antibody or a heavy or light chain thereof, the method comprising: culturing a population of the host cell of embodiment 37 under conditions suitable for expressing the heavy chain variable region and/or the light chain variable region or the antigen-binding protein.

[0070] Embodiment 40 is the method of embodiment 39, further comprising: recovering from the host cells the expressed heavy chain variable region and/or the light chain variable region or the antigen-binding protein.

[0071] Embodiment 41 is a method for treating a subject having or suspected of having a coronavirus infection, the method comprising: administering to the subject an effective amount of the antigen-binding protein of any one of embodiments 1-25 or the pharmaceutical composition of any one of embodiments 26-29.

[0072] Embodiment 42 is a method for treating a subject having or suspected of having a coronavirus infection, comprising: administering a pharmaceutical composition of any one of embodiments 26-29 to the subject, wherein said administration is by pulmonary delivery by inhalation.

[0073] Embodiment 43 is the method of embodiment 42, wherein said pulmonary delivery is by a nebulizer.

[0074] Embodiment 44 is a method for treating a subject having or suspected of having a coronavirus infection, comprising: administering a pharmaceutical composition of any one of embodiments 26-29 to the subject, wherein said administration is by intranasal delivery.

[0075] Embodiment 45 is the method of any of embodiments 41-44, further comprising administering to the subject an anti-viral agent.

[0076] Embodiment 46 is the method of any one of embodiments 39-45, wherein the subject has a coronavirus infection.

[0077] Embodiment 47 is the method of any one of embodiments 39-45, wherein the coronavirus infection is a SARS-CoV-2 infection.

[0078] Embodiment 48 is a method for detecting the presence of a coronavirus in a sample, comprising: contacting the sample with the antigen-binding protein of any one of claims 1-25 under conditions suitable to form an antibody-antigen complex, wherein the sample contains a target antigen; and detecting the presence of the antibody-antigen complex.

[0079] Embodiment 49 is the method of embodiment 48, wherein the sample comprises phlegm, saliva, blood, cheek scraping, tissue biopsy, hair or semen.

[0080] Embodiment 50 is the method of embodiment 48 or 49, wherein the antibody-antigen complex is detected using a radioactive, colorimetric, antigenic, enzymatic, a detectable bead (such as a magnetic or electrodense (e.g., gold) bead), biotin, streptavidin or protein A-based mode of detection.

[0081] Embodiment 51 is a method for diagnosing a subject suspected of having a coronavirus infection using the method of any one of embodiments 48-50.

[0082] Embodiment 52 is the antigen-binding protein of any one of embodiments 1-25 for use in the method of any one of embodiments 42-51.

[0083] Embodiment 53 is the use of the antigen-binding protein of any one of embodiments 1-25 for the manufacture of a medicament for treating, detecting, or diagnosing according to the method of any one of embodiments 42-51.

BRIEF DESCRIPTION OF THE DRAWINGS

[0084] **Figure 1** is a schematic illustrating the phenomenon where (left panel ‘Infected’): coronaviruses are shown binding to the ACE2 protein on the surface of a subject’s target cells where the subject is not treated with a neutralizing antibody (“untreated”), leading to infection; (middle panel ‘Infected due to ADE’): the subject is treated with an antibody that binds the coronavirus S protein, but the Fc region of the antibody is bound by the Fc receptor on the surface of antigen-presenting immune cells (e.g., macrophages), leading to antibody-dependent-enhancement (ADE) of infection; and finally (rightmost panel, ‘Covi-Guard Treated, Protected’): a neutralizing antibody with a mutation in the Fc region that reduces ADE (“LALA”) binds the S protein of the coronavirus but does not bind to the Fc receptor on antigen presenting cells. In this scenario, there is no significant ADE, but the blockade of coronavirus binding to and infecting ACE2-expressing target cells is maintained (“Full Viral Blockade”).

[0085] **Figure 2** is a bar graph showing the results of an ELISA in which thirteen IgG antibodies engineered as fully human antibodies and an isotype control were assayed for binding to (shown in the bars proceeding from left to right for each of the tested antibodies): the S1 subunit protein of SARS-CoV-2, the RBD of the S protein, the S protein in trimeric form, and no antigen (control). Binding is measured as absorbance at 450 nm.

[0086] **Figure 3** is an IC₅₀ curve for the S1D2 antibody inhibition of binding of the SARS-CoV-2 S1 protein binding to the ACE2 protein in an ELISA format. The table below provides the IC₅₀ as 1.867×10^{-9} M.

[0087] **Figure 4** provides a sensorgram for S1D2 binding to the RBD of the SARS-CoV-2 S protein and a table of the binding parameters.

[0088] **Figure 5** is an image of binding of the S1D2 antibody to the S1 subunit fixed to nitrocellulose. The two leftmost spots are binding of S1D2 to the native (untreated) S1 protein, the middle two spots are binding of S1D2 to heat treated S1 protein, and the rightmost two spots (not visible) show no binding of S1D2 to reduced and heat treated S1.

[0089] **Figure 6** is a bar graph providing the results of an ELISA testing S1D2 binding to the D614G mutant S1 protein as well as to non-mutant and mutant forms of the RBD of the SARS-CoV-2 S protein.

[0090] **Figure 7** provides sensorgrams of the S1D2 antibody binding to the non-mutated S1 protein of the WA strain of SARS-CoV-2 and the S1 protein having the D614G mutation and a table that provides binding parameters.

[0091] **Figure 8** provides sensorgrams of the S1D2 antibody binding to the non-mutated RBD of SARS-CoV-2 and mutant forms of the RBD having the mutations 357D, 364Y; v367F; and W436R, as well as a table that provides binding parameters.

[0092] **Figure 9** shows the results of flow cytometry in which the neutralizing antibody S1D2_{LALA} (“COVI-GUARD™”, having the LALA mutation in the Fc region) binds with high specificity to the full-length SARS-CoV-2 spike protein expressed by transfected HEK283T cells.

[0093] **Figure 10** provides data from binding for the binding of the S1D2_{LALA} antibody to cells expressing the wild-type SARS-CoV-2 S1 protein (“Spike WT”, lower curve) and the SARS-CoV-2 spike protein having the D614G mutation (“Spike D614G”, upper curve). The table below the graph provides the EC₅₀.

[0094] **Figure 11** shows binding data in which the neutralizing antibody S1D2_{LALA} inhibits interaction between recombinant ACE2 and SARS-CoV-2 S1 spike protein (IC₅₀ of 4.88 nM).

[0095] **Figure 12** shows the results of an infection neutralization assay in which the neutralizing antibody S1D2_{LALA} provides potent inhibition of CPE in SARS-CoV-2 infected cells (IC₅₀ of 3.13 µg/ml).

[0096] **Figure 13** provides a graph of inhibition of the cytopathic effect (CPE) of the WA strain of SARS-CoV-2 and the 2020001 strain of SARS-CoV-2 having an S1 protein with the D614G mutation in response to antibody concentration.

[0097] **Figure 14** is a bar graph demonstrating ADCC of S1D2 antibodies is not significantly different from a no antibody control.

[0098] **Figure 15** is a schematic of an assay for assessing ADE.

[0099] **Figure 16** is a graph of antibody serum concentration over time in mice administered the S1D2_{LALA} antibody.

[00100] **Figure 17A-B** provides graphs of biodistribution of the COVI-GUARD™ antibody in mice **(A)** intravenously injected or **(B)** intranasally inoculated with the antibody. In **(A)**, for each tissue type, bars from left to right provide the concentration of S1D2_{LALA} present in the tissue in mice injected with no antibody, 0.5 mg/kg, 0.05 mg/kg, and 0.005 mg/kg S1D2_{LALA}. In **(B)**, for each tissue type, bars from left to right provide the concentration of S1D2_{LALA} present in the tissue in mice inoculated with no antibody, 2.5 mg/kg, 0.5 mg/kg, 0.05 mg/kg, and 0.005 mg/kg S1D2_{LALA}.

[00101] **Figure 18A-D:** **(A)** provides a schematic of the infection, injection, and clinical observation schedule for a pre-clinical in vivo hamster study on treatment with S1D2_{LALA} (STI-1499). **(B)** provides graphs showing the % weight change recorded over the study for members of the treatment groups. **(C)** provides a graph in which the average % weight change over the course of the study for each treatment group is shown. The upper curve represents the average values for the uninfected isotype control group and the lower curve represents the average values for the infected isotype control group. **(D)** provides a graph in which the titers of virus present in the lungs of animals sacrificed at day five are represented individually.

DESCRIPTION

[00102] Headings provided herein are solely for the convenience of the reader and do not limit the various aspects of the disclosure, which aspects can be understood by reference to the specification as a whole.

[00103] The disclosures of all publications, patents, and patent applications cited herein are hereby incorporated by reference in their entireties into this application. To the extent any incorporated material conflicts with any of the express content of this application, the express content controls.

Definitions

[00104] Unless defined otherwise, technical and scientific terms used herein have meanings that are commonly understood by those of ordinary skill in the art unless defined otherwise. Generally, terminologies pertaining to techniques of cell and tissue culture, molecular biology, immunology, microbiology, genetics, transgenic cell production, protein chemistry and nucleic acid chemistry and hybridization described herein are well known and commonly used in the art. The methods and techniques provided herein are generally performed according to conventional

procedures well known in the art and as described in various general and more specific references that are cited and discussed herein unless otherwise indicated. See, e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992). A number of basic texts describe standard antibody production processes, including, Borrebaeck (ed) *Antibody Engineering, 2nd Edition* Freeman and Company, NY, 1995; McCafferty et al. *Antibody Engineering, A Practical Approach* IRL at Oxford Press, Oxford, England, 1996; and Paul (1995) *Antibody Engineering Protocols* Humana Press, Towata, N.J., 1995; Paul (ed.), *Fundamental Immunology*, Raven Press, N.Y., 1993; Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, NY; Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY; Stites et al. (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, Calif., and references cited therein; *Coding Monoclonal Antibodies: Principles and Practice* (2nd ed.) Academic Press, New York, N.Y., 1986, and Kohler and Milstein *Nature* 256: 495-497, 1975. All of the references cited herein are incorporated herein by reference in their entireties. To the extent any material incorporated by reference is inconsistent with the express content of this disclosure, the express content controls. Enzymatic reactions and enrichment/purification techniques are also well known and are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation and delivery, and treatment of patients.

[00105] Unless otherwise required by context herein, singular terms shall include pluralities. Singular forms “a”, “an” and “the”, and singular use of any word, include plural referents unless expressly and unequivocally limited on one referent.

[00106] It is understood the use of the alternative (e.g., “or”) herein is taken to mean either one or both or any combination thereof of the alternatives.

[00107] The term “and/or” used herein is to be taken mean specific disclosure of each of the specified features or components with or without the other. For example, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A”

(alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[00108] As used herein, terms “comprising”, “including”, “having” and “containing”, and their grammatical variants, as used herein are intended to be non-limiting so that one item or multiple items in a list do not exclude other items that can be added to the listed items. It is understood that wherever aspects are described herein with the language “comprising,” otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.

[00109] As used herein, the term “about” refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, i.e., the limitations of the measurement system. For example, “about” or “approximately” can mean within one or more than one standard deviation per the practice in the art. Alternatively, “about” or “approximately” can mean a range of up to 10% (i.e., $\pm 10\%$) or more depending on the limitations of the measurement system. For example, about 5 mg can include any number between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of “about” or “approximately” should be assumed to be within an acceptable error range for that particular value or composition.

[00110] The terms “peptide”, “polypeptide” and “protein” and other related terms used herein are used interchangeably and refer to a polymer of amino acids that is not limited to any particular length. Polypeptides may comprise natural and non-natural amino acids. Polypeptides include recombinant and chemically-synthesized polypeptides. Polypeptides include precursor molecules and mature (e.g., processed) molecules. Precursor molecules include those that have not yet been subjected to cleavage, for example cleavage of a secretory signal peptide or by enzymatic or non-enzymatic cleavage at certain amino acid residue(s). Polypeptides include mature molecules that have undergone cleavage. These terms encompass native proteins, recombinant proteins, and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, chimeric proteins and fusion proteins) of a protein sequence as well as post-

translationally, or otherwise covalently or non-covalently, modified proteins. Polypeptides that bind the S protein of a coronavirus and that are produced using recombinant procedures are described herein.

[00111] The terms “nucleic acid”, “nucleic acid molecule”, “polynucleotide” and “oligonucleotide” and other related terms used herein are used interchangeably and refer to polymers of nucleotides that are not limited to any particular length. Nucleic acids include recombinant and chemically-synthesized forms. Nucleic acids include DNA molecules (e.g., cDNA or genomic DNA, expression constructs, DNA fragments, etc.), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and hybrids thereof, as well as peptide nucleic acids, locked nucleic acids, and other synthetic nucleic acid analogs and hybrids thereof. A nucleic acid molecule can be single-stranded or double-stranded. In one embodiment, the nucleic acid molecules of the disclosure comprise a contiguous open reading frame encoding an antibody, or a fragment or scFv, derivative, mutein, or variant thereof. In some embodiments, nucleic acids comprise one type of polynucleotides or a mixture of two or more different types of polynucleotides. Nucleic acids encoding anti-S protein antibodies or antigen-binding portions thereof are described herein.

[00112] The term “recover” or “recovery” or “recovering”, and other related terms, refer to obtaining a protein (e.g., an antibody or an antigen binding portion thereof), from host cell culture medium or from host cell lysate or from the host cell membrane. In one embodiment, the protein is expressed by the host cell as a recombinant protein fused to a secretion signal peptide sequence (e.g., leader peptide sequence) which mediates secretion of the expressed protein. The secreted protein can be recovered from the host cell medium. In one embodiment, the protein is expressed by the host cell as a recombinant protein that lacks a secretion signal peptide sequence which can be recovered from the host cell lysate. In one embodiment, the protein is expressed by the host cell as a membrane-bound protein which can be recovered using a detergent to release the expressed protein from the host cell membrane. In one embodiment, irrespective of the method used to recover the protein, the protein can be subjected to procedures that remove cellular debris from the recovered protein. For example, the recovered protein can be subjected to chromatography, gel electrophoresis and/or dialysis. In one embodiment, the chromatography comprises any one or any combination or two or more procedures including affinity

chromatography, hydroxyapatite chromatography, ion-exchange chromatography, reverse phase chromatography and/or chromatography on silica. In one embodiment, affinity chromatography comprises protein A or protein G (cell wall components from *Staphylococcus aureus*).

[00113] The term "isolated" refers to a protein (e.g., an antibody or an antigen binding portion thereof) or polynucleotide that is substantially free of other cellular material. The term isolated also refers in some embodiments to protein or polynucleotides that are substantially free of other molecules of the same species, for example other proteins or polynucleotides having different amino acid or nucleotide sequences, respectively. The purity or homogeneity of the desired molecule can be assayed using techniques well known in the art, including low resolution methods such as gel electrophoresis and high resolution methods such as HPLC or mass spectrometry. In various embodiments any of the anti-S antibodies or antigen binding protein thereof disclosed herein are isolated.

[00114] Antibodies can be obtained from sources such as serum or plasma that contain immunoglobulins having varied antigenic specificity. If such antibodies are subjected to affinity purification, they can be enriched for a particular antigenic specificity. Such enriched preparations of antibodies usually are made of less than about 10% antibody having specific binding activity for the particular antigen. Subjecting these preparations to several rounds of affinity purification can increase the proportion of antibody having specific binding activity for the antigen. Antibodies prepared in this manner are often referred to as "monospecific." Monospecific antibody preparations can be made up of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or 99.9% antibody having specific binding activity for the particular antigen. Antibodies can be produced using recombinant nucleic acid technology as described below.

[00115] The term "leader sequence" or "leader peptide" or "[peptide] signal sequence" or "signal peptide" or "secretion signal peptide" refers to a peptide sequence that is located at the N-terminus of a polypeptide. A leader sequence directs a polypeptide chain to a cellular secretory pathway and can direct integration and anchoring of the polypeptide into the lipid bilayer of the cellular membrane. Typically, a leader sequence is about 10-50 amino acids in length and is cleaved from the polypeptide upon secretion of the mature polypeptide or insertion of the mature polypeptide into the membrane. Thus, proteins provided herein such as membrane proteins and antibodies having signal peptides that are identified by their precursor sequences

that include a signal peptide sequence are also intended to encompass the mature forms of the polypeptides lacking the signal peptide, and proteins provided herein such as membrane proteins and antibodies having signal peptides that are identified by their mature polypeptide sequences that lack a signal peptide sequence are also intended to encompass forms of the polypeptides that include a signal peptide, whether native to the protein or derived from another secreted or membrane-inserted protein.. In one embodiment, a leader sequence includes signal sequences comprising CD8 α , CD28 or CD16 leader sequences. In one embodiment, the signal sequence comprises a mammalian sequence, including for example mouse or human Ig gamma secretion signal peptide. In one embodiment, a leader sequence comprises a mouse Ig gamma leader peptide sequence MEWSWVFLFFLSVTTGVHS (SEQ ID NO:17).

[00116] An "antigen-binding protein" and related terms used herein refer to a protein comprising a portion that binds to an antigen and, optionally, a scaffold or framework portion that allows the antigen binding portion to adopt a conformation that promotes binding of the antigen-binding protein to the antigen. Examples of antigen-binding proteins include antibodies, antibody fragments (e.g., an antigen binding portion of an antibody), antibody derivatives, and antibody analogs. As used herein an "antigen-binding protein derived from [a referenced] antibody" is an antigen-binding protein that includes the variable light chain sequence and variable heavy chain sequence of the referenced antibody. The antigen binding protein can comprise, for example, an alternative protein scaffold or artificial scaffold with grafted CDRs or CDR derivatives. Such scaffolds include, but are not limited to, antibody-derived scaffolds comprising mutations introduced to, for example, stabilize the three-dimensional structure of the antigen binding protein as well as wholly synthetic scaffolds comprising, for example, a biocompatible polymer. See, for example, Korndorfer et al., 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129; Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as scaffolds based on antibody mimetics utilizing fibronectin components as a scaffold. Antigen binding proteins that bind the spike protein of SARS-CoV-2 are described herein.

[00117] An antigen binding protein can have, in some examples, the structure of an immunoglobulin. In one embodiment, an "immunoglobulin" refers to a tetrameric molecule composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain

includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The heavy and/or light chains may or may not include a leader sequence for secretion. The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two antigen binding sites. In one embodiment, an antigen binding protein can be a synthetic molecule having a structure that differs from a tetrameric immunoglobulin molecule but still binds a target antigen or binds two or more target antigens. For example, a synthetic antigen binding protein can comprise antibody fragments, 1-6 or more polypeptide chains, asymmetrical assemblies of polypeptides, or other synthetic molecules.

[00118] The variable regions of immunoglobulin chains exhibit the same general structure of three hypervariable regions, also called complementarity determining regions or CDRs, joined by relatively conserved framework regions (FR). From N-terminus to C-terminus, both light and heavy chains comprise the segments FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

[00119] One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

[00120] The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991 (e.g., "Kabat numbering"). Other numbering systems for the amino acids in immunoglobulin chains include IMGT.RTM. (international ImMunoGeneTics information system; Lefranc et al, *Dev. Comp. Immunol.* 29:185-203; 2005) and AHo (Honegger and Pluckthun, *J. Mol. Biol.* 309(3):657-670; 2001);

Chothia (Al-Lazikani et al., 1997 *J. Mol. Biol.* 273:927-948; Contact (Maccallum et al., 1996 *J. Mol. Biol.* 262:732-745, and Aho (Honegger and Pluckthun 2001 *J. Mol. Biol.* 309:657-670.

[00121] An "antibody" and "antibodies" and related terms used herein refers to an intact immunoglobulin or to an antigen binding portion thereof (or an antigen binding fragment thereof) that binds specifically to an antigen. Antigen binding portions (or the antigen binding fragment) may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions (or antigen binding fragments) include, *inter alia*, Fab, Fab', F(ab')₂, Fv, single domain antibodies (dAbs), and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies, triabodies, tetrabodies, nanobodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

[00122] Antibodies include recombinantly produced antibodies and antigen binding portions. Antibodies include non-human, chimeric, humanized and fully human antibodies. Antibodies include monospecific, multispecific (e.g., bispecific, trispecific and higher order specificities). Antibodies include tetrameric antibodies, light chain monomers, heavy chain monomers, light chain dimers, heavy chain dimers. Antibodies include F(ab')₂ fragments, Fab' fragments and Fab fragments. Antibodies include single domain antibodies, monovalent antibodies, single chain antibodies, single chain variable fragment (scFv), camelized antibodies, affibodies, disulfide-linked Fvs (sdFv), anti-idiotypic antibodies (anti-Id), minibodies. Antibodies include monoclonal and polyclonal antibody populations.

