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(54) Title: COMPOSITIONS AND METHODS FOR THE DIAGNOSIS AND TREATMENT OF SARS-COV-2 VIRUS INFECTION

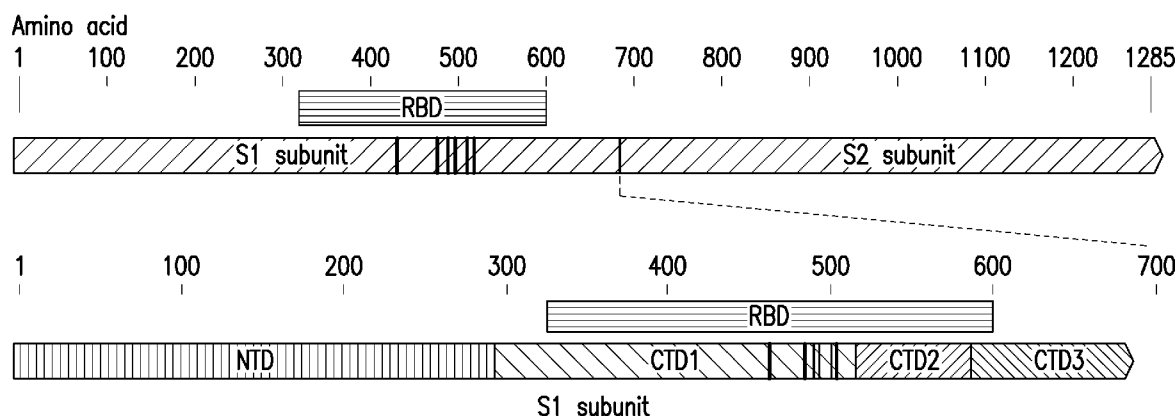


FIG.1A

(57) Abstract: Compositions that selectively bind to coronaviruses, including but not limited to SARS-COV-2 and methods of use thereof are provided. The disclosed antibodies and antigen-binding fragments were developed against SARS-CoV-2. The antibodies bind to the spike protein of coronaviruses. The disclosed antibodies are useful for diagnosing, detecting, preventing, and treating coronavirus infections.

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COMPOSITIONS AND METHODS FOR THE DIAGNOSIS AND TREATMENT OF SARS-CoV-2 VIRUS INFECTION

FIELD OF THE INVENTION

The invention is generally directed to compositions and methods of use thereof for the diagnosis, detection, and treatment of SARS-CoV-2 infections.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Award No. 75D30119P05589 from the Centers for Disease Control and Prevention. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The novel coronavirus, SARS-CoV-2, has caused a global pandemic of COVID-19 respiratory disease, with millions infected, hundreds of thousands of deaths, and many survivors dealing with multiple long-term adverse health effects.

Coronaviruses, including SARS-CoV-2 and two other zoonotic infections to have recently appeared called severe acute respiratory syndrome-1 (SARS-1) and Middle East Respiratory Syndrome (MERS). These viruses are spherical, enveloped, RNA viruses displaying prominent glycosylated spikes known as the ectodomain and are composed of two subunits (S1 and S2). The S1 subunit contains the key receptor binding domain (RBD), responsible for binding to the human angiotensin converting enzyme 2 (ACE2) receptor that mediates viral entry. The ectodomain is a trimeric assembly in which part of the S1 subunit can adopt two different conformations, “up” and “down,” thought to be important in ACE2 recognition.

Because of its key role in viral infectivity, the RBD is a logical target for antibody development. Antibodies to the SARS-CoV-1 RBD were found to strongly inhibit viral infection (thereby designated as neutralizing), and some antibodies have been reported against SARS-CoV-2 with the same or similar properties. However, it has also been observed that other antibodies enhance ACE2 binding and thus speed viral uptake by cells, presumably by binding in such a way as to increase binding affinity with the receptor either directly or allosterically. The critical nature of this interaction has been further highlighted by the dominance of the D614G mutation, which strongly stabilizes the RBD-ACE2 interaction.

Furthermore, epidemiological control of virus spread among the human population requires fast and accurate testing. Current genome-detecting assays based on reverse

transcription polymerase chain reaction (RT-PCR) are relatively expensive and can only be made fast using expensive and technically demanding equipment. Direct detection and quantification of viral particles in patient and environmental samples with sensitivity to rival that of RT-PCR would represent a breakthrough in the ability to identify and help those infected. Such detection requires, first and foremost, a molecule capable of binding selectively and tightly to the virus.