[00123] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible spontaneous mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies include monoclonal antibodies produced using hybridoma methods that provide a cell line producing a population of identical antibody molecules, and also include chimeric, hybrid, and recombinant antibodies produced by cloning methods such that a cell transfected with the construct or constructs that include the antibody-encoding sequences and the progeny of the transfected cell

produce a population of antibody molecules directed against a single antigenic site. For example, variable regions of an antibody (variable heavy chain and light chain regions or variable heavy and light chain CDRs) may be cloned into an antibody framework that includes constant regions of any species, including human constant regions, where expression of the construct in a cell can produce a single antibody molecule or antigen-binding protein that is referred to herein as monoclonal.

[00124] The modifier "monoclonal" thus indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The "monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990), for example.

[00125] An "antigen binding domain," "antigen binding region," or "antigen binding site" and other related terms used herein refer to a portion of an antigen binding protein that contains amino acid residues (or other moieties) that interact with an antigen and contribute to the antigen binding protein's specificity and affinity for the antigen. For an antibody that specifically binds to its antigen, this will include at least part of at least one of its CDR domains.

[00126] The terms "specific binding", "specifically binds" or "specifically binding" and other related terms, as used herein in the context of an antibody or antigen binding protein or antibody fragment, refer to non-covalent or covalent preferential binding to an antigen relative to other molecules or moieties (e.g., an antibody specifically binds to a particular antigen relative to other available antigens). In various embodiments, an antibody specifically binds to a target antigen if it binds to the antigen with a dissociation constant (K_d) of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less, or 10^{-11} or less, or 10^{-12} or less.

[00127] Binding affinity of an antigen-binding protein for a target antigen can be reported as a dissociation constant (K_d) which can be measured using a surface plasmon resonance (SPR) assay. Surface plasmon resonance refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor

matrix, for example using a BIAcore system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[00128] An "epitope" and related terms as used herein refers to a portion of an antigen that is bound by an antigen binding protein (e.g., by an antibody or an antigen binding portion thereof). An epitope can comprise portions of two or more antigens that are bound by an antigen binding protein. An epitope can comprise non-contiguous portions of an antigen or of two or more antigens (e.g., amino acid residues that are not contiguous in an antigen's primary sequence but that, in the context of the antigen's tertiary and quaternary structure, are near enough to each other to be bound by an antigen binding protein). Generally, the variable regions, particularly the CDRs, of an antibody interact with the epitope.

[00129] With respect to antibodies, the term "antagonist" and "antagonistic" refers to a blocking antibody that binds its cognate target antigen and inhibits or reduces the biological activity of the bound antigen. The term "agonist" or "agonistic" refers to an antibody that binds its cognate target antigen in a manner that mimics the binding of the physiological ligand which causes antibody-mediated downstream signaling.

[00130] An "antibody fragment", "antibody portion", "antigen-binding fragment of an antibody", or "antigen-binding portion of an antibody" and other related terms used herein refer to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')₂, Fd; and Fv fragments, as well as dAb; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide. Antigen binding portions of an antibody may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen binding portions include, inter alia, Fab, Fab', F(ab')₂, Fv, domain antibodies (dAbs), and complementarity determining region (CDR) fragments, chimeric antibodies, diabodies, triabodies, tetrabodies, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer antigen binding properties to the antibody fragment.

[00131] The terms "Fab", "Fab fragment" and other related terms refers to a monovalent fragment comprising a variable light chain region (V_L), constant light chain region (C_L), variable heavy chain region (V_H), and first constant region (C_{H1}). A Fab is capable of binding an antigen.

An F(ab')₂ fragment is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region. A F(Ab')₂ has antigen binding capability. An Fd fragment comprises V_H and C_{H1} regions. An Fv fragment comprises V_L and V_H regions. An Fv can bind an antigen. A dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain (U.S. Patents 6,846,634 and 6,696,245; U.S. published Application Nos. 2002/02512, 2004/0202995, 2004/0038291, 2004/0009507, 2003/0039958; and Ward et al., *Nature* 341:544-546, 1989).

[00132] A single-chain antibody (scFv) is an antibody in which a V_L and a V_H region are joined via a linker (e.g., a synthetic sequence of amino acid residues) to form a continuous protein chain. In one embodiment, the linker is long enough to allow the protein chain to fold back on itself and form a monovalent antigen binding site (see, e.g., Bird et al., 1988, *Science* 242:423-26 and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-83).

[00133] Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (see, e.g., Holliger et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-48, and Poljak et al., 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different. Diabody, tribody and tetrabody constructs can be prepared using antigen binding portions from any of the anti-Spike protein antibodies described herein.

[00134] A “humanized antibody” refers to an antibody originating from a non-human species that has one or more variable and constant regions that has been sequence modified to conform to corresponding human immunoglobulin amino acid sequences. For example, the constant regions of a humanized antibody may be human constant region sequences, where the amino acid sequence of a variable domains may be from an antibody sequence of another species, such as a mouse (in which the antibody may have been generated). A humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as

compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In some embodiments, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In some embodiments, one or more amino acid residues in one or more CDR sequences of a non-human antibody is changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[00135] In some embodiments, an antibody can be a “fully human” antibody in which all of the constant and variable domains (optionally excepting from the CDRs) are derived from human immunoglobulin sequences. A fully human antibody as disclosed herein may have one or more mutations (which may be, for example amino acid substitutions, deletions, or insertions) in the constant regions, such as for example the Fc constant regions of the heavy chain, with respect to a wild type human antibody sequence. For example, a fully human antibody can have one or more mutation in the constant regions of either the light or heavy chain of the antibody, where the sequence of either or both of the light chain constant region or heavy chain constant regions (CH1, CH2, and CH3) of the fully human antibody are greater than 95%, greater than 96%, greater than 97%, and preferably greater than 98% or at least 99% identical to the sequence of the non-mutant human constant regions. Humanized and fully human antibodies may be prepared in a variety of ways, examples of which are described below, including through recombinant methodologies or through immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes, e.g., the “Xenomouse II” that, when challenged with an antigen, generates high affinity fully human antibodies Mendez et al. ((1997) *Nature Genetics* 15: 146-156). This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion of the endogenous J_H region. The antibodies produced in these mice closely

resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire.

[00136] Alternatively, phage display technology (McCafferty et al., *Nature* 348, 552-553 [1990]) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from immunized or nonimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3, 564-571 (1993). Any of a number of sources of V-gene segments can be used for phage display, e.g., the spleens of immunized mice (Clackson et al., *Nature* 352, 624-628 (1991)) or blood cells of nonimmunized human donors can be used to generate antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., *J. Mol. Biol.* 222, 581-597 (1991) or Griffith et al., *EMBO J.* 12, 725-734 (1993).

[00137] The term “chimeric antibody” and related terms used herein refers to an antibody that contains one or more regions from a first antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human antibody. In another embodiment, all of the CDRs are derived from a human antibody. In another embodiment, the CDRs from more than one human antibody are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human antibody, a CDR2 and a CDR3 from the light chain of a second human antibody, and the CDRs from the heavy chain from a third antibody. In another example, the CDRs originate from different species such as human and mouse, or human and rabbit, or human and goat. One skilled in the art will appreciate that other combinations are possible.

[00138] Further, the framework regions of a chimeric antibody may be derived from one of the same antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light

chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (i.e., the ability to specifically bind a target antigen).

[00139] As used herein, the term “variant” polypeptides and “variants” of polypeptides refers to a polypeptide comprising an amino acid sequence with one or more amino acid residues inserted into, deleted from and/or substituted into the amino acid sequence relative to a reference polypeptide sequence. Polypeptide variants include fusion proteins. In the same manner, a variant polynucleotide comprises a nucleotide sequence with one or more nucleotides inserted into, deleted from and/or substituted into the nucleotide sequence relative to another polynucleotide sequence. Polynucleotide variants include fusion polynucleotides.

[00140] As used herein, the term “derivative” of a polypeptide is a polypeptide (e.g., an antibody) that has been chemically modified, e.g., via conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (e.g., human serum albumin), phosphorylation, and glycosylation.

[00141] Unless otherwise indicated, the term “antibody” includes, in addition to antibodies comprising full-length heavy chains and full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

[00142] The term “hinge” refers to an amino acid segment that is generally found between two domains of a protein and may allow for flexibility of the overall construct and movement of one or both of the domains relative to one another. Structurally, a hinge region comprises from about 10 to about 100 amino acids, *e.g.*, from about 15 to about 75 amino acids, from about 20 to about 50 amino acids, or from about 30 to about 60 amino acids. In one embodiment, the hinge region is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length. The hinge region can be derived from is a hinge region of a naturally-occurring protein, such as a CD8 hinge region or a fragment thereof, a CD8 α hinge region, or a fragment thereof, a hinge region of an antibody (*e.g.*, IgG, IgA, IgM, IgE, or IgD antibodies), or a hinge region that joins the constant domains CH1 and CH2 of an antibody. The hinge region can be derived from an antibody and may or may not comprise one or more constant regions of the antibody, or the hinge region comprises

the hinge region of an antibody and the CH3 constant region of the antibody, or the hinge region comprises the hinge region of an antibody and the CH2 and CH3 constant regions of the antibody, or the hinge region is a non-naturally occurring peptide, or the hinge region is disposed between the C-terminus of the scFv and the N-terminus of the transmembrane domain. In one embodiment, the hinge region comprises any one or any combination of two or more regions comprising an upper, core or lower hinge sequences from an IgG1, IgG2, IgG3 or IgG4 immunoglobulin molecule. In one embodiment, the hinge region comprises an IgG1 upper hinge sequence EPKSCDKTHT (SEQ ID NO: 28). In one embodiment, the hinge region comprises an IgG1 core hinge sequence CPXC, wherein X is P, R or S (SEQ ID NO: 29). In one embodiment, the hinge region comprises a lower hinge/CH2 sequence PAPELLGGP (SEQ ID NO:18). In one embodiment, the hinge is joined to an Fc region (CH2) having the amino acid sequence SVFLFPPKPKDT (SEQ ID NO:19). In one embodiment, the hinge region includes the amino acid sequence of an upper, core and lower hinge and comprises EPKSCDKTHTCPPCPAP ELLGGP (SEQ ID NO:20). In one embodiment, the hinge region comprises one, two, three or more cysteines that can form at least one, two, three or more interchain disulfide bonds.

[00143] The term “Fc” or “Fc region” as used herein refers to the portion of an antibody heavy chain constant region beginning in or after the hinge region and ending at the C-terminus of the heavy chain. The Fc region comprises at least a portion of the CH2 and CH3 regions and may, or may not, include a portion of the hinge region. An Fc domain may bind Fc cell surface receptors and some proteins of the immune complement system. An Fc region may bind a complement component C1q. An Fc domain may exhibit effector function, including any one or any combination of two or more activities including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent phagocytosis (ADP), opsonization and/or cell binding. An Fc domain may bind an Fc receptor, including FcγRI (e.g., CD64), FcγRII (e.g., CD32) and/or FcγRIII (e.g., CD16a). In one embodiment, the Fc region may include a mutation that increases or decreases any one or any combination of these functions. In one embodiment, the Fc domain comprises Fc region comprises one or more mutations selected from N297A, N297Q, N297D, L234A, L235A, L235E, P329A, and P329G (e.g., according to Kabat numbering). In one embodiment, the Fc domain comprises a LALA mutation (e.g., equivalent to L234A, L235A according to Kabat numbering) which reduces effector function. In one embodiment, the Fc domain comprises a LALA-PG mutation (e.g.,

equivalent to L234A, L235A, P329G according to Kabat numbering) which reduces effector function. In one embodiment, the Fc domain mediates serum half-life of the protein complex, and a mutation in the Fc domain can increase or decrease the serum half-life of the protein complex. In one embodiment, the Fc domain affects thermal stability of the protein complex, and mutation in the Fc domain can increase or decrease the thermal stability of the protein complex. In one embodiment, the Fc region comprises one or more mutations selected from M252Y, T256D, T307Q, T307W, M252Y, S254T, T256E, M428L, and N434S (e.g., according to Kabat numbering). In one embodiment, the Fc region comprises the mutations M252Y, S254T, and T256E (YTE) (e.g., according to Kabat numbering).

[00144] The term “labeled” or related terms as used herein with respect to a polypeptide refers to joiner antibodies and their antigen binding portions thereof that are unlabeled or joined to a detectable label or moiety for detection, wherein the detectable label or moiety is radioactive, colorimetric, antigenic, enzymatic, a detectable bead (such as a magnetic or electrodense (e.g., gold) bead), biotin, streptavidin or protein A. A variety of labels can be employed, including, but not limited to, radionuclides, fluorescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and ligands (e.g., biotin, haptens). Any of the anti-PD-1 antibodies described herein can be unlabeled or can be joined to a detectable label or moiety.

[00145] The term “labeled” or related terms as used herein with respect to a polypeptide refers to joiner thereof to a detectable label or moiety for detection. Exemplary detectable labels or moieties include radioactive, colorimetric, antigenic, enzymatic labels/moieties, a detectable bead (such as a magnetic or electrodense (e.g., gold) bead), biotin, streptavidin or protein A. A variety of labels can be employed, including, but not limited to, radionuclides, fluorescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors and ligands (e.g., biotin, haptens). Any of the anti-spike protein antibodies described herein or antigen-binding portions thereof that described herein can be unlabeled or can be joined to a detectable label or detectable moiety.

[00146] A “neutralizing antibody” and related terms refers to an antibody that is capable of specifically binding to a target antigen (e.g., a coronavirus spike protein) and substantially inhibiting or eliminating the biological activity of the target antigen. In the present context, a neutralizing antibody binds to a coronavirus and inhibits infection of susceptible cells by the coronavirus. An antibody that blocks binding of the coronavirus to a target cell is a neutralizing

antibody as binding is required for infection of the target cell. As provided herein, a "neutralizing antibody", an "antibody with neutralizing activity", or "inhibitory antibody" is an antibody that neutralizes 100 times the tissue culture infectious dose required to infect 50% of cells ($100 \times \text{TCID}_{50}$) of a virus, for example, a SARS coronavirus, such as for example, SARS-CoV-1 or SARS-CoV-2. In some embodiments, a "neutralizing antibody" is an antibody that neutralizes 200 times the tissue culture infectious dose required to infect 50% of cells ($200 \times \text{TCID}_{50}$) of a virus, for example, a SARS Corona virus, such as SARS-CoV-1 or SARS-CoV-2. Neutralizing antibodies such as those disclosed herein are effective at antibody concentrations of less than 20 $\mu\text{g/ml}$, less than 15 $\mu\text{g/ml}$, less than 12.5 $\mu\text{g/ml}$, less than 10 $\mu\text{g/ml}$, less than 5 $\mu\text{g/ml}$, less than 3.5 $\mu\text{g/ml}$, less than 2 $\mu\text{g/ml}$ or less than 1 $\mu\text{g/ml}$. In some preferred embodiments, neutralizing antibodies are effective at antibody concentrations of $<0.8 \mu\text{g/ml}$. For the S1D2 antibody, a concentration of 50 $\mu\text{g/ml}$ corresponds to 333 nM. In some preferred embodiments, neutralizing antibodies are effective at antibody concentrations of less than 0.5 $\mu\text{g/ml}$ and in some further preferred embodiments, neutralizing antibodies are effective at antibody concentrations of less than 0.2 $\mu\text{g/ml}$ or less than 0.1 $\mu\text{g/ml}$.

[00147] The term " TCID_{50} " or "median tissue culture infective dose" refers to the amount of virus necessary to infect 50% of cells in tissue culture. The 100x and 200x refer to 100 and 200 times the TCID_{50} concentration of virus.

[00148] The "percent identity" or "percent homology" and related terms used herein refers to a quantitative measurement of the similarity between two polypeptide or between two polynucleotide sequences. The percent identity between two polypeptide sequences is a function of the number of identical amino acids at aligned positions that are shared between the two polypeptide sequences, taking into account the number of gaps, and the length of each gap, which may need to be introduced to optimize alignment of the two polypeptide sequences. In a similar manner, the percent identity between two polynucleotide sequences is a function of the number of identical nucleotides at aligned positions that are shared between the two polynucleotide sequences, taking into account the number of gaps, and the length of each gap, which may need to be introduced to optimize alignment of the two polynucleotide sequences. A comparison of the sequences and determination of the percent identity between two polypeptide sequences, or between two polynucleotide sequences, may be accomplished using a mathematical algorithm. For example, the "percent identity" or "percent homology" of two

polypeptide or two polynucleotide sequences may be determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys, San Diego, Calif.)) using its default parameters. Expressions such as “comprises a sequence with at least X% identity to Y” with respect to a test sequence mean that, when aligned to sequence Y as described above, the test sequence comprises residues identical to at least X% of the residues of Y.

[00149] In one embodiment, the amino acid sequence of a test antibody may be similar but not necessarily identical to any of the amino acid sequences of the polypeptides that make up any of the anti-spike protein antibodies, or antigen binding protein thereof, described herein. The similarities between the test antibody and the polypeptides can be at least 95%, or at or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical, to any of the polypeptides that make up any of the anti-spike protein antibodies, or antigen binding protein thereof, described herein. In one embodiment, similar polypeptides can contain amino acid substitutions within a heavy and/or light chain. In one embodiment, the amino acid substitutions comprise one or more conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307-331, herein incorporated by reference in its entirety. 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 are cysteine and methionine.

[00150] A "vector" and related terms used herein refers to a nucleic acid molecule (e.g., DNA or RNA) which can be operably linked to foreign genetic material (e.g., nucleic acid transgene).

Vectors can be used as a vehicle to introduce foreign genetic material into a cell (e.g., host cell). Vectors can include at least one restriction endonuclease recognition sequence for insertion of the transgene into the vector. Vectors can include at least one gene sequence that confers antibiotic resistance or a selectable characteristic to aid in selection of host cells that harbor a vector-transgene construct. Expression vectors can include one or more origin of replication sequences. Vectors can be single-stranded or double-stranded nucleic acid molecules. Vectors can be linear or circular nucleic acid molecules. One type of vector is a "plasmid," which refers to a linear or circular double stranded extrachromosomal DNA molecule which can be linked to a transgene, and is capable of replicating in a host cell, and transcribing and/or translating the transgene. A viral vector typically contains viral RNA or DNA backbone sequences which can be linked to the transgene. The viral backbone sequences can be modified to disable infection but retain insertion of the viral backbone and the co-linked transgene into a host cell genome. Examples of viral vectors include retroviral, lentiviral, adenoviral, adeno-associated viral, baculoviral, papovaviral, vaccinia viral, herpes simplex viral and Epstein Barr viral vectors. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors comprising a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

[00151] An "expression vector" is a type of vector that can contain one or more regulatory sequences, such as inducible and/or constitutive promoters and enhancers. Expression vectors can include ribosomal binding sites and/or polyadenylation sites. Expression vectors can include one or more origin of replication sequences. Regulatory sequences direct transcription, or transcription and translation, of a transgene linked to or inserted into the expression vector which is transduced into a host cell. The regulatory sequence(s) can control the level, timing and/or location of expression of the transgene. The regulatory sequence can, for example, exert its effects directly on the transgene, or through the action of one or more other molecules (e.g., polypeptides that bind to the regulatory sequence and/or the nucleic acid). Regulatory sequences can be part of a vector. Further examples of regulatory sequences are described in, for example, Goeddel, 1990, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. and Baron et al., 1995, *Nucleic Acids Res.* 23:3605-3606.

[00152] A transgene is “operably linked” to a regulatory sequence (e.g., a promoter) when the regulatory sequence affects the expression (e.g., the level, timing, or location of expression) of the transgene.

[00153] The terms "transfected" or "transformed" or "transduced" or other related terms used herein refer to a process by which exogenous nucleic acid (e.g., transgene) is transferred or introduced into a host cell, such as an antibody production host cell. A "transfected" or "transformed" or "transduced" host cell is one which has been introduced with exogenous nucleic acid (transgene). The host cell includes the primary subject cell and its progeny. Exogenous nucleic acids encoding at least a portion of any of the anti-spike protein antibodies described herein can be introduced into a host cell. Expression vectors comprising at least a portion of any of the anti-spike protein antibodies described herein can be introduced into a host cell, and the host cell can express polypeptides comprising at least a portion of the anti-spike protein antibody.

[00154] In this context, a host cell can be a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell. The phrase “transgenic host cell” or "recombinant host cell" can be used to denote a host cell that has been introduced (e.g., transduced, transformed or transfected) with a nucleic acid either to be expressed or not to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, e.g., mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00155] Thus the terms "host cell" or “or a population of host cells” or related terms as used herein may refer to a cell (or a population thereof or a plurality of host cells) to be used for production of the antibody or fragment thereof, is a cell or cells into which foreign (exogenous or transgene) nucleic acids have been introduced, for example, to direct production of the anti-spike protein antibody by the production host cell. The foreign nucleic acids can include an expression vector operably linked to a transgene, and the host cell can be used to express the nucleic acid and/or polypeptide encoded by the foreign nucleic acid (transgene). A host cell (or a population

thereof) can be a cultured cell, can be extracted from a subject, or can be the cell of an organism, including a human subject. The host cell (or a population of host cells) includes the primary subject cell and its progeny without any regard for the number of generations or passages. The host cell (or a population thereof) includes immortalized cell lines. Progeny cells may or may not harbor identical genetic material compared to the parent cell. In one embodiment, a production host cell describes any cell (including its progeny) that has been modified, transfected, transduced, transformed, and/or manipulated in any way to express an antibody, as disclosed herein. In one example, the host cell (or population thereof) can be transfected or transduced with an expression vector operably linked to a nucleic acid encoding the desired antibody, or an antigen binding portion thereof, as described herein. Production host cells and populations thereof can harbor an expression vector that is stably integrated into the host's genome or can harbor an extrachromosomal expression vector. In one embodiment, host cells and populations thereof can harbor an extrachromosomal vector that is present after several cell divisions or is present transiently and is lost after several cell divisions.

[00156] In other contexts, the disclosure may use the term “host cell” or “host cells” to refer to a cell or cells that are infected with a virus (such as a coronavirus), cells capable of being infected by a virus (e.g., lung cells of a subject), or cells used in assays or experiments testing their ability to be infected by a virus. Other terms for virally-infected cells, cells capable of being infected by a virus, or cells used in assays that include viral infection procedures, may include, as nonlimiting examples, “target cells”, “susceptible cells”, “test cells”, “virus propagating cells”, “infected cells”, and the like.

[00157] The term “subject” as used herein refers to human and non-human animals, including vertebrates, mammals and non-mammals. In one embodiment, the subject can be human, non-human primates, simian, ape, murine (e.g., mice), bovine, porcine, equine, canine, feline, caprine, lupine, ranine, or piscine.

[00158] The term “administering”, “administered” and grammatical variants refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” as used herein means modes of administration other than

enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation. In one embodiment, the formulation is administered via a non-parenteral route, e.g., orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods. Any of the anti-spike protein antibodies described herein (or antigen binding protein thereof) can be administered to a subject using art-known methods and delivery routes.

[00159] The terms "effective amount", "therapeutically effective amount" or "effective dose" or related terms may be used interchangeably and refer to an amount of antibody or an antigen binding protein (e.g., any of the anti-spike protein antibodies described herein or antigen binding protein thereof) that when administered to a subject, is sufficient to effect a measurable improvement or prevention of a disease or disorder associated with tumor or cancer antigen expression. Therapeutically effective amounts of antibodies provided herein, when used alone or in combination, will vary depending upon the relative activity of the antibodies and combinations (e.g., in inhibiting cell growth) and depending upon the subject and disease condition being treated, the weight and age and sex of the subject, the severity of the disease condition in the subject, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

[00160] In one embodiment, a therapeutically effective amount will depend on certain aspects of the subject to be treated and the disorder to be treated and may be ascertained by one skilled in the art using known techniques. In general, the polypeptide is administered to a subject at about 0.01 g/kg - 50 mg/kg per day, about 0.01 mg/kg - 30 mg/kg per day, or about 0.1 mg/kg - 20 mg/kg per day. The polypeptide may be administered daily (e.g., once, twice, three times, or four times daily) or less frequently (e.g., weekly, every two weeks, every three weeks, monthly, or quarterly). In addition, as is known in the art, adjustments for age as well as the body weight, general health, sex, diet, time of administration, drug interaction, and the severity of the disease may be necessary.

[00161] The present disclosure provides methods for treating a subject testing positive for a coronavirus infection, such as an infection with SARS-CoV or SARS-CoV-2. The present disclosure also provides methods for treating a subject suspected of being infected or at risk of being infected with a coronavirus, such as SARS-CoV or SARS-CoV-2.

Antigen Binding Proteins that Specifically Bind the Coronavirus S1 Protein

[00162] The present disclosure provides antigen-binding proteins that specifically bind the spike (S) protein of a beta coronavirus, such as but not limited to the S protein of HCoV-NL63, SARS-CoV, or SARS-CoV-2. The term spike protein or S protein, as used herein, includes both the precursor form of an S protein that includes the N-terminal leader sequence and the processed or mature form that lacks the N-terminal leader sequence. Thus an antigen-binding protein that binds an S protein can bind either or both of the precursor that includes the leader sequence and the mature S protein that lacks the N-terminal leader sequence. (The leader sequence of the SARS-CoV-2 S protein is not precisely defined, but may extend from the N-terminus to amino acid 11, 12, 13, 14, 15, or 16 of SEQ ID NO:1, for example from the N-terminus to amino acid 12, 13, 14, or 15 of SEQ ID NO:1.) In some embodiments, an antigen-binding protein as provided herein binds the S protein of SARS-CoV-2. An antigen-binding protein as provided herein can specifically bind the spike protein of SARS-CoV-2 having the amino acid sequence of SEQ ID NO:1 or comprising the amino acid sequence of SEQ ID NO:2, and/or in various embodiments can specifically bind an S protein of a coronavirus where the S protein comprises an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2. The spike protein, a transmembrane protein of the coronavirus having an ectodomain that extends from the N-terminus of the mature protein to amino acid 1208 of SEQ ID NO:1, has two regions or domains, referred to as S1 and S2, that are cleaved into the S1 and S2 subunits after binding of the S1 domain, which includes the receptor binding domain (RBD) of the S protein, to the ACE2 protein on target cells. In general, the term “S1 subunit” or “S1 protein” will refer to the cleaved S1 domain or region of the spike or “S” protein of a coronavirus.

[00163] In various embodiments the antigen-binding protein disclosed herein specifically binds the S1 subunit of the spike (S) protein. For example, an antigen-binding protein as provided herein can specifically bind the S1 domain or subunit of a spike protein of SARS-CoV-2 having the amino acid sequence of SEQ ID NO:4 and in various embodiments can specifically

bind an S1 subunit having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:4.

[00164] The antigen-binding proteins provided herein that bind the S protein of a coronavirus such as SARS-CoV-2 can comprise an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6 (the heavy chain variable region of antibody S1D2) and an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7 (the light chain variable region of antibody S1D2). Alternatively or in addition, the antigen-binding proteins provided herein that specifically bind the S protein of a betacoronavirus, such as but not limited to the S protein of SARS-CoV-2, include the heavy chain complementarity-determining regions (CDRs) of SEQ ID NO:8 (heavy chain CDR1), SEQ ID NO:9 (heavy chain CDR2), and SEQ ID NO:10 (heavy chain CDR3) and further include the light chain complementarity-determining regions (CDRs) of SEQ ID NO:11 (light chain CDR1), SEQ ID NO:12 (light chain CDR2), and SEQ ID NO:13 (light chain CDR3). Antigen-binding proteins as provided herein that include the CDR sequences of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13 can in some embodiments have a heavy chain variable region sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the amino acid sequence of SEQ ID NO:6 and a light chain variable region sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to the amino acid sequence of SEQ ID NO:7. In some exemplary embodiments of the antigen-binding proteins provided herein that specifically bind the S protein of a betacoronavirus, such as the S protein of SARS-CoV-2, the antigen-binding proteins include the heavy chain variable region amino acid sequence of SEQ ID NO:6 and the light chain variable region amino acid sequence of SEQ ID NO:7.

[00165] The antigen-binding proteins provided herein having an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:6 and an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7 and/or comprising the heavy chain CDR1 sequence of SEQ ID NO:8, the heavy chain CDR2 sequence of SEQ ID NO:9, the heavy chain CDR3 sequence of SEQ ID NO:10, the light chain CDR1 sequence of SEQ ID NO:11, the light chain CDR2 sequence of SEQ ID NO:12, and the light chain CDR3 sequence of SEQ ID NO:13 can bind the S protein of a coronavirus such as the

S protein of SARS-CoV-2 with a binding affinity (K_d) of 10^{-5} M (10 μ M) or less, 10^{-6} M (1 μ M) or less, 10^{-7} M (100 nM) or less, 5×10^{-8} M (50 nM) or less, 10^{-8} M (10 nM) or less, 10^{-9} M (1 nM) or less, or 10^{-10} M (0.1 nM) or less. For example, the antigen-binding protein can bind the S protein of a coronavirus such as SARS-Cov-2 with a K_d of between 10^{-5} M and 10^{-6} M, or between 10^{-6} M and 10^{-7} M, or between 10^{-7} M and 10^{-8} M, or between 10^{-8} M and 10^{-9} M, between 10^{-9} M and 10^{-10} M, or between 10^{-10} M and 10^{-11} M.

[00166] For example, an antigen-binding protein as provided herein that specifically binds the S protein of SARS-CoV-2, which can be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein comprising or derived from any thereof, can specifically bind a coronavirus spike protein (e.g., a spike protein comprising the amino acid sequences of SEQ ID NO:1, comprising amino acids 16-1273 of SEQ ID NO:1, or comprising SEQ ID NO:2, or a spike protein of a coronavirus comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:1, amino acids 16-1273 of SEQ ID NO:1, or SEQ ID NO:2) with a K_d of between about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In some embodiments, the antigen-binding protein is the S1D2 antibody having a heavy chain variable sequence of SEQ ID NO:6 and a light chain variable sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, having a K_d for binding the S protein of SARS-CoV-2 of between about 100 nM and about 1 nM.

[00167] In some embodiments the antigen-binding protein can bind the S1 subunit of the S protein of a coronavirus such as the S1 subunit of SARS-CoV-2 with a binding affinity (K_d) of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less. For example, the antigen-binding protein can bind the S1 subunit of an S protein of a coronavirus such as SARS-CoV-2 with a K_d of between 10^{-5} M and 10^{-6} M, or between 10^{-6} M and 10^{-7} M, or between 10^{-7} M and 10^{-8} M, or between 10^{-8} M and 10^{-9} M, or between 10^{-9} M and 10^{-10} M. For example, an antigen-binding protein provided herein, which can in various embodiments be an antibody, such as a fully human antibody, and may be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein derived from any thereof, specifically binds the S1 subunit of a coronavirus S protein (e.g., SEQ ID NO:4 or an S1 subunit of a spike protein of a coronavirus comprising a sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:4) with a K_d of between

about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In some embodiments, the antibody is the fully human S1D2 antibody or antibody fragment, or an antigen-binding protein derived therefrom, having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, that binds the S1 subunit of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM. In some embodiments the antibody is a fully humanized IgG having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7 that optionally comprises one or more mutations in the Fc region. In some embodiments the one or more mutations in the Fc region is a mutation that reduces ADE, such as the LALA mutations. Exemplary Fc region sequences comprising the LALA mutations are SEQ ID NOs: 14 and 16.

[00168] In some embodiments the antigen-binding protein can bind the RBD of the S protein of a coronavirus (which is localized to the S1 domain) such as the RBD of the SARS-CoV S protein or SARS-CoV-2 S protein with a binding affinity (K_d) of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less. For example, the antigen-binding protein can bind the RBD of an S protein of a coronavirus such as SARS-CoV-2 with a K_d of between 10^{-5} M and 10^{-6} M, or between 10^{-6} M and 10^{-7} M, or between 10^{-7} M and 10^{-8} M, or between 10^{-8} M and 10^{-9} M, or between 10^{-9} M and 10^{-10} M. In some embodiments, an antigen-binding protein provided herein, which can be in various embodiments be an antibody, such as a fully human antibody, and may be, as nonlimiting examples, an IgG, a Fab fragment, or a single chain antibody, or can be an antigen-binding protein derived from any thereof, specifically binds the receptor binding domain (RBD) of a coronavirus S protein (e.g., SEQ ID NO:5 or an RBD of a spike protein of a coronavirus comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO:5) with a K_d of between of between about 200 nM and about 0.01 nM, or between 100 nM and 0.1 nM, or between 100 nM and 1 nM. In some embodiments, the antigen binding protein is the fully human S1D2 antibody having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the RBD of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM or between about 60 nM and about 2 nM. For example, the antigen binding protein may be the fully human S1D2 antibody disclosed herein having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally having L234A and L235A

mutations in the Fc region, where the antibody binds the RBD of the S protein of SARS-CoV-2 with a K_d of between about 100 nM and about 1 nM, or between about 80 nM and about 5 nM, as measured by SPR.

[00169] Binding affinity of an antigen-binding protein as provided herein to an S protein, S1 subunit, or RBD can be calculated based on binding assays performed using methods known in the art such as surface plasmon resonance (SPR). The sequences of many coronavirus proteins are publicly available such that the proteins can be produced using recombinant methods and many, including for example coronavirus S proteins, S1 subunits, and the RBDs of S proteins, are commercially available. Commercial sources for coronavirus proteins include for example Sino Biological US (Wayne, PA) and Acrobiosystems (San Jose, CA, USA).

[00170] In various embodiments provided herein, an antigen-binding protein that specifically binds the S protein, S1 subunit, or S protein RBD of a coronavirus as disclosed herein is a neutralizing antibody that it is able to inhibit infection of a target cell by a coronavirus such as the SARS-CoV virus or the SARS-CoV-2 virus. In various embodiments, the antigen-binding proteins described herein block binding between the S protein of a coronavirus (such as HCoV-NL63, SARS-CoV, or SARS-CoV-2) and the ACE2 protein, for example, block binding of the ectodomain of the human ACE2 protein (hACE2) by the S protein of a coronavirus. In various embodiments, the antigen-binding proteins described herein can block binding between the S protein of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of between about 0.001 $\mu\text{g/ml}$ and about 200 $\mu\text{g/ml}$, between about 0.01 $\mu\text{g/ml}$ and about 100 $\mu\text{g/ml}$, between about 0.05 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, between about 1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, or between about 1 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 5 $\mu\text{g/ml}$, or between about 0.1 $\mu\text{g/ml}$ and about 1 $\mu\text{g/ml}$. In some embodiments, the antigen binding proteins described herein block binding between the S1 domain or subunit of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of between about 0.001 $\mu\text{g/ml}$ and about 200 $\mu\text{g/ml}$, between about 0.01 $\mu\text{g/ml}$ and about 100 $\mu\text{g/ml}$, between about 0.05 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, between about 1 $\mu\text{g/ml}$ and about 10 $\mu\text{g/ml}$, or between about 1 $\mu\text{g/ml}$ and about 50 $\mu\text{g/ml}$, between about 0.1 $\mu\text{g/ml}$ and about 5 $\mu\text{g/ml}$, or between about 0.1 $\mu\text{g/ml}$ and about 1 $\mu\text{g/ml}$. For example, an antigen-binding protein as disclosed herein can block the binding of the S1 subunit of SARS-CoV-2 and the human ACE2 protein with an IC_{50} of less than 200 nM, less than 100 nM, less than 50 nM, less than 10 nM,

less than 5 nM, or less than 1 nM, for example, with an IC_{50} of between about 100 nM and about 0.1 nM or between about 50 nM and about 0.5 nM, or between about 50 nM and about 1 nM, for example, between about 10 nM and about 1 nM, or between about 5 nM and about 0.5 nM. In some embodiments, any IC_{50} described herein of an antigen-binding protein is determined in an assay in which the S1 subunit of SARS-CoV-2 (e.g., SEQ ID NO: 4) is present at 1.25 μ g/ml or 3 μ g/ml, and/or an assay performed as an ELISA in which a solid substrate is coated with an ACE2 protein, e.g., an ACE2-Fc fusion (e.g., SEQ ID NO: 23 fused to SEQ ID NO: 25). In some embodiments, any IC_{50} described herein of an antigen-binding protein is determined as described in Example 2 or Example 7.

[00171] In various embodiments, an antigen-binding protein that specifically binds the S protein of a coronavirus as disclosed herein is a neutralizing antigen-binding protein that can block infection of target cells by a coronavirus, such as but not limited to SARS-CoV-2. For example, a neutralizing antigen-binding protein as provided herein can inhibit a cytopathic effect (CPE) by a coronavirus such as SARS-CoV or SARS-CoV-2 on susceptible cells with an IC_{50} of between about 0.001 μ g/ml and about 200 μ g/ml, or between about 0.01 μ g/ml and about 100 μ g/ml, or between about 0.1 μ g/ml and about 100 μ g/ml, or between about 0.1 μ g/ml and about 50 μ g/ml, or between about 0.5 μ g/ml and about 50 μ g/ml, or between about 0.1 μ g/ml and about 20 μ g/ml, or between about 0.1 μ g/ml and about 10 μ g/ml, or between about 1 μ g/ml and about 10 μ g/ml. For example, a neutralizing antigen-binding protein as provided herein can inhibit a cytopathic effect (CPE) by a coronavirus such as SARS-CoV or SARS-CoV-2 on susceptible cells with an IC_{50} of between about 0.01 nM and about 100 nM, or between about 0.1 nM and about 100 nM, or between about 0.5 nM and about 100 nM, or between about 0.1 nM and about 50 nM. For example, in some embodiments the antigen binding protein is the fully human S1D2 antibody having a variable heavy chain sequence of SEQ ID NO:6 and a variable light chain sequence of SEQ ID NO:7, and optionally comprising one or more mutations in the Fc region, that binds the S1 subunit of the S protein of SARS-CoV-2 and can inhibit CPE with an IC_{50} of between about 0.01 μ g/ml and about 100 μ g/ml between about 0.1 μ g/ml and about 50 μ g/ml, between about 0.5 μ g/ml and about 50 μ g/ml, between about 0.1 μ g/ml and about 20 μ g/ml, between about 0.5 μ g/ml and about 20 μ g/ml, or between about 0.5 μ g/ml and about 10 μ g/ml. For example, the antibody can inhibit CPE with an IC_{50} between about 0.1 nM and about 200 nM, between about 0.5 nM and about 200 nM, between about 1 nM and about 200 nM, between

about 0.1 nM and about 100 nM, between about 0.5 nM and about 100 nM, between about 1 nM and about 100 nM, between about 0.1 nM and about 50 nM, between about 0.5 nM and about 50 nM, or between about 1 nM and about 50 nM. Cytopathic effect can be assessed for coronavirus infection by live cell staining (e.g., using LysoTracker™ dyes, see for example Gorshkov et al. (2020) *bioRxiv* doi.org/10.1101/2020.05.16.091520), plaque assays (Singh et al. (2018) *J. Virol. Methods* 262:32-37), MTT assays (e.g., Singh et al. (2018) *J. Virol. Methods* 262:32-37), using assays that report on the presence of ATP in the cells (e.g., Severson et al. (2007) *J. Biomol. Screening* 12:33-40; doi: 10.1177/1087057106296688); Gorshkov et al. (2020) *bioRxiv* doi.org/10.1101/2020.05.16.091520), or using impedance-based assays (xCELLigence, Acea Biosciences, San Diego, CA), for example. An assay for cytopathic effect can be formatted as a plaque assay in multiwell plates, where a confluent monolayer of susceptible cells is incubated with the virus and increasing concentrations of inhibitor (e.g., neutralizing antibody), where each inhibitor concentration is added to multiple replicate wells. At the end of the assay, which can be, for example, after 24, 48, or 72 hours, the cells are stained with crystal violet, and the concentration of inhibitor that results in 50% of the wells demonstrating disruption of the monolayer (for example, the appearance of plaques or gaps in the monolayer resulting from cell death due to infection) is the IC₅₀ concentration for the inhibitor. In some embodiments, an assay for blocking infection is performed as described in Example 8.