SUMMARY

Compositions that selectively bind to coronaviruses, including but not limited to SARS-CoV-2 and methods of use thereof are provided. One embodiment provides a panel of 37 antibodies and antigen-binding fragments thereof that were developed against SARS-CoV-2, as derived from these sequences [including F(ab), F(ab')₂, single-chain variable (scFv) fragments, and others]. The disclosed compositions are useful in diagnostic and therapeutic applications.

One embodiment is based in part on a variety of antibodies to SARS-CoV-2 spike protein (S protein) and their use in the detection, diagnosis, and treatment during active COVID-19 infection. Another embodiment provides monoclonal antibodies (mAbs) from BALB/c mice immunized with mammalian recombinant S1, mFc-RBD, and mFc-RBD displayed on virus-like particles. Characterization of the antibodies using multiple methods (ELISA, bio-layer interferometry (BLI), split-GFP assay for receptor binding, and immunofluorescence) with active virus and tissue from infected patients revealed some of the mAbs of this disclosure to be SARS-CoV-2-specific and others to be strongly reactive with both SARS-CoV-1 and SARS-CoV-2. Immunoassays were developed using optimal mAb pairings with high sensitivity for spike protein, viral culture supernatant, and infected tissue samples. Thus, the mAbs of this disclosure are capable of recognizing spike protein in a variety of environments with potential uses in immunoassay development and as immunodiagnostic reagents for clinical sample and tissue confirmation of SARS-CoV-2.

Still another embodiment provides an antibody or antigen-binding fragments thereof that bind, preferably specifically, to a SARS-CoV-2 spike protein. Optionally, the antibody is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, single-chain antibody or antibody that competitively inhibits the binding of an anti-SARS-CoV protein antibody or antigen-binding fragment thereof to its respective antigenic epitope. In one embodiment, the disclosed antibodies can optionally be produced in Chinese hamster ovary (CHO) cells or in bacterial cells and preferably inhibit, reduce or prevent the binding of coronaviruses, including but not limited to SARS-CoV-2 spike protein to cell surface proteins

in a subject. In some embodiments, the disclosed antibodies and antigen-binding fragments thereof reduce or inhibit the growth or proliferation of or induce the death of a cell infected with a coronavirus to which they bind.

In another embodiment, the disclosed antibodies and antigen-binding fragments thereof are detectably labeled, attached to a solid support, or the like, such as a lateral flow assay device which provides for point-of-care detection of SARS-CoV-2 and/or diagnosis.

Yet another embodiment provides vectors comprising nucleic acids, including but not limited to DNA, encoding any of the herein described antibodies and antigen-binding fragments thereof. Host cells comprising any such vector are also provided. By way of example, the host cells may be CHO cells, bacterial cells (such as *E. coli*) used for recombinant protein expression, or yeast cells. A process for producing any of the herein described antibodies is further provided and comprises culturing host cells under conditions suitable for expression of the desired antibody and recovering the desired antibody from the cell culture.

One embodiment provides a composition, for example a pharmaceutical composition, including an anti-SARS-CoV-2 antibody or antigen-binding fragment thereof, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier. Another embodiment provides an article of manufacture including a container and a composition contained within the container, wherein the composition of matter comprises an anti-SARS-CoV-2 antibody or antigen-binding fragment thereof as described herein. The article may optionally include a label affixed to the container, or a package insert included with the container, that refers to the use of the composition for the therapeutic treatment or diagnostic detection of a SARS-CoV-2 infection.

Still another embodiment provides the use of an anti-SARS-CoV-2 polypeptide antibody or antigen-fragment thereof as described herein, for the preparation of a medicament useful in the treatment of a condition which is responsive to the anti-SARS-CoV-2 protein antibody.

Yet another embodiment provides any isolated antibody having one or more of the complementarity determining regions (CDRs), including a CDR-kappa1, CDR-kappa2, CDR-kappa3, CDR-H1, CDR-H2, or CDR-H3 sequence disclosed herein.

Another embodiment provides a method of therapeutically treating a mammal having a SARS-CoV-2 infection by administering to the mammal a therapeutically effective amount of an antibody that binds to the SARS-CoV-2 spike protein, thereby resulting in the effective therapeutic treatment of the infection in the mammal. In these therapeutic methods, the antibody may be a monoclonal antibody, antibody fragment, chimeric antibody, humanized

antibody, or single-chain antibody. Antibodies employed in these methods may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin.