[00172] An antigen-binding protein as provided herein can be or can be derived from an antibody, such as an IgG, IgA, IgD, IgE, or IgM antibody that specifically binds the S protein of SARS-CoV-2. In some embodiments, an IgG, IgA, IgD, IgE, or IgM antibody as provided herein, or an antigen-binding protein derived therefrom, can specifically bind the S protein of a coronavirus (such as but not limited to SARS-CoV or SARS-CoV-2 or a variant of SARS-CoV or SARS-CoV-2) having an S protein with at least 65%, at least 70%, at least at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to SEQ ID NO:1 or SEQ ID NO:2. In various embodiments an IgG, IgA, IgD, IgE, or IgM antibody as provided herein, or an antigen-binding protein derived therefrom, can specifically bind the S1 subunit of a coronavirus (such as but not limited to SARS or SARS-CoV-2 or a variant of SARS or SARS-CoV-2) having an S1 subunit with at least 65%, at least 70%, at least at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity

to SEQ ID NO:4. In some embodiments the antigen-binding protein specifically binds the RBD that is within the S1 domain of the S protein.

[00173] An antigen-binding protein as provided herein that specifically binds the S protein of a coronavirus can be or comprise an antibody, such as for example an IgG, IgA, IgD, IgE, IgM, or a single chain antibody and/or can be or can comprise antibody fragments, such as but not limited to Fab, Fab', or F(ab')₂ antibody fragments. In some embodiments, an antigen-binding protein as provided herein comprises an IgG or IgM antibody. In some embodiments, an antigen-binding protein comprises an IgG1, IgG2, IgG3, or IgG4 antibody. In some embodiments, an antigen-binding protein comprises an IgG1 or IgG4 antibody. For example, an antigen-binding protein as provided herein can be an IgG1 or IgG4 antibody having an ADE-reducing mutation, such as the LALA mutation, in the Fc region, or can be single chain antibody (ScFv) that optionally includes an Fc region that can optionally include an ADE-reducing mutation, such as the LALA mutation. In further embodiments, an antigen-binding protein as provided herein can be a Fab, Fab', or F(ab')₂ antibody fragment.

[00174] An antigen-binding protein as provided herein that is "derived from" an IgG, IgA, IgD, IgE, or IgM antibody has heavy chain CDR amino acid sequences of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10 and light chain CDR amino acid sequences of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13 or has a heavy chain sequence having at least 95% identity to SEQ ID NO:6 and a light chain sequence having at least 95% identity to SEQ ID NO:7. In various embodiments the S protein-binding protein as provided herein comprises or is derived from an IgG1, IgG2, IgG3, or IgG4 antibody. For example, the antigen-binding protein that binds an S protein can comprise or be derived from an IgG1 or IgG4 class antibody. In further embodiments, an antigen-binding protein as provided herein can be or comprise an antibody fragment, such as but not limited to a Fab fragment, a Fab' fragment, or F(ab')₂ fragment. In additional embodiments an antigen-binding protein as provided herein can be or comprise a single chain antibody (*e.g.*, an ScFv). An antigen binding protein as provided herein that may be or may not be an immunoglobulin molecule can comprise more than one polypeptide chain where the two or more polypeptide chains assemble together, for example, can be an immunoglobulin having two heavy and two light chains, or can be a multi-subunit protein having one heavy and one light chain, or can be a non-immunoglobulin protein that comprises multiple polypeptide chains that may comprise, for example, one or more Fab fragments or ScFvs, etc.

[00175] Antigen-binding proteins as provided herein can comprise or be derived from antibodies, antibody fragments, and single chain antibodies. For example, an antigen-binding protein as provided herein can include heavy chain variable region and light chain variable region sequences of an antibody disclosed herein, such as the S1D2 antibody, or sequences having at least 95% amino acid sequence identity thereto, or can include CDR sequences of an antibody disclosed herein, where the antigen-binding protein can have a framework that is not an antibody framework, or the antigen binding protein can include additional protein moieties that may be, as nonlimiting examples, active domains or additional binding domains.

[00176] In some nonlimiting embodiments, the antigen-binding protein provided herein is or comprises a fully human antibody or a fully human antibody fragment, for example, a fully human IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, or IgM, or a fully human single chain antibody, fully human Fab fragment, or fully human single chain antibody, or is or comprises an antigen binding protein derived from or comprising any of these.

[00177] In some embodiments the antigen-binding protein is an IgG, IgA, IgD, IgE, or IgM antibody having one or more mutations in the Fc region, for example one or more mutations that decreases antibody dependent enhancement (ADE) and/or one or more mutations that increases antibody half-life. For example, the presence of virus-specific antibodies can enhance pathogenicity of a virus (see, for example, Khandia et al. (2018) *Frontiers Immunol.* 9; doi:103389/fimmu.2018.00597). This phenomenon occurs when virus-specific antibodies enhance the entry of virus due to binding of IgG Fc regions to Fc-receptors on the surface of cells that naturally bind Fc regions of antibodies such as macrophages, monocytes, and dendritic cells. Mutations that reduce or eliminate interaction of the Fc region of antibody with its receptor (e.g., FcγRs) on such cells can reduce or eliminate ADE.

[00178] In some embodiments the antibody has one or more mutations in the Fc region selected from L234A; L235A or L235E; N297A, N297Q, or N297D; and P329A or P329G. For example, the anti-S antibody can include the mutations L234A and L235A (LALA). An antibody having one or more mutations in the Fc region selected from L234A; L235A or L235E; N297A, N297Q, or N297D; and P329A or P329G can demonstrate reduced ADE with respect to the same antibody without the mutation(s). For example, an antibody as provided herein that includes the LALA mutations can have reduced ADE with respect to the antibody without the LALA mutations.

[00179] Alternatively or in addition, the antibody may have one or more mutations in the Fc region that increase half-life of the antibody in human serum. For example, mutations such as the M252Y/S254T/T256E (YTE) mutation in the Fc region of an antibody can increase serum half-life of an antibody by as much as four-fold in cynomolgus monkeys (e.g., US 2011/0158985, incorporated by reference in its entirety). Extending the half-life a neutralizing antibody as provided herein that binds the S protein of a coronavirus such as SARS-CoV-2 can improve its usefulness as a prophylactic to confer prolonged passive immunity to those that are regularly exposed to the virus (e.g. healthcare workers) or individuals at particular risk following an exposure event (e.g. elderly, patients with hypertension, or immune-compromised patients). In various examples, an neutralizing antibody the specifically binds the S protein of a coronavirus such as SARS-CoV-2, can have an Fc region mutation that extends the half-life of the antibody such as any selected from M252Y, S254T, T256D or T256E, T307Q or T307W, M428L, and N434S. For example, the anti-S antibody can include the mutations M252Y, S254T, and T256E (YTE). Exemplary Fc region sequences that include the YTE mutations are SEQ ID NOs: 15 and 16.

[00180] An antigen-binding protein as provided herein that specifically binds a coronavirus S protein, including an antibody or antibody fragment that specifically binds a coronavirus S protein, can include a label, such as, for example, a fluorophore. An antigen-binding protein as disclosed herein can further include one or more additional amino acid sequences in addition to antibody-derived sequences (e.g., heavy chain and/or light chain sequences), such as but not limited to peptide tags, localization sequences, linkers, fluorescent protein sequences, or functional domains, including additional binding domains or enzymatic domains. Peptide or protein tags that can be used for detection and/or purification of the antigen-binding protein can include, without limitation, his, FLAG, a myc, HA, V5, polyarginine, calmodulin, a maltose binding protein tag, a GST tag, and a Halo tag.

[00181] In some examples an antigen-binding protein as provided herein that specifically binds the S protein of the SARS-CoV-2 coronavirus has a heavy chain variable domain having the sequence of SEQ ID NO:6 and a light chain variable domain having the sequence of SEQ ID NO:7. In some embodiments an antigen binding protein as provided herein is or comprises an IgG antibody having the heavy chain variable region of SEQ ID NO:6 and the light chain variable region of SEQ ID NO:7. In some embodiments an antigen binding protein as provided

herein is or comprises a monoclonal fully human IgG antibody having the heavy chain variable region of SEQ ID NO:6 and the light chain variable region of SEQ ID NO:7. The antibody can specifically bind the S1 subunit of the coronavirus S protein, and in some embodiments specifically binds the RBD of the S protein. In some embodiments, the present disclosure provides a fully human antibody comprising both heavy and light chains, wherein the heavy/light chain variable region amino acid sequences have at least 95% sequence identity, or at least 96% sequence identity, or at least 97% sequence identity, or at least 98% sequence identity, or at least 99% sequence identity to SEQ ID NO:6 and SEQ ID NO:7 (herein called S1D2).

[00182] As disclosed herein, antibody S1D2 is a fully human recombinant monoclonal IgG1 antibody having a heavy chain variable domain having the sequence of SEQ ID NO:6 and a light chain variable domain having the sequence of SEQ ID NO:7. The S1D2 antibody has a heavy chain CDR1 with the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 with the amino acid sequence of SEQ ID NO:9, a heavy chain CDR3 with the amino acid sequence of SEQ ID NO:10, a light chain CDR1 with the amino acid sequence of SEQ ID NO:11, a light chain CDR2 with the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 with the amino acid sequence of SEQ ID NO:13. As disclosed in the examples herein, the S1D2 antibody specifically binds the RBD of the S protein of the SARS-CoV-2 coronavirus (SEQ ID NO:5) with a K_d of between about 100 nM and about 1 nM, for example, of between about 30 nM and about 60 nM, or about 46 nM. As demonstrated in the Examples herein, S1D2 is a neutralizing antibody that blocks infection of susceptible cells by SARS-CoV-2 (as measured by CPE) with an IC_{50} of between about 100 nM and about 1 nM, for example, of between about 5 nM and about 40 nM, or about 21 nM. In some embodiments the S1D2 antibody includes the mutations L234A and L235A in the Fc region (S1D2_{LALA}).

[00183] The present disclosure also provides a Fab fully human antibody fragment, comprising a heavy variable region from a heavy chain and a variable region from a light chain, wherein the sequence of the variable region from the heavy chain is at least 95% identical, or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical to the amino acid sequence of SEQ ID NO:6. The sequence of the variable region from the light chain can be at least 95% identical, or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical to the amino acid sequence of SEQ ID NO:7.

[00184] The present disclosure further provides a single chain fully human antibody comprising a polypeptide chain having a variable region from a fully human heavy chain and a variable region from a fully human light chain, and optionally a linker joining the variable heavy and variable light chain regions, wherein the variable heavy chain region comprises at least 95% sequence identity, or at least 96% sequence identity, or at least 97% sequence identity, or at least 98% sequence identity, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6, and the variable light chain region comprises at least 95% sequence identity, or at least 96% sequence identity, or at least 97% sequence identity, or at least 98% sequence identity, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7. In one embodiment, the linker comprises a peptide linker having the sequence (GGGGS)_N wherein 'N' is 1-6 (SEQ ID NO: 30). In some embodiments, the linker comprises, consists of, or consists essentially of the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO:21).

Nucleic Acid Molecules, Production Hosts and Methods

[00185] The present disclosure provides nucleic acid molecules, such as vectors, encoding one or more polypeptides of an antigen-binding protein as disclosed herein that specifically binds the S protein of a coronavirus. In some embodiments, the one or more nucleic acid molecules, such as one or more vectors, encode an antigen-binding protein as disclosed herein that specifically binds the S protein of a coronavirus.

[00186] In some embodiments, the present disclosure provides a composition that includes a first nucleic acid molecule encoding a first polypeptide comprising a heavy chain variable region having a heavy chain CDR1 having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO:10, and a second nucleic acid molecule encoding a second polypeptide comprising the light chain variable region having a light chain CDR1 having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:13. The first and second nucleic acid molecules can encode a heavy chain and light chain of an antibody, for example. Alternatively, the disclosure provides a single nucleic acid molecule encoding a first polypeptide that comprises a heavy chain variable region having a heavy chain CDR1 having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 region having

the amino acid sequence of SEQ ID NO:10, and a second polypeptide comprising the light chain variable region having a light chain CDR1 having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:13. In a further alternative, the disclosure provides a nucleic acid molecule that encodes a polypeptide having a heavy chain CDR1 having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 region having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 region having the amino acid sequence of SEQ ID NO:10, a light chain CDR1 having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 region having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 region having the amino acid sequence of SEQ ID NO:13. An antigen-binding protein encoded by one or more nucleic acid molecules as provided herein can be, as nonlimiting examples, an IgG or a single chain antibody.

[00187] In further embodiments, the present disclosure provides a first nucleic acid molecule encoding a first polypeptide comprising a heavy chain variable region having a heavy chain variable region having at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity to SEQ ID NO:6, and a second nucleic acid molecule encoding a second polypeptide comprising the light chain variable region having at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity to SEQ ID NO:7. The first and second nucleic acid molecules can encode a heavy chain and light chain of an antibody, for example. Alternatively, the disclosure provides a single nucleic acid molecule encoding a first polypeptide that comprises a heavy chain variable region having a heavy chain CDR1 having an amino acid having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to SEQ ID NO:6, and a second polypeptide comprising a light chain variable region having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% to SEQ ID NO:7. In further alternatives, the disclosure provides a nucleic acid molecule that encodes a polypeptide having a heavy chain amino acid sequence having at least 95% identity to SEQ ID NO:6 and a light chain amino acid sequence having at least 95% identity to SEQ ID NO:7. An antigen-binding protein encoded by one or more nucleic acid molecules as provided herein can be, as nonlimiting examples, an IgG or a single chain antibody.

[00188] A nucleic acid molecule encoding an antigen-binding protein that specifically binds the S protein of a coronavirus as disclosed herein can be an expression vector that includes a promoter operably linked to the protein-encoding sequence. A promoter operably linked to the polypeptide-encoding sequence(s) can be a eukaryotic or prokaryotic promoter but is preferably a eukaryotic promoter that is active in a mammalian cell. The expression vector(s) can direct transcription and/or translation of the transgene in the host cell and can include ribosomal binding sites and/or polyadenylation sites. The polypeptide(s) that include heavy and light chain sequences as disclosed above can be displayed on the surface of the transgenic host cell or secreted into the cell culture medium. In various embodiments, the host cell, or population of host cells, harbor one or more expression vectors that can direct transient introduction of the transgene into the host cells or stable insertion of the transgene into the host cells' genome, where the transgene comprises nucleic acids encoding any of the first and/or second polypeptides described herein. In embodiments where a nucleic acid molecule encodes two polypeptides (e.g., a first polypeptide having homology to SEQ ID NO:6 and a second polypeptide having homology to SEQ ID NO:7), the two polypeptide-encoding sequences can be regulated by the same promoter and can be linked by an IRES or 2A sequence (Shao et al. (2009) *Cell Research* 19:296-306) or the two polypeptide-encoding sequences can be operably linked to different promoters.

[00189] A production host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (e.g., a yeast or other fungus), a plant cell (e.g., a tobacco or tomato plant cell), a mammalian cell (e.g., a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. In one embodiment, a production host cell can be transfected with an expression vector operably linked to a nucleic acid encoding a desired antigen-binding protein thereby generating a transfected/transformed host cell which is cultured under conditions suitable for expression of the antigen-binding protein by the transfected/transformed host cell, and optionally recovering the antibody from the transfected/transformed host cells (e.g., recovery from host cell lysate) or recovery from the culture medium. In one embodiment, production host cells comprise non-human cells including CHO, BHK, NS0, SP2/0, and YB2/0. In one embodiment, host cells comprise human cells including HEK293, HT-1080, Huh-7 and PER.C6. Examples of host cells include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (see Gluzman *et al.*, 1981, *Cell* 23: 175), L cells,

C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum- free media (see Rasmussen et al., 1998, *Cytotechnology* 28:31) or CHO strain DX-B 11, which is deficient in DHFR (see Urlaub et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-20), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (see McMahan et al., 1991, *EMBO J.* 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo 205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. In some embodiments, host cells can be lymphoid cells such as YO, NS0 or Sp20. In some embodiments, a host cell is a mammalian host cell, but is not a human host cell.

[00190] Polypeptides of the present disclosure (e.g., antibodies and antigen binding proteins) can be produced using any methods known in the art. In one example, the polypeptides are produced by recombinant nucleic acid methods by inserting a nucleic acid sequence (e.g., DNA) encoding the polypeptide into a recombinant expression vector which is introduced into a host cell and expressed by the host cell under conditions promoting expression.

[00191] The recombinant DNA can also encode any type of protein tag sequence that may be useful for purifying the protein. Examples of protein tags include but are not limited to a histidine (his) tag, a FLAG tag, a myc tag, an HA tag, or a GST tag. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts can be found in *Cloning Vectors: A Laboratory Manual*, (Elsevier, N.Y., 1985).

[00192] The expression vector construct can be introduced into a host cell, e.g., a production host cell, using a method appropriate for the host cell. A variety of methods for introducing nucleic acids into host cells are known in the art, including, but not limited to, electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; viral transfection; non-viral transfection; microprojectile bombardment; lipofection; and infection (e.g., where the vector is a viral vector).

[00193] Suitable bacteria include gram negative or gram positive organisms, for example, *E. coli* or *Bacillus* spp. Yeast, for example from the *Saccharomyces* species, such as *S. cerevisiae*, may also be used for production of polypeptides. Various mammalian or insect cell culture systems can also be employed to express recombinant proteins. Baculovirus systems for

production of heterologous proteins in insect cells are reviewed by Luckow and Summers, (*Bio/Technology*, 6:47, 1988). Examples of suitable mammalian host cell lines include endothelial cells, COS-7 monkey kidney cells, CV-1, L cells, C127, 3T3, Chinese hamster ovary (CHO), human embryonic kidney cells, HeLa, 293, 293T, and BHK cell lines. Purified polypeptides are prepared by culturing suitable host/vector systems to express the recombinant proteins. For many applications, *E. coli* host cells are suitable for expressing small polypeptides. The protein can then be purified from culture media or cell extracts.

[00194] Antibodies and antigen binding proteins disclosed herein can also be produced using cell-translation systems. For such purposes the nucleic acids encoding the polypeptide must be modified to allow *in vitro* transcription to produce mRNA and to allow cell-free translation of the mRNA in the particular cell-free system being utilized (eukaryotic such as a mammalian or yeast cell-free translation system or prokaryotic such as a bacterial cell-free translation system).

[00195] Nucleic acids encoding any of the various polypeptides disclosed herein may be synthesized chemically or using gene synthesis methods (available for example through commercial entities such as Blue Heron, DNA 2.0, GeneWiz, etc.). Codon usage may be selected so as to improve expression in a cell. Such codon usage will depend on the production host cell type. Specialized codon usage patterns have been developed for *E. coli* and other bacteria, as well as mammalian cells, plant cells, yeast cells and insect cells. See for example: Mayfield et al., *Proc. Natl. Acad. Sci. USA*. 2003 100(2):438-42; Sinclair et al. *Protein Expr. Purif.* 2002 (1):96-105; Connell N D. *Curr. Opin. Biotechnol.* 2001 12(5):446-9; Makrides et al. *Microbiol. Rev.* 1996 60(3):512-38; and Sharp et al. *Yeast*. 1991 7(7):657-78.

[00196] Antibodies and antigen binding proteins described herein can also be produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984, The Pierce Chemical Co., Rockford, Ill.). Modifications to the protein can also be produced by chemical synthesis.

[00197] Antibodies and antigen binding proteins described herein can be purified by isolation/purification methods for proteins generally known in the field of protein chemistry. Non-limiting examples include extraction, recrystallization, salting out (e.g., with ammonium sulfate or sodium sulfate), centrifugation, dialysis, ultrafiltration, adsorption chromatography, ion exchange chromatography, hydrophobic chromatography, normal phase chromatography, reversed-phase chromatography, gel filtration, gel permeation chromatography, affinity

chromatography, electrophoresis, countercurrent distribution or any combinations of these. After purification, polypeptides may be exchanged into different buffers and/or concentrated by any of a variety of methods known to the art, including, but not limited to, filtration and dialysis.

[00198] The purified antibodies and antigen binding proteins described herein can be at least 65% pure, at least 75% pure, at least 85% pure, at least 95% pure, or at least 98% pure.

Regardless of the exact numerical value of the purity, the polypeptide is sufficiently pure for use as a pharmaceutical product. Any of the anti-spike protein antibodies, or antigen binding protein thereof, described herein can be expressed by transgenic host cells and then purified to about 65-98% purity or high level of purity using any art-known method.

[00199] In certain embodiments, the antibodies and antigen binding proteins herein can further comprise post-translational modifications. Exemplary post-translational protein modifications include phosphorylation, acetylation, methylation, ADP-ribosylation, ubiquitination, glycosylation, carbonylation, sumoylation, biotinylation or addition of a polypeptide side chain or of a hydrophobic group. As a result, the modified polypeptides may contain non-amino acid elements, such as lipids, poly- or mono-saccharide, and phosphates. In one embodiment, a form of glycosylation can be sialylation, which conjugates one or more sialic acid moieties to the polypeptide. Sialic acid moieties improve solubility and serum half-life while also reducing the possible immunogenicity of the protein. See Rajuetal. *Biochemistry* 2001 31; 40:8868-76.

[00200] In some embodiments, the antibodies and antigen binding proteins described herein can be modified to increase their solubility and/or serum half-life which comprises linking the antibodies and antigen binding proteins to non-proteinaceous polymers. For example, polyethylene glycol ("PEG"), polypropylene glycol, or polyoxyalkylenes can be conjugated to antigen-binding proteins, for example in the manner as set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; or 4,179,337.

[00201] The term "polyethylene glycol" or "PEG" is used broadly to encompass any polyethylene glycol molecule, without regard to size or to modification at an end of the PEG, and can be represented by the formula: $X-O(CH_2CH_2O)_n-CH_2CH_2OH$ (1), where n is 20 to 2300 and X is H or a terminal modification, e.g., a C₁₋₄ alkyl. In one embodiment, the PEG terminates on one end with hydroxy or methoxy, i.e., X is H or CH₃ ("methoxy PEG"). A PEG can contain further chemical groups which are necessary for binding reactions; which results from the

chemical synthesis of the molecule; or which is a spacer for optimal distance of parts of the molecule. In addition, such a PEG can consist of one or more PEG side-chains which are linked together. PEGs with more than one PEG chain are called multiarmed or branched PEGs.

Branched PEGs can be prepared, for example, by the addition of polyethylene oxide to various polyols, including glycerol, pentaerythritol, and sorbitol. Branched PEG molecules are described in, for example, EP-A 0 473 084 and U.S. Pat. No. 5,932,462. One form of PEGs includes two PEG side-chains (PEG2) linked via the primary amino groups of a lysine (Monfardini et al., *Bioconjugate Chem.* 6 (1995) 62-69).

Pharmaceutical Compositions

[00202] The present disclosure provides pharmaceutical compositions comprising any of the anti-spike protein antibodies or antigen-binding proteins described herein and a pharmaceutically acceptable excipient. An excipient encompasses carriers and stabilizers. In one embodiment, the pharmaceutical compositions comprise an anti-spike protein antibody or antigen binding protein as disclosed herein, comprising a heavy chain variable region with an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6 (the heavy chain variable region of antibody S1D2) and an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7 (the light chain variable region of antibody S1D2). Alternatively or in addition, a pharmaceutical composition can comprise an anti-spike protein antibody or antigen binding protein as disclosed herein that comprises a heavy chain variable region comprising a heavy chain CDR1 sequence having the amino acid sequence of SEQ ID NO:8, a heavy chain CDR2 sequence having the amino acid sequence of SEQ ID NO:9, and a heavy chain CDR3 sequence having the amino acid sequence of SEQ ID NO:10, and further comprises a light chain variable region comprising a light chain CDR1 sequence having the amino acid sequence of SEQ ID NO:11, a light chain CDR2 sequence having the amino acid sequence of SEQ ID NO:12, and a light chain CDR3 sequence having the amino acid sequence of SEQ ID NO:13.

[00203] The pharmaceutical compositions can be produced to be sterile and stable under the conditions of manufacture and storage. The antigen-binding proteins provided herein can be in powder form, for example for reconstitution in the appropriate pharmaceutically acceptable excipient before or at the time of delivery. Alternatively, the antigen-binding proteins can be in

solution with an appropriate pharmaceutically acceptable excipient or a pharmaceutically acceptable excipient can be added and/or mixed before or at the time of delivery, for example to provide a unit dosage injectable or inhalable form. Preferably, the pharmaceutically acceptable excipient used in the present invention is suitable to high drug concentration, can maintain proper fluidity and, in some embodiments, can delay absorption.

[00204] Examples of pharmaceutically acceptable excipients includes for example inert diluents or fillers (e.g., sucrose and sorbitol), lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Additional examples include buffering agents, stabilizing agents, preservatives, non-ionic detergents, anti-oxidants and isotonicifiers.

[00205] Therapeutic compositions and methods for preparing them are well known in the art and are found, for example, in *“Remington: The Science and Practice of Pharmacy”* (20th ed., ed. A. R. Gennaro A R., 2000, Lippincott Williams & Wilkins, Philadelphia, Pa.). Therapeutic compositions can be formulated for parenteral administration may, and can for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the antibody (or antigen binding protein thereof) described herein. Nanoparticulate formulations (e.g., biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the antibody (or antigen binding protein thereof). Other potentially useful parenteral delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The concentration of the antibody (or antigen binding protein thereof) in the formulation varies depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

[00206] Any of the anti-spike protein antibodies disclosed herein (or antigen binding portions thereof) may be optionally administered as a pharmaceutically acceptable salt, such as non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pantoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid,

carboxymethyl cellulose, or the like; and inorganic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. In one example, the antibody (or antigen binding portions thereof) is formulated in the presence of sodium acetate to increase thermal stability.

[00207] Any of the anti-spike protein antibodies disclosed herein (or antigen binding portions thereof) may be formulated for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium.

[00208] Also provided is a kit comprising an anti-S protein as disclosed herein. In one embodiment, the kit comprises an antigen binding protein that specifically binds the S protein of a coronavirus as disclosed herein, such as an anti-S protein comprising a heavy chain variable region having at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity to SEQ ID NO:6 and a light chain variable region having at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 98% identity, or at least 99% identity to SEQ ID NO:7. In one embodiment, the kit comprises an antigen binding protein that specifically binds the S protein of a coronavirus as disclosed herein, such as an anti-S protein comprising heavy chain CDRs having the amino acid sequences of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, and further comprising light chain CDRs having the amino acid sequences of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13. The antibody can be provided in solution or as a solid, for example, a powder for reconstitution. The kit can further include one or more sterile pharmaceutically acceptable solutions for resuspension or dilution of the antibody, and may include one or more additional pharmaceutical formulations, which may be, as nonlimiting examples, any of an additional antibody, an analgesic, an antibiotic, an anti-inflammatory drug, a bronchodilator, or an antiviral drug. The kit can be used for treating a subject having a coronavirus infection, or for providing prophylaxis against infection with a coronavirus. The components of the kit of can be provided in suitable containers and labeled for diagnosis, prophylaxis and/or treatment of coronavirus infection. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile, solution or as a lyophilized,

preferably sterile, formulation for reconstitution. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, HBSS, Tyrode's solution, Ringer's solution, or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts.

[00209] In some embodiments, such as but not limited to embodiment in which the antibody is formulated for pulmonary delivery, the antibody can be provided as a dry powder, which may be formulated with one or more suitable excipients, or as a liquid formulation. The kit can further include: solutions for resuspension or dilution of the antibody and a means for dispensing the pharmaceutical composition comprising the antibody into a nebulizer or metered dose inhaler. The kit may in some embodiments include a metered dose inhaler. In one embodiment, the kit can be used for treating a subject having a coronavirus-associated infection or disease.

[00210] Associated with the kits can be instructions customarily included in commercial packages of therapeutic, prophylactic or diagnostic products, that contain information about, for example, the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic, prophylactic or diagnostic products.

[00211] Also included herein are pharmaceutical compositions that include nucleic acid molecules that may be administered to a subject, such as a human subject for treatment or prevention of a coronavirus infection. A nucleic acid molecule that encodes a neutralizing antigen binding protein as provided herein can be an RNA molecule or a DNA molecule and can include one or more non-naturally occurring linkages (e.g., backbone linkages) or nucleobases.

[00212] A nucleic acid molecule provided in a pharmaceutical composition can be, for example, a DNA molecule encoding a neutralizing antigen-binding protein as described hererinabove. A pharmaceutical composition can include one or more nucleic acid molecules, for example, can include a nucleic acid molecule that encodes a heavy chain of an antibody and a second nucleic molecule that encodes a light chain of an antibody, or a pharmaceutical composition can include a single nucleic acid construct that includes two open reading frames or genes, each operably linked to its own promoter, for example, encoding a light chain of an

antibody and a heavy chain of an antibody. In some embodiments the composition is formulated for intramuscular injection, and a first gene encoding a light chain of an antibody and a second gene encoding a heavy chain of the antibody are each independently linked to a promoter active in muscle cells. The heavy and light chain genes can be on the same or different nucleic acid molecules. The nucleic acid molecule(s) in the pharmaceutical composition can be a plasmid, for example, a nanoplasmid having fewer than 500 base pairs of sequence of a bacterial plasmid, ten or fewer, five or fewer, or three or fewer CpG sequences, and/or can lack an antibiotic resistance marker such as any disclosed in US 9,550,998; US 10,047,365; or US 10,844,388, all of which are incorporated herein by reference in their entireties.

[00213] A pharmaceutical composition that comprises one or more nucleic acid molecules as provided herein can include compounds that enhance delivery of nucleic acid molecules into cells, such as for example a cationic lipid or amphiphilic block copolymers, for example, linear and/or X-shaped copolymers, and can include one or more poloxamers or poloxamines, or of any of an ethylene oxide/propylene oxide copolymer, synperonics®, pluronics®, kolliphor®, poloxamer 181, poloxamer 188, or poloxamer 407, poloxamines, tetronics®, T/908, or T/1301, for example. The pharmaceutical composition can further include any of alginate, or a PEG polymer or copolymer, e.g., DSPE-PEG, as nonlimiting examples, and can be formulated for injection and can include a buffer such as PBS, TBS, HBSS, Ringer's, or Tyrode's. The pharmaceutical composition that comprises one or more nucleic acid molecules can be formulated for injection and can be provided in a vial or other container as a liquid solution or solid (e.g., a lyophilate).

Methods of Treatment

[00214] The present disclosure provides methods for treating a subject having a coronavirus infection or suspected of having a coronavirus infection, the method comprising: administering to the subject an effective amount of a therapeutic composition comprising an anti-S1 antigen-binding protein as described herein, e.g., an antibody as described herein. In some embodiments, the (suspected or actual) coronavirus infection is a SARS-CoV-2 infection. The subject can be a human subject or an animal. The subject may be a subject who has tested positive for the coronavirus, a subject who has had close and/or prolonged contact with another individual or animal that has tested positive for coronavirus, and/or can be a subject exhibiting symptoms associated with coronavirus infection. The antigen-binding protein or antibody is preferably a

fully human neutralizing antigen-binding protein or antibody and may be a fully human neutralizing antibody having one or more mutations in the Fc region that result in reduced Fc effector function. In some embodiments, the subject is infected with or suspected of being infected with HCoV-NL63, SARS-CoV, or SARS-CoV-2, for example, the subject may be a human subject infected with or suspected of being infected with SARS-CoV-2.

[00215] The present disclosure also provides methods for preventing a coronavirus infection in a subject, the method comprising: administering to a subject at risk of becoming infected with a coronavirus an effective amount of a therapeutic composition comprising an anti-S1 antigen-binding protein as described herein, which may be an antibody or antibody fragment as described herein. The subject can be a human subject or an animal. The subject may be a health care worker, a first responder, a transportation worker, a delivery person, a worker in a meat-packing plant, or a warehouse. The subject may be an incarcerated subject in a jail or prison. The subject may be a person in an assisted living facility. The subject may live in an area with a high rate of increase of people testing positive for the coronavirus. The antigen-binding protein or antibody is preferably a fully human neutralizing antigen-binding protein or antibody as disclosed herein and may be a fully human neutralizing antibody having one or more mutations in the Fc region that result in reduced Fc effector function. In some embodiments, the subject is at risk of being infected with HCoV-NL63, SARS-CoV, or SARS-CoV-2, for example, the subject may be a human subject at risk of becoming infected with SARS-CoV-2.

[00216] In some embodiments, administration of the antigen binding protein that specifically binds the S protein of a coronavirus, such as SARS-CoV-2, can be by oral delivery. Oral dosage forms can be formulated for example as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard capsules, soft gelatin capsules, syrups or elixirs, pills, dragees, liquids, gels, or slurries. These formulations can include pharmaceutically excipients including, but not limited to, inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents such as corn starch or alginic acid; binding agents such as starch, gelatin or acacia; lubricating agents such as calcium stearate, glyceryl behenate, hydrogenated vegetable oils, magnesium stearate, mineral oil, polyethylene glycol, sodium stearyl, fumarate, stearic acid, talc, zinc stearate; preservatives such as n-propyl-p-hydroxybenzoate; coloring, flavoring or sweetening agents such as sucrose, saccharine, glycerol, propylene glycol or sorbitol; vegetable

oils such as arachis oil, olive oil, sesame oil or coconut oil; mineral oils such as liquid paraffin; wetting agents such as benzalkonium chloride, docusate sodium, lecithin, poloxamer, sodium lauryl sulfate, sorbitan esters; and thickening agents such as agar, alginic acid, beeswax, carboxymethyl cellulose calcium, carageenan, dextrin or gelatin.

[00217] Alternatively, administration can be by injection or intravenous or intra-arterial delivery, and may be, for example, by epidermal, intradermal, subcutaneous, intramuscular, intraperitoneal, intrapleural, intra-abdominal, or intracavitary delivery. Formulations for parenteral administration can be inter alia in the form of aqueous or non-aqueous isotonic sterile non-toxic injection or infusion solutions or suspensions. Preferred parenteral administration routes include intravenous, intra-arterial, intraperitoneal, epidural, and intramuscular injection or infusion. Intravenous delivery can be by infusion or by bolus ("push") injection. The solutions or suspensions may comprise agents that are non-toxic to recipients at the dosages and concentrations employed such as 1,3-butanediol, Ringer's solution, Hank's solution, isotonic sodium chloride solution, oils such as synthetic mono- or diglycerides or fatty acids such as oleic acid, local anesthetic agents, preservatives, buffers, viscosity or solubility increasing agents, water-soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like, oil-soluble antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like, and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, etc.

[00218] In various embodiments, an antibody (or antibody fragment) that specifically binds an epitope of a coronavirus S1 subunit, such as any of the neutralizing antibodies disclosed herein, can be incorporated into a pharmaceutical composition suitable for pulmonary administration to a subject. For example, an antibody as provided herein antibody that specifically binds an epitope of a coronavirus S1 subunit can be formulated into a liquid pharmaceutical composition that includes a pharmaceutical excipient, where pharmaceutical composition is suitable for inhalation by a subject. The neutralizing antibody can comprise a heavy chain variable region having at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:6 and can comprise a light chain variable region having 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:7. In some embodiments, the neutralizing antibody comprises a heavy

chain variable region comprising the sequence of SEQ ID NO:6 or a sequence having at least 99% identity to SEQ ID NO:6. In various embodiments the antigen-binding proteins have a heavy chain CDR1 sequence of SEQ ID NO:8, a heavy chain CDR2 sequence of SEQ ID NO:9, a heavy chain CDR3 sequence of SEQ ID NO:10, a light chain CDR1 sequence of SEQ ID NO:11, a light chain CDR2 sequence of SEQ ID NO:12, and a light chain CDR3 sequence of SEQ ID NO:13. In some embodiments, the neutralizing antibody comprises a light chain variable region comprising the sequence of SEQ ID NO:7 or a sequence having at least 99% identity to SEQ ID NO:7. The neutralizing antibody can be an immunoglobulin molecule that optionally includes one or more mutations in the Fc region, for example one or both of a LALA mutation and a YTE mutation as described hereinabove.

[00219] A liquid composition that comprises an anti-neutralizing antibody that specifically binds an epitope of a coronavirus S protein as disclosed herein formulated for pulmonary administration comprises a neutralizing antibody formulated into a solution or suspension, e.g., an isotonic saline solution, which is optionally buffered, at an appropriate concentration for pulmonary administration as an aerosol, mist, or vapor. Preferably, a solution or suspension that includes the neutralizing antibody is isotonic with respect to pulmonary fluids and of about the same pH, for example, has a pH of from about pH 4.0 to about pH 8.5 or from pH 5.5 to pH 7.8, or, for example, from about 7.0 to about 8.2. Suitable buffering agents that can be present in a liquid pharmaceutical composition for pulmonary delivery include, but are not limited to, citrate buffer, phosphate buffer, and succinate buffer. Alternatively or in addition, imidazole, histidine, or another compound that maintains pH in the range of about pH 4.0 to about 8.5 can be used. For example, Ringer's solution, isotonic sodium chloride, and phosphate buffered saline may be used. One of skill in the art can determine an appropriate saline content and pH for an aqueous solution for pulmonary administration.

[00220] Additional compounds that may be present in a liquid formulation for pulmonary delivery include, without limitation, sugars, sugar alcohols, alcohols (e.g., benzyl alcohol), polyols, amino acids, salts, polymers, surfactants, and preservatives (e.g., ethyl or n-propyl p-hydroxybenzoate). Other possible ingredients include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone, and gum tragacanth, and a wetting agent such as lecithin. The compositions can include any of a variety of compounds to aid in solubility,

stability, or delivery, where the added compounds do not negatively affect the coronavirus S-protein binding activity of the neutralizing antibody.

[00221] For example, a liquid pharmaceutical composition for pulmonary delivery of an S1-binding neutralizing antibody as provided herein may include an excipient or stabilizer including but not limited to a sugar, alcohol, sugar alcohol, or an amino acid. Preferred sugars include sucrose, trehalose, raffinose, stachyose, sorbitol, glucose, lactose, dextrose, or any combination thereof. A sugar can optionally be present in the range of about 0% to about 9.0% (w/v), preferably about 0.5% to about 5.0%, for example about 1.0%. An amino acid, for example, can be optionally be present in the range of about 0% to about 1.0% (w/v), preferably about 0.3% to about 0.7%, for example about 0.5%.

[00222] A buffering agent such as phosphate, citrate, succinate, histidine, imidazole, or Tris can also optionally be present in the liquid neutralizing antibody formulation. EDTA may be present as a stabilizer. Any of various surfactants may also be present, such as for example, polyoxyethylene sorbitol esters such as polysorbate 80 (Tween 80) and polysorbate 20 (Tween 20); polyoxypropylene-polyoxyethylene esters such as Poloxamer 188; polyoxyethylene alcohols such as Brij 35; a mixture of polysorbate surfactants with phospholipids (such as phosphatidylcholine and derivatives), dimyristolglycerol and other members of the phospholipid glycerol series; lysophosphatidylcholine and derivatives thereof; mixtures of polysorbates with lysolecithin or cholesterol; bile salts and their derivatives such as sodium cholate, sodium deoxycholate, sodium glycodeoxycholate, sodium taurocholate, etc. Additional examples of suitable surfactants include L-alpha-phosphatidylcholine dipalmitoyl ("DPPC"), diphosphatidyl glycerol (DPPG), 1,2-Dipalmitoyl-sn-glycero-3-phospho-L-serine (DPPS), 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), fatty alcohols, polyoxyethylene-9-lauryl ether, surface active fatty acids, sorbitan trioleate (Span 85), glycocholate, surfactin, poloxomers, sorbitan fatty acid esters, tyloxapol, phospholipids, and alkylated sugars.

[00223] Such pharmaceutical compositions may be administered for example, as a propellant-free inhalable solution comprising a soluble S protein-binding neutralizing antibody and may be administered to the subject via a nebulizer. Other suitable preparations include, but are not limited to, mist, vapor, or spray preparations so long as the particles comprising the protein composition are delivered in a size range consistent with that described for the delivery device.

[00224] Therapeutic compositions are preferably sterile and stable under the conditions of manufacture and storage. The formulation can be formulated as a solution, microemulsion, dispersion, or suspension. Sterile inhalable solutions can be prepared by incorporating the active compound (i.e., a soluble S1-binding neutralizing antibody as provided herein) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. Fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and/or by the use of surfactants.

[00225] The concentration of S protein-binding neutralizing antibody in a liquid formulation for pulmonary delivery can range for example, from about 1 µg per ml to about 500 mg per ml, and may be in the range of, for example, from about 10 µg per ml to about 200 mg per ml, or from about 20 µg per ml to about 100 mg per ml, although these ranges are not limiting.

[00226] In some embodiments, a pharmaceutical composition as provided herein is formulated for nasal delivery. “Nasal delivery” is used herein to refer to deposition of the pharmaceutical composition within one or preferably both nares (nostrils or nasal passages) of a subject; “nasal delivery” and “intranasal delivery” are used interchangeably herein. Nasal delivery may be topical administration, i.e., deposition or application of a liquid, gel, paste, powder, or particles within the nasal passages, for example using a dropper, squeeze bottle, or applicator. In some instances, nasal delivery may be by inhalation, including by means of a dry powder inhaler, metered-dose inhaler, or nebulizer that generates aerosols for delivery of particles or droplets to the lung. Contemplated herein are methods of topical nasal delivery that do not require an inhalation device such as a dry powder inhaler, metered dose inhaler, or nebulizer, although the formulations and methods provided herein are not limited to such methods.