Another embodiment provides a method of determining the presence of a SARS-CoV-2 spike protein in a sample suspected of containing the SARS-CoV-2 spike protein, by exposing the sample to an antibody that binds to the SARS-CoV-2 spike protein and determining binding of the antibody to the SARS-CoV-2 spike protein in the sample, wherein the presence of such binding is indicative of the presence of the SARS-CoV-2 spike protein in the sample. Optionally, the sample may contain cells (which may be fibroblasts, epithelial cells, mucosal cells, and the like) suspected of expressing the SARS-CoV-2 spike protein. The antibody employed in these methods may optionally be detectably labeled, attached to a solid support, or the like.

This disclosure is further directed to mAbs and related binding proteins that bind specifically to the spike protein of the SARS-CoV-2 virus and to the use of those mAbs and related binding proteins in epitope blocking ELISAs. Thus, one embodiment provides methods for detecting SARS-CoV-2 virus spike protein in a biological sample by contacting the sample with an antigen which contains an epitope of a spike protein and determining whether an antibody in the sample binds to the epitope. Preferably the binding determination is made in an epitope blocking ELISA. These methods thereby provide highly sensitive and specific epitope blocking ELISAs (EB ELISA) for detecting SARS-CoV-2 spike subtypes.

A further embodiment provides a method of diagnosing the presence of a SARS-CoV-2 infection in a mammal, by detecting the level of expression of a gene encoding a SARS-CoV-2 spike protein in a test sample of tissue cells obtained from the mammal, wherein detection of expression of the SARS-CoV-2 spike protein in the test sample is indicative of the presence of SARS-CoV-2 infection in the mammal from which the test sample was obtained.

Another embodiment provides a method of diagnosing the presence of a SARS-CoV-2 infection in a mammal, by contacting a test sample comprising tissue cells obtained from the mammal with an antibody that binds to a SARS-CoV-2 spike protein and detecting the formation of a complex between the antibody and the SARS-CoV-2 spike protein in the test sample, wherein the formation of a complex is indicative of the presence of a SARS-CoV-2 infection in the mammal. Optionally, the antibody employed is detectably labeled, attached to a solid support, or the like. In these methods, the test sample of tissue cells may be obtained from an individual suspected of having a viral infection.

Another embodiment provides a method of binding an antibody to a cell that expresses a SARS-CoV-2 spike protein, by contacting a cell that expresses a SARS-CoV-2 spike protein

with the antibody of this disclosure under conditions which are suitable for binding of the antibody to the SARS-CoV-2 spike protein and allowing binding therebetween. In preferred embodiments, the antibody is labeled with a molecule or compound that is useful for qualitatively and/or quantitatively determining the location and/or amount of binding of the antibody to the cell.

Other embodiments of this disclosure include the use of a SARS-CoV-2 spike protein, a nucleic acid encoding a SARS-CoV-2 spike protein, or a vector or host cell comprising that nucleic acid, or an anti-SARS-CoV-2 spike protein antibody in the preparation of a medicament useful for (i) the therapeutic treatment or diagnostic detection of a SARS-CoV-2 infection, or (ii) the therapeutic treatment or prevention of a SARS-CoV-2 infection-related disorder.

Another embodiment provides a method for inhibiting the production of additional viral particles in a SARS-CoV-2-infected cell, wherein the growth of the SARS-CoV-2 infected cell is at least in part dependent upon the expression of a SARS-CoV-2 spike protein (wherein the SARS-CoV-2 spike protein may be expressed either within the infected cell itself or a cell that produces polypeptide(s) that have a growth potentiating effect on the infected cells), by contacting the SARS-CoV-2 spike protein with an antibody that binds to the SARS-CoV-2 spike protein, thereby antagonizing the growth-potentiating activity of the SARS-CoV-2 spike protein and, in turn, inhibiting the growth of the infected cell. Preferably the growth of the infected cell is completely inhibited. More preferably, binding of the antibody to the SARS-CoV-2 spike protein induces the death of the infected cell. Optionally, the antibody is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, or single-chain antibody. Antibodies employed in these methods may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin, or the like. The antibodies employed in the methods of this disclosure may optionally be produced in CHO cells or bacterial cells.