[00227] For example, the subject may use a dropper or squeeze bottle to deposit one, two, or more drops of a pharmaceutical composition for nasal delivery in one or both nostrils. The user (or another person) may administer the nasal drops when the subject is lying down, or has the head tilted back to avoid having the composition drain from the nostrils. The subject may remain supine (or with head tilted back) for several seconds to two minutes, for example.

[00228] A coronavirus-neutralizing antigen-binding protein as provided herein can be formulated with any suitable excipient(s) for nasal delivery. Reference may be made to standard handbooks, such as for example Remington's Introduction to the Pharmaceutical Sciences, 2nd Ed., Lippincott Williams and Wilkins, USA (2011) or Remington, the Science and Practice of Pharmacy, 23rd Edition, Academic Press (2020). Preferably a neutralizing antibody for nasal administration is formulated as a composition or pharmaceutical composition comprising at least a therapeutically effective amount of a neutralizing antibody, such as a neutralizing antibody disclosed herein, and at least one pharmaceutically acceptable nasal carrier, and optionally one or more additional pharmaceutically acceptable additives and/or agents. A "nasal carrier" as set forth in the present invention is a carrier that is suitable for application through the nasal route, i.e. deposition within the nostril or application to the nasal mucosa. The nasal carrier may be a solid, semi-liquid, or liquid filler, diluent, or encapsulating material, for example. The nasal composition can be provided in a variety of forms, including fluid or semi-liquid or viscous solutions, gels, creams, pastes, powders, microspheres, and films for direct application to the nasal mucosa including application as liquid drops into the nasal passage. The nasal carrier should be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the formulation and not eliciting an unacceptable deleterious effect in the subject. Preferred nasal carriers are those which maintain the solubility of the neutralizing antibody (when applied as a liquid or in a gel or paste) and those that improve the contact of the pharmaceutical composition with the nasal mucus or nasal mucosa. A carrier provided in the composition may also facilitate the diffusion of the antibody from the composition to the nasal mucosa and/or may prolong the nasal residence time of the composition allowing dissemination to the lungs via respiration, for example.

[00229] Suitable nasal carriers are known to those skilled in the art of pharmacology. A carrier used in a composition for intranasal delivery can be a liquid or solvent or dispersion medium containing, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof. Other preferred liquid carriers are aqueous saline, e.g. physiological saline, or an aqueous buffer, e.g. a phosphate/citric acid buffer. Further components of the composition, which can be soluble, in semi-solid or gelatinous form, sparingly soluble, or in solid form can include, without limitation, additional salts, one or more sugars, or polymers, for example, mannitol, lactose, starch, magnesium

stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. Further carriers include polyacrylates, sodium carboxy methyl cellulose, starches and their derivatives, alginic acid and salts, hyaluronic acid and salts, pectic acid and salts, gelatin and its derivatives, gums, polylactic acid and its copolymers, polyvinyl acetate, celluloses and their derivatives, coated celluloses, crosslinked dextrans, polylactic acid and its copolymers, and polyvinyl acetate. Non-limiting examples of other solid nasal carriers are described in US 5,578,5674, WO 04/093917 and WO 05/120551, incorporated herein by reference.

[00230] In some embodiments, the neutralizing antibody may be released from the composition by diffusion or by disintegration of the nasal carrier. In some circumstances, the neutralizing antibody is dispersed in microcapsules (microspheres) or nanocapsules (nanospheres) prepared from a suitable polymer, e.g., isobutyl 2-cyanoacrylate (see, e.g., Michael et al., *J. Pharmacy Pharmacol.* 1991; 43: 1-5). These particular microspheres not only demonstrate mucoadhesion properties, but also protect against enzymatic degradation. They may further allow manipulation of the rate of release of the neutralizing antibody or antibodies to provide sustained delivery and biological activity over a protracted time (Morimoto et al., *Eur. J. Pharm. Sci.* 2001 May; 13(2): 179-85).

[00231] The pharmaceutical composition can include a bioadhesive nasal carrier, e.g., a compound that adheres to the nasal mucosa by chemical or physical binding such as Van der Waals interaction, ionic interaction, hydrogen bonding or by polymer chain entanglement. The adhesion may be to the epithelial (cellular) surface or to the mucus overlying the surface (a mucoadhesive). These compounds promote binding of drugs to biological material in the nasal cavity, thereby extending residence time and allowing delivery to the lungs through respiration. Examples of bioadhesive or mucoadhesive materials include, without limitation, carhopol, cellulose and cellulose derivatives (e.g., hydroxypropyl methylcellulose, hydroxypropylcellulose) or cellulose-containing compounds, coated cellulose (e.g., microcrystalline cellulose coated with glycerol monooleate) starch, dextran, and chitosan (See, for example, Ilium, Bioadhesive formulations for nasal peptide delivery. In: E Mathiowitz, D E Chickering III, C Lehr, eds. *Bioadhesive Drug Delivery Systems*. New York: Marcel Dekker, 1999: 507-541; EP 0490806; and WO 96/03142, all incorporated herein by reference).

[00232] To further enhance mucosal delivery of a neutralizing antibody as provided herein, formulations comprising neutralizing antibody may also contain a hydrophilic low molecular

weight compound as a nasal carrier, base, or excipient. Such hydrophilic low molecular weight compounds provide a passage medium through which a water-soluble active agent, such as the neutralizing antibody of the invention, may diffuse. Exemplary hydrophilic low molecular weight compound are disclosed in WO 05/120551 and include polyol compounds, such as oligo-, di- and monosaccharides such as sucrose, mannitol, lactose, L-arabinose, D-erythrose, D-ribose, D-xylose, D-mannose, D-galactose, lactulose, cellobiose, gentibiose, glycerin, and polyethylene glycol. Other non-limiting examples of hydrophilic low molecular weight compounds useful as such carriers or bases include N-methylpyrrolidone, and alcohols (e.g. oligovinyl alcohol, ethanol, ethylene glycol, propylene glycol, etc.). These hydrophilic low molecular weight compounds can be used alone or in combination with one another or with other active or inactive components of the intranasal formulation.

[00233] A carrier in a nasal formulation may further contain pharmaceutically acceptable additives such as acidifying agents, alkalizing agents, antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, stabilizers, tonicity adjustors, wetting agents or other biocompatible materials. A tabulation of ingredients listed by the above categories can be found in the U.S. Pharmacopeia National Formulary, pp. 1857-1859, 1990. Examples of pharmaceutically acceptable preservatives include benzalkonium chloride, an alkyl p-hydroxybenzoate (paraben) such as methyl p-hydroxybenzoate and propyl p-hydroxybenzoate, or sodium methylmercurithiosalicylate (Thiomersal). Further non-limiting examples of pharmaceutically acceptable preservatives are described in U.S. Pat. No. 5,759,565, US 20100129354, and WO 04/093917. Examples of pharmaceutically acceptable antioxidants include alkali metal sulfites, alkali metal bisulfites, alkali metal pyrosulfites, sodium thiosulfate, thiodipropionic acid, cysteine in free or salt form (such as cysteine hydrochloride), ascorbic acid, citraconic acid, propyl or ethyl gallate, nordihydroguaiaretic acid, butylated hydroxyanisole or -toluene, and tocol. Further non-limiting examples of pharmaceutically acceptable antioxidants are provided in US 20100129354, WO 04/093917, and WO 05/120551.

[00234] The desired viscosity for the compositions of the invention will depend on the particular form for administration, e.g. whether administration is to be by nasal drops or nasal spray. For example, for nasal drops an appropriate viscosity may be from about 2 to about 40×10^{-3} Pa s, and for nasal sprays the viscosity may be less than 2×10^{-3} Pa s, e.g. from 1 to 2×10^{-3}

Pa s. Such values are exemplary only, and acceptable viscosities for a therapeutic or prophylactic neutralizing antibody composition can be determined empirically. Examples of pharmaceutically acceptable compounds for enhancing viscosity include, for example, methylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, (which can also serve as mucoadhesives) sucrose, PVA, PVP, polyacrylic acid, or natural polymers. Further non-limiting examples of viscosity builders are described in US 5,578,567.

[00235] Examples of pharmaceutically acceptable stabilizers include albumin, e.g. human serum albumin, aprotinin or δ -aminocaproic acid. In some instances, the activity or physical stability of proteins can also be enhanced by various additives to aqueous solutions of the neutralizing antibody or antibodies. For example, additives, such as polyols (including sugars), amino acids, and various salts may be used. Further non-limiting examples of stabilizers are provided in US 20100129354. Examples of pharmaceutically acceptable tonicity adjustors include nasally acceptable sugars, e.g. glucose, mannitol, sorbitol, ribose, mannose, arabinose, xylose or another aldose or glucosamine. Further non-limiting examples of tonicity adjustors are provided in US20100129354. Such additives may be used in suitable amounts as known in the art and as can be determined by the skilled person based on the disclosure and art, including art cited herein.

[00236] Enzyme inhibitors may also be optionally added to the composition for nasal delivery to reduce the activity of any hydrolytic enzymes in the nasal mucosa that can potentially degrade the neutralizing antibody or other components of the pharmaceutical composition. Enzyme inhibitors that can reduce degradative activities for use within the invention can be selected from a wide range of non-protein inhibitors that vary in their degree of potency and toxicity (see, e.g., L. Stryer, *Biochemistry*, WI-1: Freeman and Company, NY, N.Y., 1988). Non-limiting examples include amastatin and bestatin (O'Hagan et al., *Pharm. Res.* 1990, 7: 772-776). Various classes of enzyme inhibitors that may be considered are extensively described and exemplified in WO 05/120551. Another means to inhibit degradation is pegylation with PEG molecules, preferably low molecular weight PEG molecules (e.g. 2 kDa; Lee et al., *Calcif Tissue Int.* 2003, 73: 545-549).

[00237] When the nasal carrier is a liquid, the tonicity of the formulation, as measured with reference to the tonicity of 0.9% (w/v) physiological saline solution taken as unity, is typically adjusted to a value at which no substantial, irreversible tissue damage will be induced in the

nasal mucosa at the site of administration. Generally, the tonicity of the solution is adjusted to a value of about 1/3 to 3, more typically 1/2 to 2, and most often 3/4 to 1.7. Liquid compositions of the invention further preferably have a mildly acid pH, e.g. from about pH 3 to about pH 6.5, from about pH 3.5 to about pH 6.5, or preferably from about pH 4.5 to about pH 6.5 to minimize nasal irritation (Betel et al. *Adv. Drug Delivery Rev.* 1998; 29: 89-116). The required degree of acidity may conveniently be achieved, e.g. by the addition of a buffering agent, e.g. a mixture of citric acid and disodium hydrogen phosphate, or an acid such as HCl or another appropriate mineral or an organic acid, e.g. phosphoric acid. Solid compositions may also comprise a buffering agent when they are prepared by lyophilization of a liquid composition buffered to a pH value as indicated above. Non-limiting examples of pharmaceutically acceptable buffering agents are provided in US20100129354, WO 04/093917, and WO 05/120551. In some embodiments provided herein, a composition for topical nasal delivery that includes a neutralizing antibody as provided herein, such as the STI-2020 antibody, comprises: 20mM Histidine, 240 mM Sucrose, 0.2 - 0.3% Hydroxypropyl methyl cellulose (HPMC), and 0.05% Polysorbate 80, at a pH of about 5.8. The concentration of antibody can be, for example, from about 1 mg/mL to about 200 mg/mL, from about 2 mg/mL to about 100 mg/mL, or from about 5 mg/mL to about 80 mg/mL, or from about 10 mg/mL to about 50 mg/mL.

[00238] Alternatively, a neutralizing antibody, such as a neutralizing antibody provided herein may be formulated for intranasal delivery in the form of a powder (such as a freeze-dried or micronised powder) or mist; for example with a particle size within the ranges indicated herein. Compounds may also be included in a pharmaceutical composition for intranasal delivery to reduce or prevent aggregation of the neutralizing antigen-binding protein. Aggregation inhibitory agents include, for example, polymers such as polyethylene glycol, dextran, diethylaminoethyl dextran, and carboxymethyl cellulose, which significantly increase the stability and reduce the solid-phase aggregation of peptides and proteins. Certain additives, in particular sugars and other polyols, also impart significant physical stability to dry, e.g., lyophilized proteins. These additives can also be used within the invention to protect the proteins against aggregation not only during lyophilization but also during storage in the dry state. For example, sucrose and Ficoll 70 (a polymer with sucrose units) exhibit significant protection against peptide or protein aggregation during solid-phase incubation under various conditions. These additives may also enhance the stability of solid proteins embedded within polymer matrices. Yet additional

additives, for example sucrose, stabilize proteins against solid-state aggregation in humid atmospheres at elevated temperatures, as may occur in certain sustained-release formulations of the invention. These additives can be incorporated into polymeric melt processes and compositions within the invention. For example, polypeptide microparticles can be prepared by simply lyophilizing or spray drying a solution containing various stabilizing additives described above. Sustained release of unaggregated peptides and proteins can thereby be obtained over an extended period of time. A wide non-limiting range of suitable methods and anti-aggregation agents are available for incorporation within the compositions of the invention such as disclosed in WO 05/120551, Breslow et al. (*J. Am. Chem. Soc.* 1996; 118: 11678-11681), Breslow et al. (*PNAS USA* 1997; 94: 11156-11158), Breslow et al. (*Tetrahedron Lett.* 1998; 2887-2890), Zutshi et al. (*Curr. Opin. Chem. Biol.* 1998; 2: 62-66), Daugherty et al. (*J. Am. Chem. Soc.* 1999; 121: 4325-4333), Zutshi et al. (*J. Am. Chem. Soc.* 1997; 119: 4841-4845), Ghosh et al. (*Chem. Biol.* 1997; 5: 439-445), Hamuro et al. (*Angew. Chem. Int. Ed. Engl.* 1997; 36: 2680-2683), Alberg et al., *Science* 1993; 262: 248-250), Tauton et al. (*J. Am. Chem. Soc.* 1996; 118: 10412-10422), Park et al. (*J. Am. Chem. Soc.* 1999; 121: 8-13), Prasanna et al. (*Biochemistry* 1998; 37:6883-6893), Tiley et al. (*J. Am. Chem. Soc.* 1997; 119: 7589-7590), Judice et al. (*PNAS USA* 1997; 94: 13426-13430), Fan et al. (*J. Am. Chem. Soc.* 1998; 120: 8893-8894), Gamboni et al. (*Biochemistry* 1998; 37: 12189-12194).

[00239] Where a carrier is included in a solid nasal composition, the particle size of the components including the carriers, of the invention may be from 5 to 500 μ , preferably from 10 to 250 μ , more preferably from 20 to 200 μ . For example, the average particle size may be in the range of 50 to 100 μ .

[00240] The present disclosure provides methods for treating a subject having a coronavirus infection, the method comprising: administering to the subject an effective amount of a therapeutic composition comprising a neutralizing antibody as provided herein by inhalation. The present disclosure also provides methods of preventing infection with a coronavirus such as SARS-CoV or SARS-Cov-2. The method includes administering to a subject at risk of becoming infected with a coronavirus such as SARS-CoV or SARS-Cov-2 an effective amount of a neutralizing antibody as disclosed herein, for example in a pharmaceutical formulation as disclosed herein, to the subject. Administration is by bronchial or pulmonary delivery, such as by inhalation.

[00241] In various embodiments of treatment the composition is administered by pulmonary delivery, for example by oral inhalation. Pulmonary delivery can use any delivery device that can deliver a liquid (e.g., droplets) to the lungs, e.g., can deliver aerosols comprising a therapeutic composition such as a liquid pharmaceutical composition comprising a neutralizing antibody as provided herein to the lungs.

[00242] The subject can be a human subject and can be a patient testing positive for a coronavirus such as hCov-NL63, SARS-CoV, or SARS-CoV-2. In some embodiments the subject is a subject testing positive for SARS-CoV-2 or exhibiting symptoms of infection with SARS-CoV-2. In some embodiments, the neutralizing antibody(ies) can be administered to the subject in combination with at least one anti-viral agent and/or at least one viral entry inhibitor. One skilled in the art can routinely select an appropriate anti-viral agent or viral entry inhibitor to be administered with a neutralizing antibody. In one embodiment, the anti-viral agent and/or the viral entry inhibitor can be administered prior to, during, or after, administration of the neutralizing antibody.

[00243] Administration of a pharmaceutical formulation that include a neutralizing S1-binding antibody by pulmonary delivery via inhalation can use any device that provides respiratable droplets or particles that are able to reach the lungs by inhalation, preferably by oral inhalation. For example, pulmonary delivery can be by means of a delivery device such as but not limited to a nebulizer or a metered dose inhaler.

[00244] The formulations of the invention may include a "therapeutically effective amount" of a neutralizing S1-binding antibody as provided herein. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the neutralizing antibody may vary according to factors such as the viral load, disease state, age, sex, and weight of the individual, and the ability of the neutralizing S1-binding antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the neutralizing S1-binding antibody are outweighed by the therapeutically beneficial effects.

[00245] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined using standard pharmaceutical procedures including in vitro, in cell cultures, and experimental animals. The data obtained from these in vitro and cell culture assays and animal

studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p. 1).

[00246] Dosage amount and interval may be adjusted individually, for example, to provide serum and cell levels of the active ingredient which are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). Dosages necessary to achieve the MEC will depend on individual characteristics including the severity of the viral infection and related pathologies, the condition of the patient, and the judgment of the physician. Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[00247] The delivery device can deliver, in a single dose or in multiple doses, a pharmaceutically effective amount of the composition to the subject's lungs by pulmonary inhalation. Devices suitable for pulmonary delivery of a dry powder form of a protein composition as a nonaqueous suspension are commercially available. Examples of such devices include the Ventolin metered-dose inhaler (Glaxo Inc., Research Triangle Park, N.C.) and the Intal Inhaler (Fisons, Corp., Bedford, Mass.). See also the aerosol delivery devices described in U.S. Pat. Nos. 5,522,378, 5,775,320, 5,934,272 and 5,960,792, herein incorporated by reference. An aerosol propellant used in an aerosol delivery device may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochloro-fluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoromethane, dichlorodifluoro-methane, dichlorotetrafluoroethanol, and 1,1,1,2-tetra-fluoroethane, or combinations thereof.

[00248] Where the solid or dry powder form of the formulation is to be delivered as a dry powder form, a dry powder inhaler or other appropriate delivery device is preferably used. The dry powder form of the formulation is preferably prepared as a dry powder aerosol by dispersion in a flowing air or other physiologically acceptable gas stream. For example, the delivery device can be any of dispenser is of a type selected from the group consisting of a reservoir dry powder inhaler (RDPI), a multi-dose dry powder inhaler (MDPI), and a metered dose inhaler (MDI).

[00249] Liquid aerosol delivery by nebulizer is another form of pulmonary drug delivery that can be employed. Nebulizers as they are generally more effective for delivery to the deep lung and may be preferred for delivering protein therapeutics in active form. Nebulizers create liquid aerosols, which are forced from a small orifice at high velocity by the release of compressed air, resulting in low pressure at the exit region due to the Bernoulli effect. *See, e.g.,* U.S. Pat. No. 5,511,726. The low pressure is used to draw the fluid to be aerosolized out of a second tube. This fluid breaks into small droplets as it accelerates in the air stream. Types of nebulizers for liquid formulation aerosolization include, for example, air jet nebulizers, liquid jet nebulizers, ultrasonic nebulizers, and vibrating mesh nebulizers. Nonlimiting examples of nebulizers include the Akita™ (Activaero GmbH) (see U.S. Pat. No. 7,766,012, EP1258264 and the portable Aeroneb™ Go, Pro, and Lab nebulizers (AeroGen). The nebulizer can use a pharmaceutical composition that includes any pharmaceutically acceptable carrier, including a saline solution. A dry powder formulation can also be delivered by a nebulizer. Nebulizers can be customized for delivery of the particular protein, e.g. a neutralizing anti-S protein antibody, to reduce any denaturation, aggregation, and loss of activity during nebulization.

[00250] Ultrasonic nebulizers use flat or concave piezoelectric disks submerged below a liquid reservoir to resonate the surface of the liquid reservoir, forming a liquid cone which sheds aerosol particles from its surface (U.S. 2006/0249144 and U.S. Pat. No. 5,551,416). Since no airflow is required in the aerosolization process, high aerosol concentrations can be achieved. Smaller and more uniform liquid respirable dry particles can be obtained by passing the liquid to be aerosolized through micron-sized holes. *See, e.g.,* U.S. Pat. Nos. 6,131,570; 5,724,957; and 6,098,620.

[00251] Vibrating mesh nebulizers, which are considered less likely to cause protein denaturation (*See, Bodier-Montagutelli et al. (2018) Exp Op Drug Deliv 15:729-736*), may be used to generate aerosols for delivery of a liquid composition the includes a neutralizing anti-S1 antibody to the lungs of a subject. Vibrating mesh nebulizers force liquid through a vibrating membrane with apertures of specific sizes, resulting in droplets having diameters within a specified range, and may be customized for optimal delivery and stability of specific protein formulations. Nonlimiting examples of vibrating mesh nebulizers include the ALX-0171 Nanobody™ nebulizer, the Vectura FOS-Flamingo®, the PARI eFlow®, the Philips I-neb

AAD®, and the Aeroneb™ Pro (Rohm et al. (2017) *Intl J Pharmaceutics* 532:537-546; Bodier-Montagutelli et al. (2018)).

[00252] In various embodiments, a dosage regimen can include a single dose of a liquid formulation of the invention, of 0.001 to 500 mg neutralizing anti-S protein antibody, or about 10 µg to 200 mg neutralizing anti-S protein antibody, administered daily, every other day, or weekly, or a plurality of doses administered at least twice, 2-3 times, 2-4 times or 2-6 times daily; or a plurality of doses administered once every 36 hours, once every 36-48 hours, once every 36-72 hours, once every 2-3 days, once every 2-4 days, once every 2-5 days, or once every week; or a plurality of doses administered once every 36 hours, once every 36-48 hours, once every 36-72 hours, once every 2-3 days, once every 2-4 days, once every 2-5 days, or once every week.

[00253] In some embodiments, the method further comprises detecting reduced infection or reduced viral load of the coronavirus in a subject diagnosed as having a coronavirus infection after pulmonary delivery of a neutralizing antibody as provided herein to the subject.

[00254] The disclosure provides methods for treating coronavirus infection by delivering to the lungs of a subject having or suspected of having a coronavirus infection a composition that includes a polypeptide comprising a neutralizing anti-S protein antibody formulated for pulmonary administration. The composition can be a pharmaceutical composition that includes, in addition to a neutralizing anti-S protein antibody, at least one pharmaceutically acceptable excipient or carrier compound. The pharmaceutical formulation that includes a neutralizing anti-S1 antibody can be a formulation for delivery by aerosol inhalation such as by a nebulizer and can be in liquid form. Compositions as provided herein can be packaged in single dose units, for example, in vials or dispensers such as nebulizers that generate aerosols for delivery of particles or droplets to the lung.

[00255] Further provided herein are methods of treatment and methods of prophylaxis that include administering to a subject a composition as described that includes at least one nucleic acid construct that encodes a neutralizing antibody that binds a coronavirus. The subject can be a human subject, for example, a human subject that has a coronavirus infection, is suspected of having a coronavirus infection, or is at risk of becoming infected with a coronavirus. The subject can also be a non-human animal. The administering can be by injection, for example, intramuscular injection. Single or multiple doses, including multiple doses over weeks or

months, can be administered. The amount of DNA (e.g., plasmid or plasmids encoding a neutralizing antibody) to be delivered can be determined for example, at least in part by experiments on non-human animals.

Methods of Detecting Coronavirus

[00256] The present disclosure provides methods (e.g., *in vitro*) for detecting the presence of a coronavirus, or a protein from a coronavirus, in a sample, comprising: (a) contacting the sample (containing a target antigen) with any one or any combination of two or more of the neutralizing antibodies having increased *in vivo* serum half-life and/or reduced effector function described herein, under conditions suitable to form an antibody-antigen complex; and (b) detecting the presence of the antibody-antigen complex. In one embodiment, this method can be used to detect the presence of a coronavirus in a sample from a subject and thus can be used to diagnose a subject suspected of having a coronavirus infection. In one embodiment, the sample from the subject comprises phlegm, saliva, blood, cheek scraping, tissue biopsy, hair or semen. In one embodiment, the sample from the subject can be obtained from an acutely coronavirus infected subject or a convalescing subject. In one embodiment, the subject can be human, non-human primates, simian, ape, murine (e.g., mice and rats), bovine, porcine, equine, canine, feline, caprine, lupine, ranine or piscine. In one embodiment, the sample can comprise cells expressing a coronavirus membrane protein, or a coronavirus. In one embodiment, the neutralizing antibodies can be labeled so permit detection of an antigen-antibody complex, where the label comprises a radionuclide, fluorescer, enzyme, enzyme substrate, enzyme cofactors, enzyme inhibitors and ligands (e.g., biotin, haptens). In one embodiment, the presence of the antibody-antigen complex can be detected using any detection mode including radioactive, colorimetric, antigenic, enzymatic, a detectable bead (such as a magnetic or electrodense (e.g., gold) bead), biotin, streptavidin or protein A.

[00257] The present disclosure further provides methods (e.g., *in vitro*) for identifying a compound that modulates (increases or decreases) binding between a coronavirus S protein and an ACE2 target receptor (or cells expressing ACE2 e.g., Vero E6 cells), comprising: (a) contacting (i) a candidate compound with (ii) a coronavirus S protein and with (iii) any one or any combination of two or more of the neutralizing antibodies having increased *in vivo* serum half-life and/or reduced effector function described herein, under conditions suitable to form an

antibody-S1 complex; and (b) detecting the presence or absence of the antibody-S protein complex. In one embodiment, the lack of formation of the complex may indicate that the candidate compound competes for the same or overlapping epitope on the S protein subunit as the neutralizing antibody. In one embodiment, the concentration of the candidate compound and/or the neutralizing antibody can be increased to obtain a dose response curve. In one embodiment, the neutralizing antibodies can be labeled so permit detection of an antigen-antibody complex, where the label comprises a radionuclide, fluorescer, enzyme, enzyme substrate, enzyme cofactors, enzyme inhibitors and ligands (e.g., biotin, haptens). In one embodiment, the presence of the antibody-antigen complex can be detected using any detection mode including radiation, fluorescence, colorimetric, absorption wavelength, electron density antigenic, enzymatic, a detectable bead (such as a magnetic or electrode (e.g., gold bead), biotin, streptavidin or protein A.

SEQUENCES

SEQ ID NO:1

Protein

SARS-Cov-2 spike (S) protein (including leader sequence) with S1 and S2 subunits

Genbank ACCESSION QHD43416

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVVYPDKVFRSSVLHSTQDLFLPFFS
 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLILV
 NNATNVVIKVCEFQFCNDPFLGVYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMDLE
 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPPQGSALPLVDLPIGINITRFQT
 LLALHRSYLTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRKISN
 CVADYSVLYNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIAD
 YNYKLDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN
 FNFENGLTGTGVLTESNKKFLPFQGFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNSY
 ECDIPIGAGICASYQTQTNPRRARSVASQSI IAYTMSLGAENSVAYSNNIAIPTNFTI
 SVTTEILPVSMTKTSVDCTMYICGDSTECNNLLQYGSFCTQLNRALTGIAVEQDKNTQE
 VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLENKVTADAGFIKQYGDC
 LGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM
 QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALN
 TLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA
 SANLAATKMSECVLGQSKRVDFCGKGYHLSMFPQSAPHGVVFLHVTYVPAQEKNFTTAPA
 ICHDGKAHFPREGVFSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVIGIVNNTVYDP
 LQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL
 QELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDD
 SEPVLKGVKLHYT

SEQ ID NO:2

Protein

SARS-Cov-2 spike protein without leader sequence, that includes the S1 subunit and the S2 subunit up to amino acid 1213 (amino acids 16-1213 of SEQ ID NO:1)

VNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWTFHAIHVSGTNGTKRFDNPVLP
 FNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLIIVNNATNVVIKVCEFCNDPFLGVYYHKNNKSWMES
 EFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEP
 LVDLPIGINITRFQTLALHRSYLT PGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDP
 LSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADY
 SVLYNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKL PDDFTGCVIA
 WNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGY
 QPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDA
 VRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQT
 RAGCLIGAEHVNNSECDIPIGAGICASYQTQTNSPRRARSVASQSI IAYTMSLGAENSVAYSNNIAIP
 TNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQV
 KQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKF
 NGLTVLPPLLTDemiaQYTSALLAGTITSGWTFGAGALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN
 QFNSAIGKIQDLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDR
 LITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHV
 TYVPAQEKNFTTAPAICHGKAHFPREGVFSNGTHWFTQRNFYEPQIITDNTFVSGNCDVVIGIVNN
 TVYDLPQPELDSFKEELDKY FKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGK
 YEQYIKWP

SEQ ID NO:3

SARS-Cov-2 spike S1 subunit (including leader sequence)

MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
 NVTWTFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLIIV
 NNATNVVIKVCEFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMDLE
 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL
 LALHRSYLT PGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISN
 CVADYSVLYNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIAD
 YNYKL PDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEIQAGSTPC
 NGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVN
 FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITP
 GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSE
 CDIPIGAGICASYQTQTNSPRRAR

SEQ ID NO:4

SARS-Cov-2 spike S1 subunit (no leader sequence)

Amino acids 16-685 of SEQ ID NO:1

VNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWTFHAIHVSGTNGTKRFDNPVLP
 FNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLIIVNNATNVVIKVCEFCNDPFLGVYYHKNNKSWMES

EFVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEP
 LVDLPIGINITRFQTLALLHRSYLTTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDP
 LSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADY
 SVLYNSASFSTFKCYGVSPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDFTGCVIA
 WNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG
 QPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDA
 VRDPQTLILDITPCSFSGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQT
 RAGCLIGAELHVNNSYECDIPIGAGICASYQTQTNSPRRAR

SEQ ID NO:5

SARS-Cov-2 spike protein RBD

amino acids 319-537 of SEQ ID NO:1

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLN
 DLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDFTGCVIAWNSNNLDSKVGGNYNLYRLFRK
 SNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYPYRVVLSFELLHAPATVCGPK
 KSTNLVKNK

SEQ ID NO:6

mAb S1D2 VH

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIIPGGSTNYADSVKGRFTIS
 RDSRNTLYLQMNSLRAEDTAVYYCARELGYYGMDVWGQGTTVTVSS

SEQ ID NO:7

mAb S1D2 VL

DIQMTQSPSSVSASVGDRTITCRASQGISTWLWVYQQKPGKAPNLLIYGASSLQSGVPSRFRSGSGSGTD
 FTLTISSLQPEDFATYYCQQANSFPYTFGQGTKLEIK

SEQ ID NO:8

mA S1D2 Heavy chain CDR1

SNYMS

SEQ ID NO:9

mA S1D2 Heavy chain CDR2

IIPGGSTNYADSVKG

SEQ ID NO:10

mA S1D2 Heavy chain CDR3

ELGYYGMDV

SEQ ID NO:11

mA S1D2 Light chain CDR1

RASQGISTWLV

SEQ ID NO:12

mA S1D2 Light chain CDR2

GASSLQS

SEQ ID NO:13
mA S1D2 Light chain CDR3
QQANSFPYT

SEQ ID NO:14
Human Fc region with LALA mutations (L234A, L235A)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:15
Human Fc region with triple mutation YTE: M252Y, S254T, T256E

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREP
EVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:16
Human Fc region with LALA and YTE mutations:
(L234A, L235A) (M252Y, S254T, T256E)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREP
EVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:17
mouse Ig gamma leader peptide sequence
MEWSWVFLFFLSVTTGVHS

SEQ ID NO:18
lower hinge/CH2 sequence
PAPELLGGP

SEQ ID NO:19
Fc region sequence (CH2)

SVFLFPPKPKDT

SEQ ID NO:20

hinge region

EPKSCDKTHTCPPCPAPELLGGP

SEQ ID NO:21

linker

GGGGSGGGSGGGGS

SEQ ID NO:22

Protein

Homo sapiens

angiotensin-converting enzyme 2 (ACE2) precursor

NCBI Reference Sequence: NP_068576.1 UniProtKB Q9BYF1

Signal peptide is underlined

805 amino acids

MSSSSWLLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWS
AFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQE
CLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVN
GVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYS
LTVPFQKPNIDVTDAMVDQAWDAQRI FKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWD
LGKGD FRI LMCTKVTMD DFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKS
IGLLSPDFQEDNETEINFLKQALTI VGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEMKREIVGVVEP
VPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRL
GKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISLKSALGD
KAYEWNNDNEMYLFRRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRISFNFFVTAPKNVSDIIPRTEV
EKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPNPQPPVSIWLVFVGMGVIVVGIVILIFTGIRDRAK
KNKARSGENPYASIDISKGNNPGFQNTDDVQTSF

SEQ ID NO:23

Homo sapiens

protein

ACE2 receptor polypeptide soluble ectodomain, no signal peptide (amino acids 18-740)

QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQE
IQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLD
YNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE
HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFQKPNIDVTDAM
VDQAWDAQRI FKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGD FRI LMCTKVTMD
DFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEIN
FLKQALTI VGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEMKREIVGVVEPVP HDETYCDPASLFHVS
NDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLALENVVGAK
NMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRRSSV

AYAMRQYFLKVKNQMILFGEEDVRVANLKPRI SFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRL
NDNSLEFLG

SEQ ID NO:24

Homo sapiens

protein

ACE2 receptor polypeptide soluble ectodomain, no signal peptide (amino acids 18-615)

QSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNAGDKWSAFLKEQSTLAQMYPLQE
IQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLD
YNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVE
HTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFQKPNIDVTDAM
VDQAWDAQRI FKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMD
DFLTAHHEMIGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIIGLLSPDFQEDNETEIN
ELLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWEMKREIVGVVEPVPHDETYCDPASLFHVS
NDYSFIRYYTRTLYQFQFQEAALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTALENVVGA
NMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

SEQ ID NO:25

Protein

human IgG1 Fc region (amino acids 100-330 of Genbank P01857) ("Fc tag")

PKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSTIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:26

DNA

Cytomegalovirus

CMV enhancer plus promoter

CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATA
ATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
AAACTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG
TAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTAC
GTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTG
ACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACG
GGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAG
GTCTATATAAGCAGAGCT

SEQ ID NO:27

Protein

SARS-Cov-2 spike S1 subunit (no leader sequence), polyhistidine tag

VNLTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLP
FNDGVYFASTEKSNIIIRGWI FGTTLDSTQSLLI VNNATNVVIVKVEFQFCNDPFLGVYYHKNNKSWMES
EFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFNKLNRE FVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEP

LVDLPIGINITRFQTLALHRSYLTTPGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDP
 LSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADY
 SVLYNSASFSTFKCYGVSPKTLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLDDFTGCVIA
 WNSNNLDSKVGGNYNYLYRLFRKSNLKPFRDISTEYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGY
 QPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDA
 VRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQT
 RAGCLIGAETHVNNSECDIPIGAGICASYQTQTNSPRRARHHHHHH

SEQ ID NO: 28

Protein

IgG1 upper hinge sequence

EPKSCDKTHT

SEQ ID NO: 29

Protein

IgG1 core hinge sequence

CPXC wherein X is P, R or S

SEQ ID NO: 30

Protein

peptide linker

(GGGGS)_N wherein 'N' is 1-6

EXAMPLES

[00258] The following examples are meant to be illustrative and can be used to further understand embodiments of the present disclosure and should not be construed as limiting the scope of the present teachings in any way.

Example 1. Identification of a Neutralizing Anti-S1 Antibody.

[00259] Fully human IgG1 antibodies were tested by ELISA for their ability to bind the spike (S) protein of SARS-CoV-2, the S1 subunit of SARS-CoV-2, and the RBD of the S protein. Each of the antigens tested included a C-terminal poly histidine tag (his tag). The SARS-CoV-2 spike protein used in the ELISA had the amino acid sequence of SEQ ID NO:2 (which includes all of the extracellular portion of the mature S protein) with a C-terminal polyhistidine tag. The SARS-CoV-2 S1 subunit used in the ELISA included amino acid 16 to amino acid 685 of SEQ ID NO:1 (i.e., SEQ ID NO:4) and a carboxy terminal polyhistidine tag (Acrobiosystems, San Jose, CA, catalog # S1N-C52H3), and the RBD used in the ELISA included amino acids 319-537 of SEQ ID NO:1 (i.e., SEQ ID NO:5) and a carboxy terminal polyhistidine tag (Acrobiosystems, San Jose, CA, catalog # SPD-C52H3).

[00260] These polypeptides were added to the wells of an Ni-NTA-coated 96 well ELISA plate (Qiagen) by adding to each well 50 μ l of 1 μ g /ml protein (polyhistidine-tagged spike protein, S1 subunit, or RBD) in PBS and the plates were incubated for one hour at room temperature. The plate was washed three times with PBS-T (phosphate buffered saline with 0.05% Tween 20) and then antibodies were added to the wells at 0.5 in casein buffer (Blocker Casein: 1% casein in PBS, ThermoFisher catalog # 37528). The antibody incubation was for one hour at room temperature. The plate was then washed three times with PBS-T, followed by the addition of mouse anti-human Fc HRP (Southern Biotech, catalog # 9042-05, diluted 1:5000 in Blocker Casein) and the plates were incubated for 30 minutes at room temperature. After the secondary antibody incubation, the plates were again washed three times with PBS-T.

[00261] To detect the binding signal, 3,3',5,5' tetramethylbenzidine (TMB) was added to each well and the plate was allowed to develop for 3-5 min. at room temperature. The reaction was stopped by adding 30 μ l of 2N H₂SO₄ to each well, and the plate was read at 450 nm on the Softmax Pro program in the Flex Station.

[00262] As shown in **Figure 2**, all thirteen fully human IgG antibodies bound the SARS-CoV-2 RBD except for antibody S3G11 which selectively bound the spike protein and S1 subunit but did not bind the RBD. The bar graph provides the degree of binding, as assessed by the OD450 reading, to each of (bars of the graph proceeding from left to right for each antibody) the S1 protein, the RBD of the S1 protein, the trimeric form of the S protein, and a negative control. An anti-RSV (respiratory syncytial virus) antibody was used as a negative control and demonstrated no binding to any of the SARS-CoV-2 proteins. Antibody S1D2 and S3G4 each showed substantially equivalent levels of binding of the spike protein, S1 subunit, and RBD in the ELISA format. S7C7, S1D8, S6H10, S2F5 and S7G5 appeared to have relatively higher binding to the RBD than to the spike and S1 proteins.

Example 2. Identification of Anti-S1 Antibodies that block binding to ACE2.

[00263] Antibodies that were screened for binding to the RBD and S1 subunit of SARS-CoV-2 were tested for their ability to block binding of the S1 subunit to the ACE2 ectodomain. The ACE2 ectodomain polypeptide used in the assay was a polypeptide that included the amino acid sequence of SEQ ID NO:23 fused to an Fc region sequence (SEQ ID NO:25).

[00264] A 96-well ELISA plate (CORNING 3690) was coated with 3 μ g/mL ACE2-Fc overnight at 4°C. The plate was then washed with PBS-T three times. Antibodies were serially

diluted two-fold starting at a concentration of 100 µg/mL. For each dilution, the antibody was mixed 1:1 with 1.25 µg/mL SARS-CoV-2 S1 subunit protein (SEQ ID NO:4) with a carboxy terminal his tag (Acrobiosystems catalog # S1N-C52H3). The antibody-S1 subunit protein mixtures (25 µl) were transferred to the wells of the ELISA plate and incubated 30 min with shaking. The plate was then washed three times with PBS-T. Rabbit anti-His polyclonal antibody-HRP diluted in Blocker Casein in PBS (1:5000) was added to each well and then the TMB substrate was added. The reaction was allowed to develop for 30 min. 2M H₂SO₄ was used to stop the reaction and the OD was read at 450 nm.

[00265] **Figure 3** provides the results of the blocking assay. Antibody S1D2 was able to block the interaction between the SARS-CoV-2 S1 subunit and ACE2 with a half-maximal inhibitory concentration (IC₅₀) of 1.87 nM.

Example 3. S1D2 Antibody Binding Affinity.