Yet another embodiment provides a method of therapeutically treating a viral infection in a mammal, wherein the infection is at least in part dependent upon the expression of a SARS-CoV-2 spike protein, by administering to the mammal a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds to the SARS-CoV-2 spike protein, thereby antagonizing the activity of the SARS-CoV-2 spike protein and resulting in the effective therapeutic treatment of the infection in the mammal. Optionally, the antibody is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, or single-chain antibody. Antibodies employed in these methods may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin, or the like. The antibodies employed in the methods of this disclosure may optionally be produced in CHO cells or bacterial cells.

One embodiment provides a bispecific antibody that binds to SARS-CoV-2 spike protein and a cell-surface protein of an immune cell. The immune cell can be a T cell, for example a cytotoxic T cell, a macrophage, a dendritic cell, or a neutrophil.

Further embodiments of the present invention will be evident to the skilled artisan upon a reading of the present specification.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a diagram showing recombinant spike subunit 1 protein (His-S1, residues 1-701) or S1 Receptor Binding Domain (mouse FcS1-RBD, residues 391-600, PDBID: 6vxx) antigens. Figures 1B-1C are structural models of recombinant spike protein. Figure 1D is a schematic showing the vaccine schedule and strategy. Figure 1E shows the VLP display of Fc-tagged antigens using the PP7 particle displaying 120 ZZ-domains per particle; a 1:1 mass ratio of mFc-S1 and VLP provides a Fc:ZZ molar ratio of 0.82. Rh = hydrodynamic radius measured by dynamic light scattering in phosphate buffer. Figure 1F is a line graph showing ELISA responses for serum dilutions against plated His-S1 protein from the sacrificed mice at day 30. Figure 1G is a line graph showing titer values from ELISA analysis as in panel d, against plated His-S1 or spike ectodomain protein. S1, S2, and S3 designate immunization series in panel b; a and b designate different mice.

Figure 2A is a pie chart showing total number of IgG-secreting hybridomas chosen from Series 1-3 immunization schedules. Figures 2B-2D are pie charts showing antigen specificity of antibody binding from the selected clones. Figure 2E is a scatter plot showing distribution of affinities of the 37 selected clones as measured by BLI.

Figures 3A-3B are graphs showing single-concentration (Fig. 3A) and representative (mAb 3G7) dose-dependent (Fig. 3B) antibody-mediated inhibition or enhancement of ecto-Spike binding to human ACE2 receptor as measured by BLI. Figure 3C is a histogram showing *in vitro* neutralization of SARS-CoV-2 virus by mAbs. Figure 3D is a histogram showing binding of antibodies to biotinylated peptide (amino acids 486-501) plated on streptavidin-coated plates as measured by ELISA. Dashed line represents absorbance of negative control wells. Figure 3E is a representative immunocytochemistry image showing mAb staining of SARS-CoV-2 in infected COVID-19 patient lung tissue by IHC (antigen retrieval method) (mAb 3D5, 1:25 dilution, 40x magnification).

Figure 4 is a chart showing epitope binding patterns of a subset of selected antibodies. Values represent signals from biolayer interferometry (BLI) in which two antibodies (or the same antibody twice) is added to the instrument probe functionalized with immobilized spike

ectodomain. The reported value represents the observed signal after the second antibody is added (arbitrary units). Thus, if the first antibody occupies the same binding sites as the second, little to no increase in signal is observed after the second addition. Asterisks mark the antibodies identified as superior neutralizers in Figure 3C.

Figure 5A-5B are heat maps showing amino acid sequence similarities of selected clones.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

As used herein, a molecule is said to be able to “immunospecifically bind” a second molecule if such binding exhibits the specificity and affinity of an antibody to its cognate antigen. Antibodies are said to be capable of immunospecifically binding to a target region or conformation (“epitope”) of an antigen if such binding involves the antigen recognition site of the immunoglobulin molecule. An antibody that immunospecifically binds to a particular antigen may bind to other antigens with lower affinity if the other antigen has some sequence or conformational similarity that is recognized by the antigen recognition site as determined by, *e.g.*, immunoassays, BIAcore® assays, or other assays known in the art, but would not bind to a totally unrelated antigen. In some embodiments, however, antibodies (and their antigen binding fragments) will not cross-react with other antigens. Antibodies may also bind to other molecules in a way that is not immunospecific, such as to FcR receptors, by virtue of binding domains in other regions/domains of the molecule that do not involve the antigen recognition site, such as the Fc region.

As used herein, a molecule is said to “physiospecifically bind” a second molecule if such binding exhibits the specificity and affinity of a receptor to its cognate binding ligand. A molecule can be capable of physiospecifically binding to more than one other molecule.

As used herein, the term “antibody” is intended to denote an immunoglobulin molecule that possesses a “variable region” antigen recognition site. The term “variable region” is intended to distinguish such domain of the immunoglobulin from domains that are broadly shared by antibodies (such as an antibody Fc domain). The variable region includes a “hypervariable region” whose residues are responsible for antigen binding. The hypervariable region includes amino acid residues from a “Complementarity Determining Region” or “CDR” (*i.e.*, typically at approximately residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and at approximately residues 27-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991))

and/or those residues from a “hypervariable loop” (*i.e.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917). “Framework Region” or “FR” residues are those variable domain residues other than the hypervariable region residues as herein defined. The term antibody includes monoclonal antibodies, multi-specific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, camelized antibodies (*See e.g.*, Muyldermans *et al.*, 2001, *Trends Biochem. Sci.* 26:230; Nuttall *et al.*, 2000, *Cur. Pharm. Biotech.* 1:253; Reichmann and Muyldermans, 1999, *J. Immunol. Meth.* 231:25; International Publication Nos. WO 94/04678 and WO 94/25591; U.S. Patent No. 6,005,079), single-chain Fvs (scFv) (*see, e.g., see* Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994)), single chain antibodies, disulfide-linked Fvs (sdFv), intrabodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id and anti-anti-Id antibodies to antibodies). In particular, such antibodies include immunoglobulin molecules of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass.

As used herein, the term “antigen binding fragment” of an antibody refers to one or more portions of an antibody that contain the antibody’s Complementarity Determining Regions (“CDRs”) and optionally the framework residues that include the antibody’s “variable region” antigen recognition site and exhibit an ability to immunospecifically bind antigen. Such fragments include Fab', F(ab')₂, Fv, single chain (ScFv), and mutants thereof, naturally occurring variants, and fusion proteins including the antibody’s “variable region” antigen recognition site and a heterologous protein (*e.g.*, a toxin, an antigen recognition site for a different antigen, an enzyme, a receptor, or receptor ligand, *etc.*).

As used herein, the term “fragment” refers to a peptide or polypeptide including an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues.

As used herein the term “modulate” relates to a capacity to alter an effect, result, or activity (e.g., signal transduction). Such modulation can be agonistic or antagonistic. Antagonistic modulation can be partial (*i.e.*, attenuating, but not abolishing) or it can completely abolish such activity (*e.g.*, neutralizing). Modulation can include internalization of a receptor following binding of an antibody or a reduction in expression of a receptor on the target cell. Agonistic modulation can enhance or otherwise increase or enhance an activity (e.g., signal transduction). In a still further embodiment, such modulation can alter the nature of the interaction between a ligand and its cognate receptor so as to alter the nature of the elicited signal transduction. For example, the molecules can, by binding to the ligand or receptor, alter the ability of such molecules to bind to other ligands or receptors and thereby alter their overall activity. In some embodiments, such modulation will provide at least a 10% change in a measurable immune system activity, at least a 50% change in such activity, or at least a 2-fold, 5-fold, 10-fold, or at least a 100-fold change in such activity.

The term “substantially,” as used in the context of binding or exhibited effect, is intended to denote that the observed effect is physiologically or therapeutically relevant. Thus, for example, a molecule can substantially block an activity of a ligand or receptor if the extent of blockage is physiologically or therapeutically relevant (for example if such extent is greater than 60% complete, greater than 70% complete, greater than 75% complete, greater than 80% complete, greater than 85% complete, greater than 90% complete, greater than 95% complete, or greater than 97% complete). Similarly, a molecule is said to have substantially the same immunospecificity and/or characteristic as another molecule, if such immunospecificities and characteristics are greater than 60% identical, greater than 70% identical, greater than 75% identical, greater than 80% identical, greater than 85% identical, greater than 90% identical, greater than 95% identical, or greater than 97% identical).

As used herein, the “activating” or “stimulatory” signals encompass signals that result in enhancing an activity or enhancing signal transduction.