[00266] Binding kinetics of anti-spike protein antibodies with the RBD of the SARS-CoV-2 S protein was measured using surface plasmon resonance (SPR). S1D2 IgG antibody was immobilized on a BIAcore CM5 sensor chip to approximately 500 RU using standard N-hydroxysuccinimide/N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (NHS/EDC) coupling methodology. Recombinant SARS-CoV2 RBD (SEQ ID NO:5) having a carboxy terminal his tag was serially diluted in a running buffer of 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20 (HBS-EP+). All measurements were conducted in HBS-EP+ buffer with a flow rate of 30 µL/minute. The affinity of S1D2 was analyzed with BIAcore T200 Evaluation software 3.1. A 1:1 (Langmuir) binding model was used to fit the data. All BIAcore assays were performed at room temperature.

[00267] The SPR sensorgram of anti-S1 antibody S1D2 binding to the SARS-CoV2 spike protein RBD is shown in **Figure 4**. As shown in the table in **Figure 4**, the S1D2 antibody was found to have a binding affinity (K_D) of 46 nM for the SARS-CoV2 S protein RBD.

Example 4. Epitope Characterization of S1D2 Antibody.

[00268] Three aliquots of 18 µl of 0.1 mg/ml SARS-CoV-2 S1 protein (SEQ ID NO:4) were distributed into separate tubes. PBS (0.6 µl) was added to the Sample 1 (Native S1) tube and the tube was kept on ice. PBS (0.6 µl) was also added to the Sample 2 (Heated) tube, and the tube was heated for 5 min at 90°C. A reducing agent (0.6 µl NUPAGE™ 10X Sample Reducing Agent (ThermoFisher) that included 500 mM dithiothreitol was added to the Sample 3 (Heated +

Reduced) tube and the tube was heated for 5 min at 90°C. Following these treatments, 1.5 µL of each sample was slowly pipetted onto a nitrocellulose membrane. The membrane was allowed to dry for 30 minutes at room temperature and then blocked using Blocker Casein (1% casein in PBS, ThermoFisher Blocker Casein catalog # 37528) in PBS for 1 hour at room temperature. The Blocker Casein solution was removed and 1 µg/ml antibody in Blocker Casein was added to the membrane. The membrane was then incubated at room temperature on a shaker for 1 hour. The membrane was washed three times with PBS-T after which Goat anti-Human Fc HRP (Kirkegaard Perry Laboratories, catalog # 04-10-20) diluted 1:2500 in Blocker Casein was added and the membrane was incubated at room temperature on the shaker for one hour. Following the secondary antibody incubation, the membrane was washed three times with PBS-T and then enhanced chemiluminescence (ECL) solution A and B (Bio-Rad, catalog # 1705060) mixed at a 1:1 ratio was pipetted onto the blot. After development of the signal, the blot was demonstrated that the S1D2 antibody recognizes a conformational epitope with no detectable binding to the completely denatured S1 subunit (**Figure 5**).

Example 5. Cross-reactivity of S1D2 antibody with S1 and RBD Mutants.

[00269] D614G is a mutant of the S1 subunit that has been recently disclosed in the literature (e.g., Korber et al. (2020) *Cell* S0092-8674(20)30820-5; doi:10.1016/j.cell.2020.06.043). Additional S1 mutants V367F; W436R; and N354D, D364Y are all localized to the RBD. ELISAs were performed to determine whether the novel S1D2 antibody is able to bind S1 or RBD proteins having these mutations. All mutant proteins had his tags at the carboxy terminus and were purchased from Acrobiosystems (San Jose, CA). The S1 subunit protein having the D614G mutation (Acrobiosystems catalog # S1N-C5256) extended from amino acid 16 to amino acid 685 of the S protein (SEQ ID NO:4). The RBD V367F mutant protein (Acrobiosystems catalog # SPD-S52H4), RBD W436R mutant protein (Acrobiosystems catalog # SPD-S52H7), and N354D, D364Y mutant protein (Acrobiosystems catalog # SPD-S52H3) each had an amino acid sequence that extended from amino acid 319 to 541 of the S protein, i.e., comprised the RBD of the S protein, followed by a his tag, with the respective mutations incorporated into the RBD amino acid sequence. The his-tagged non-mutant S1 subunit protein (Acrobiosystems catalog # S1N-C52H6) included amino acid 16 to amino acid 685 of SEQ ID NO:1 (i.e., SEQ ID NO:4) and the his-tagged non-mutant RBD (Acrobiosystems catalog # SDP-C52H3) included amino acid 319 to amino acid 537 of SEQ ID NO:1 protein (i.e., SEQ ID NO:5). The proteins

were bound to an Ni-NTA ELISA plate by adding to each well 50 μ l of 1 μ g/ml SARS-CoV-2 RBD mutant protein or S1 D614G mutant protein in PBS and incubating the plate for one hour at room temperature. The plate was then washed three times with PBS-T. Antibody (“COVI-GUARD™”) diluted to 0.5 μ g/ml in casein buffer was added to each well (50 μ L/well) and the plate was incubated at room temperature for one hour. The plate was then washed three times with PBS-T, after which mouse anti-human Fc HRP (1:5000) diluted in Blocker Casein was added. TMB was added as the substrate and the plate was allowed to develop for 30 min. 2M H_2SO_4 was used to stop the reaction and the OD was read at 450 nm. **Figure 6** provides the results of the ELISA and shows that the S1D2 antibody bound to all of the tested mutant forms of RBD and to the mutant S1 protein.

[00270] Binding of the “COVI-GUARD™” S1D2 antibody that included the Fc region with the L234A, L235A (LALA) mutation to the D614G mutant of the S1 protein as well as to mutant forms of the RBD were also analyzed by SPR. Kinetic interactions between the antibodies and his-tagged S1 proteins were measured at 25°C using Biacore T200 surface plasmon resonance (GE Healthcare). Anti-human Fc antibody was covalently immobilized on a CM5 sensor chip to approximately 8000 resonance units (RU) using standard N hydroxysuccinimide/N Ethyl-N’-(3-dimethylaminopropyl) carbodiimide hydrochloride (NHS/EDC) coupling methodology. Antibody (1-2 μ g/ml) was captured for 60 seconds at a flow rate of 10 μ l/minute. The his-tagged antigen proteins were run at six different dilutions (range 3.125 nM–200 nM) in a running buffer of 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% v/v Surfactant P20 (HBS EP+). The 25 nM antigen runs were measured twice. Blank flow cells were used for correction of the binding response. All measurements were conducted in HBS-EP+ buffer with a flow rate of 30 μ l/minute, and a 1:1 binding model and was used to fit the data.

[00271] **Figure 7** shows sensorgrams of S1D2 binding to the S1 protein and the S1 protein having the D614G mutation. A table below the sensorgrams provides binding kinetics parameters. The K_D for binding of the S1D2 antibody (having the LALA mutation) to the S1 protein was determined to be 2.58×10^{-8} M, and the K_D for binding of the S1D2 antibody (having the LALA mutation) to the S1 protein with the LALA mutation was determined to be 1.82×10^{-8} M. **Figure 8** shows sensorgrams of S1D2 (+LALA) binding to (proceeding left to right) the RBD with no mutations; the RBD with the 357D and D364Y mutations; the RBD with the V367F mutation, and the RBD with the W436R mutation. Below the sensorgrams is a table

providing the kinetic binding parameters for each. The K_d for S1D2 binding to the RBD (no mutations) was found to be 2.25×10^{-8} M, the K_d for S1D2 binding to the RBD with the 357D and D364Y mutations) was found to be 1.54×10^{-9} M, the K_D for S1D2 binding to the RBD with the V367F mutation was found to be 1.51×10^{-9} M, and the K_D for S1D2 binding to the RBD with the W436R mutation was found to be 1.56×10^{-9} M.

Example 6. Binding of S1D2 to SARS-Cov-2 Spike protein Expressed on Cell Surface.

[00272] For transfection of HEK293T cells with a gene encoding the SARS-CoV-2 spike protein, 4×10^6 HEK293T cells were seeded in a T25 flask and 7-8 hours later transfected with an expression vector encoding the full-length spike protein of SARS-Cov2 (SEQ ID NO:1). The gene encoding the spike protein of SARS-Cov2 was synthesized by IDTech (San Diego, CA) and cloned into an expression vector having an hCMV promoter (SEQ ID NO:26). A transfection complex was formed by mixing 3 μ g of FuGeneHD transfection reagent (Promega, Cat # E2311) per μ g DNA in DMEM media according to the manufacturer's protocol. Approximately 48 hours after transfection, the HEK293T cells were detached from the flask using non-enzymatic Cell Dissociation Buffer (Thermo Fisher, catalog No. 13151014), and resuspended in FACS buffer (DPBS+2% FBS) at cell density of 4×10^6 per ml.

[00273] To determine whether the S1D2 antibody could specifically bind the spike protein expressed on HEK293T cells surface by FACS, 25 μ L of the cells (10^5 cells) were aliquoted into the wells of a 96 well plate. First, a solution of anti-S1 antibody S1D2 having LALA mutation in the Fc region, SEQ ID NO:14) was added to the cells at two concentrations of 1 μ g/mL or 10 μ g/mL and the cells were maintained for 45 minutes on ice. The cells were then washed once with FACS buffer and stained with Goat Anti-Human IgG (H+L) F(ab')₂ fragment conjugated with allophycocyanin (APC) (Jackson ImmunoResearch, catalog No.109-136-4098) on ice for 20 minutes. The cells were washed once with FACS buffer and resuspended in 50 μ L FACS buffer for analysis in a flow cytometer. The FACS results are shown in **Figure 9**. The left column is a control where no antibody was added to the wells. The right column labeled 'COVI-GUARD' is the S1D2 antibody having the LALA mutation in the Fc region. The results show that the S1D2 antibody (with the LALA mutation) efficiently bound the SARS-CoV-2 S protein expressed on the surface of the cells when binding was performed with the concentration of antibody at either 1 μ g/mL or 10 μ g/mL. No labeled cells were observed when the S1D2 antibody was used to label HEK293T cells that had not been transfected to express the SARS-CoV-2 spike protein.

[00274] In a further experiment, the S1D2 antibody having the LALA mutation in the Fc region was serially diluted and antibody at serially diluted concentrations was mixed with HEK293 cells spike S1 protein having the D614G mutation. After binding, the cells were incubated with a secondary antibody conjugated to a fluorophore and analyzed by flow cytometry. **Figure 10** shows binding curves for S1D2 binding to the S1 protein and S1(D614G) based on the flow cytometry results. The table below the graph provides the EC₅₀ for binding of S1D2 to the wild type spike protein (0.147 µg/ml) and the EC₅₀ for binding of S1D2 to the wild type spike protein (0.130 µg/ml).

Example 7. ACE2 – S1 Interaction Inhibition ELISA.

[00275] The wells of a 96 well plate were coated with 1 µg/mL recombinant ACE2-Fc (the ACE2 polypeptide comprising the amino acid sequence of SEQ ID NO:23 fused to an Fc region (SEQ ID NO:25)) overnight at 4 °C. The plates were washed three times with PBS-Tween PBS-T and blocked for 1 hour with 170 µL Blocker Casein in PBS at room temperature. The plates were then washed three times with PBS-T. Two-fold serial dilutions of S1D2_{LALA} antibody (S1D2 with the LALA mutations in the Fc region (SEQ ID NO:14)) starting from a concentration of 60 µg/mL were mixed 1:1 with 3 µg/mL recombinant SARS-CoV-2 S1 protein (SEQ ID NO:4) that included a his tag. Twenty-five µL of the S1D2 antibody dilutions /S1 protein mixtures were added to the ELISA plate and incubated for 1 hour with shaking. The plate was washed three times with PBS-T, then rabbit anti-His polyclonal antibody-HRP (diluted at 1:5000 in casein) was added and the plate was incubated for 1 hour. Subsequently, TMB (3,3',5,5'-tetramethylbenzidine) was added as substrate and developed for 30 minutes. The reaction was stopped using 2 M H₂SO₄ and the OD was read at 450 nm. GraphPad Prism 8.3.0 software was used to analyze the data. The ELISA results are shown in **Figure 11**. The IC₅₀ of the S1D2 antibody was determined to be 4.883 nM in this assay.

Example 8. SARS-CoV-2 Infection Neutralization Assay with Live Virus

[00276] An in vitro assay was performed to assess the ability of the S1D2 antibody to block infection of susceptible cells. The day before infection, 2 x 10⁴ VeroE6 cells were plated in the wells of 96-well plates and incubated at 37 °C, 5% CO₂. The S1D2_{LALA} antibody was 2-fold serially diluted in infection media (DMEM+2% FBS), starting from a concentration of 200 µg/mL, for a total of 8 dilutions. Twenty-five µL of the serially diluted samples were incubated with 100 x 50% tissue culture infective doses (TCID₅₀) of SARS-CoV-2 in 25 µL for 1 hour at

37 °C. The antibody/virus mixtures were then used to infect monolayers of Vero E6 cells grown in the wells of 96-well plates, where each concentration sample was applied in quadruplicate wells. Virus supernatant was removed and replaced with fresh medium after 1 hour of culture at 37 °C. Cytopathic effect (CPE), i.e., the appearance of plaques or discontinuity in the cell monolayer due to cell lysis, was observed daily in each well and recorded on day 3 post-infection. At the end of the study, the media was aspirated, and the cells were then fixed with formalin and stained with 0.25% crystal violet. The concentration of antibody that completely prevented CPE in 50% of the wells (IC₅₀) were calculated following the Reed & Muench method. The results are shown in **Figure 12**, where the IC₅₀ of the S1D2 antibody in the neutralization assay was found to be 3.13 µg/mL, corresponding to 20.8 nM.

[00277] In a further experiment, CPE inhibition by the S1D2_{LALA} antibody of the non-mutated “WA” SARS-CoV-2 strain was compared to CPE inhibition of the D614G mutated “2020001” SARS-CoV-2 strain. **Figure 13** shows the results of this assay, where in vitro cell culture assays demonstrated that the IC₅₀ for S1D2_{LALA} for CPE inhibition of the WA strain was found to be 3.02 µg/ml and the IC₅₀ for S1D2_{LALA} for CPE inhibition of the 2020001 strain was found to be 4.43 µg/ml.

Example 9. Assays for ADCC and ADE Function of COVI-GUARD™.

[00278] Assays were performed to determine whether the COVI-GUARD™ antibody (S1D2_{LALA}) causes antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody-dependent enhancement (ADE) of infection that are mediated by the Fc region of antibodies.

[00279] To test for ADCC, cells that were transfected to express the spike protein on the cell membrane were used in targets in cytotoxicity assays with isolated primary human natural killer to determine whether the S1D2 antibody results in higher levels of killing of S protein expressing cells (simulating SARS-CoV-2-infected cells) by NK cells.

[00280] Two types of S1D2 antibody were tested: COVI-GUARD™ (S1D2_{LALA}, i.e., the S1D2 antibody having the LALA mutations in the Fc region) and the S1D2 antibody without the Fc mutation. Target cells expressing the SARS-CoV-2 spike protein (SEQ ID NO:1) were plated in the wells of 96 well plates (10⁴ cells per well), and antibody (COVI-GUARD™, S1D2 without the LALA mutation, Erbitux control) or no antibody was added to each well, with wells having the same conditions produced in quadruplicate. The cells were incubated with antibody for 10 min and then Natural Killer cells were added at an effector:target ratio of 20:1. The results

are shown in **Figure 14**, where it can be seen that there was no enhancement of cytotoxicity of NK cells toward spike protein-expressing cells conferred by either the S1D2 antibody lacking an Fc region mutation or the COVI-GUARD™ antibody having the LALA mutation in the Fc region.

[00281] To determine whether COVI-GUARD™ resulted in enhanced infection of cells, an ADE assay can be used as illustrated in **Figure 15**. In this assay, primary human macrophages were distributed in plate wells, and aliquots of either the SARS-CoV-2 virus mixed with varying concentrations of either the COVI-GUARD™ antibody or the S1D2 antibody without the LALA mutation in the Fc region are added to the wells. Culture medium is removed from the cells after a one hour infection period, and then the macrophages are incubated for approximately two days. At the end of the virus-macrophage incubation period, the culture supernatant is removed from the cells and used to titer the virus by assessing plaque formation on Vero cells.

Example 9. Pharmacokinetics and Biodistribution of COVI-GUARD™ in Mice.

[00282] COVI-GUARD™ was administered to mice intravenously as a bolus injection at dosages of 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, and 2.5 mg/kg and the concentration of the antibody in serum was monitored over the next 20 days as shown in **Figure 16**. The COVI-GUARD™ antibody was very stable in vivo, with a mean C_{max} of 204 +/- 16 µg per ml, and a mean half-life of 239 +/- 9 hours.

[00283] To examine biodistribution, mice were either injected intravenously or intranasally with COVI-GUARD™. Mice were intravenously injected with COVI-GUARD™ at dosages of 0.005 mg/kg, 0.05 mg/kg, and 0.5 mg/kg and sacrificed 24 hours later. Tissues were harvested and tested for virus. **Figure 17** shows that COVI-GUARD™ was detected in all tissue examined (serum, small intestine, large intestine, lungs, and lavage) of mice injected with the 0.5 mg/kg dose. Additional groups of mice were injected intranasally with 0.005 mg/kg, 0.05 mg/kg, 0.5 mg/kg, and 2.5 mg/kg of COVI-GUARD™ and these mice were also sacrificed at 24 hours. Virus was detected in serum, lungs, and lavage of mice intranasally injected with either 0.5 mg/kg, and 2.5 mg/kg of COVI-GUARD™ (**Figure 17**).

Example 10. COVI-GUARD™ Treatment of SARS-CoV-2 Infection in Hamsters.

[00284] The effectiveness of COVI-GUARD™ was tested in hamsters as a preclinical model of Covid-19 disease. Six week old male and female hamsters were intranasally inoculated with 1×10^5 TCID₅₀ of the “wild type” SARS-CoV-2 virus (USA/WA-1/2020) in 100 µl PBS. The

inoculated animals were treated one-hour post-infection with titrated doses of COVI-GUARD™ or an isotype antibody control (IsoCtl, 2000 µg) administered intravenously in up to 350 µl PBS (**Figure 18A**). Animals in COVI-GUARD™ treatment groups were administered a single dose of either 50, 500, or 2000 µg, and uninfected animals (UI) were administered 2000 µg of the isotype control IgG1 (IsoCtl) to test for any effects of antibody on growth.

[00285] The animals were monitored daily for illness and mortality for 10 days post-inoculation, and body temperatures and body weights were recorded at least once every 48 hours. Weight change as a percentage of starting weight was recorded and graphed for each animal (**Figure 18B**). Animals in the uninfected/antibody isotype control group displayed no clinical symptoms and gained weight throughout the course of the experiment. Hamsters infected with SARS-CoV-2 WA-1 isolate and administered 2000 µg of IsoCtl experienced steady weight loss over the first five days of infection, with the maximal average percent weight loss in this group reaching $5.7\% \pm 3.3$ on day 5. The duration and degree of weight loss in the 50 µg and 500 µg STI-1499 treatment groups were similar to that of animals administered IsoCtl mAb. Following administration of 2000 µg STI-1499, the animals experienced transient minor weight loss over the first two days post-infection (p.i.) followed by steady weight gain on subsequent days. By day 4 post-infection, and continuing on until the end of the experiment on day 10, the effects on SARS-CoV-2 infection on the average percentage weight change of animals treated with 2000 mg STI-1499 was significantly different than that in animals treated with control IgG (**Figure 18C**). Animals treated with 2000 mg STI-1499 began to gain weight by day 3 post-inoculation (p.i.) and had reached an average weight gain of $5.8\% \pm 4.1$ by day 10 while animals treated with IsoCtl mAb continued to lose weight each day p.i. until day 5 and, on average, only returned to day 0 weight levels by day 10 p.i..

[00286] At five days post-infection, lungs from 5 out of the 10 members of each experimental group animals (animals were designated for analysis of lung tissue prior to initiation of the experiment) were harvested and infectious virus was quantified using a CPE assay (**Figure 18D**). Treatment with 2000 mg STI-1499 resulted in an average lung titer of 1.38×10^4 TCID₅₀/g of tissue, an approximately 27-fold reduction compared to average lung titers in IsoCtl-treated animals. Notably, lung titers for three of the five animals analyzed from the 2000 mg STI-1499 group were below the limit of detection for the CPE assay. The remaining two animals displayed lung titers approximately 10-fold below that of the IsoCtl-treated group. Consistent with a partial

protective effect following a dose of 500 mg of STI-1499, the average TCID₅₀/g of lung tissue among animals in this treatment group were reduced 2.5-fold as compared to the IsoCtl treatment group.

[00287] In summary, STI-1499 demonstrated promising protective efficacy in the hamster model at a dose of 2000ug.