As used herein, “suppressive” signals refer to signals that suppress immune activity.

The term “derivative” refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to the same target of a parent or reference antibody, but which differs in amino acid sequence from the parent or reference antibody or antigen binding fragment thereof by including one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to the parent or reference antibody or antigen binding fragment thereof. In some embodiments, such derivatives will have substantially the same immunospecificity and/or characteristics, or the same immunospecificity and characteristics as

the parent or reference antibody or antigen binding fragment thereof. The amino acid substitutions or additions of such derivatives can include naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. The term “derivative” encompasses, for example, chimeric or humanized variants, as well as variants having altered CH1, hinge, CH2, CH3 or CH4 regions, to form, for example antibodies, *etc.*, having variant Fc regions that exhibit enhanced or impaired effector or binding characteristics.

As used herein, a “chimeric antibody” is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region.

As used herein, the term “humanized antibody” refers to an immunoglobulin including a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the “donor” and the human immunoglobulin providing the framework is called the “acceptor.” Constant regions need not be present, but if they are, they should be substantially identical to human immunoglobulin constant regions, *i.e.*, at least about 85-99%, or about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A humanized antibody is an antibody including a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody, because, *e.g.*, the entire variable region of a chimeric antibody is non-human.

The term “endogenous concentration” refers to the level at which a molecule is natively expressed (*i.e.*, in the absence of expression vectors or recombinant promoters) by a cell (which cell can be a normal cell, a cancer cell or an infected cell).

As used herein, the terms “treat,” “treating,” “treatment” and “therapeutic use” refer to the elimination, reduction, or amelioration of one or more symptoms of a disease or disorder. As used herein, a “therapeutically effective amount” refers to that amount of a therapeutic agent sufficient to mediate a clinically relevant elimination, reduction or amelioration of such symptoms. An effect is clinically relevant if its magnitude is sufficient to impact the health or prognosis of a recipient subject. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to delay or minimize the onset of disease, *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of a disease.

As used herein, the term “prophylactic agent” refers to an agent that can be used in the prevention of a disorder or disease prior to the detection of any symptoms of such disorder or disease. A “prophylactically effective” amount is the amount of prophylactic agent sufficient to mediate such protection. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the prevention of disease.

As used herein, an “immune cell” refers to any cell from the hemopoietic origin including, but not limited to, T cells, B cells, monocytes, dendritic cells, and macrophages.

As used herein, “inflammatory molecules” refer to molecules that result in inflammatory responses including, but not limited to, cytokines and metalloproteases such as including, but not limited to, IL-1 β , TNF- α , TGF-beta, IFN- γ , IL-18, IL-17, IL-6, IL-23, IL-22, IL-21, and MMPs.

As used herein, “valency” refers to the number of binding sites available per molecule.

As used herein, the terms “immunologic,” “immunological” or “immune” response is the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against a peptide in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules to activate antigen-specific CD4⁺ T helper cells and/or CD8⁺ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils, activation or recruitment of neutrophils or other components of innate immunity. The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4⁺ T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating antibodies and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

An “immunogenic agent” or “immunogen” is capable of inducing an immunological response against itself on administration to a mammal, optionally in conjunction with an adjuvant.

As used herein, the terms “individual,” “host,” “subject,” and “patient” are used interchangeably herein, and refer to a mammal, including, but not limited to, humans, rodents, such as mice and rats, and other laboratory animals.

As used herein, the term “polypeptide” refers to a chain of amino acids of any length, regardless of modification (*e.g.*, phosphorylation or glycosylation). The term polypeptide includes proteins and fragments thereof. The polypeptides can be “exogenous,” meaning that they are “heterologous,” *i.e.*, foreign to the host cell being utilized, such as human polypeptide produced by a bacterial cell. Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

As used herein, the term “variant” refers to a polypeptide or polynucleotide that differs from a reference polypeptide or polynucleotide, but retains essential properties. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more modifications (*e.g.*, substitutions, additions, and/or deletions). A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally.

Modifications and changes can be made in the structure of the polypeptides of the disclosure and still obtain a molecule having similar characteristics as the polypeptide (*e.g.*, a conservative amino acid substitution). For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide’s biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a

hydropathic index based on its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and cofactors. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 $\pm$ 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Trp: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include

variants having about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the polypeptide of interest.

The term “percent (%) sequence identity” is defined as the percentage of nucleotides or amino acids in a candidate sequence that are identical with the nucleotides or amino acids in a reference nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

For purposes herein, the % sequence identity of a given nucleotides or amino acids sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given sequence C that has or comprises a certain % sequence identity to, with, or against a given sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z,$$

where W is the number of nucleotides or amino acids scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides or amino acids in D. It will be appreciated that where the length of sequence C is not equal to the length of sequence D, the % sequence identity of C to D will not equal the % sequence identity of D to C.

As used herein, the term “pharmaceutically acceptable carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

II. SARS-CoV2 Binding Moieties

Previous beta coronavirus outbreaks from SARS-CoV-1 (2003) and MERS-CoV (2008) have highlighted the utility of antibody response in the quick diagnosis of infection and informed on current SARS-CoV-2 vaccine design, especially concerning the epitope motifs to target for virus neutralization. Of great interest is the ~300 amino acid receptor binding domain (RBD) of the S1 subunit of the spike protein (Fig. 1A). The S1 RBD contains the five contact residues (L455, F486, Q493, S494, N501) (Shang, J, et al., *Nature* 2020, 581, 221-224; Wan, Y., et al. *J. Virol.* 2020, 94, e00127-00120. previously shown to be important for viral entry through interactions with the human angiotensin converting enzyme 2 (hACE2) receptor present on many cell types, especially lung epithelial cells. Upon viral exposure, humoral

immunity results in antibody development that, when targeted to this region, can block viral entry.

Disclosed herein are multiple fully-defined nanomolar-affinity mouse mAbs created by an accelerated immunization and hybridoma screening process, twelve of which are capable of efficient virus neutralization *in vitro* and two which robustly stain lung tissue from COVID-19 patients.

One embodiment provides antibody or antigen-binding fragments thereof that bind, preferably specifically, to a SARS-CoV-2 spike protein. Optionally, the antibody is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, single-chain antibody or antibody that competitively inhibits the binding of an anti-SARS-CoV protein antibody or antigen-binding fragment thereof to its respective antigenic epitope. In one embodiment, the disclosed antibodies can optionally be produced in Chinese hamster ovary (CHO) cells or in bacterial cells and preferably inhibit, reduce, or prevent the binding of coronaviruses, including but not limited to SARS-CoV-2 spike protein to cell surface proteins in a subject. In some embodiments, the disclosed antibodies and antigen-binding fragments thereof reduce or inhibit the growth or proliferation of or induce the death of a cell infected with a coronavirus to which they bind.

In another embodiment, the disclosed antibodies and antigen-binding fragments thereof are detectably labeled, attached to a solid support, or the like, such as a lateral flow assay device which provides for point-of-care detection of SARS-CoV-2 and/or diagnosis.

Yet another embodiment provides vectors comprising nucleic acids, including but not limited to DNA, encoding any of the herein described antibodies and antigen-binding fragments thereof. Host cells comprising any such vector are also provided. By way of example, the host cells may be CHO cells, bacterial cells (such as *E. coli*) used for recombinant protein expression, or yeast cells. A process for producing any of the herein described antibodies is further provided and comprises culturing host cells under conditions suitable for expression of the desired antibody and recovering the desired antibody from the cell culture.

A. Exemplary Antibodies

This disclosure includes the following amino acid sequences:

SEQ ID NO	Description	Sequence
1	3C4 clone heavy chain and 3E4 clone heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHWVKQRPE QGLEWIGRIDPASGKTEYDPNFQGKATMTADTSSNTAYLQLS SLTSEDTAVYYCASGYDVNYELDYWGQGTSTVTVSS
2	3C4 clone light chain and 3E4 clone heavy chain	DIQMNQSPSSLASLGDITITCHASQNINWLSWYQQKPGNI PKLLIYKASNLHTGVPSRFSGSGSGTGFTLTISLQPEDATYY CQQGQSYPTYFGGGTKLEIK
3	4F11 clone heavy chain	QAVVTQESALTTSPGETVTLTCSRSTGAVTTSNYANWVQKEP DHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDE AIYFCALWYSNISLFFVIFGSGTKVTVL
4	4F11 clone light chain	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLYSSNQKNYLAW YQQKPGQSPKLLIYWASTRESGVDPDRFTGSGSGTDFTLTISV KAEDLAVYYCQQYYSPWTFGGGKLEIK
5	3G1 clone heavy chain	QVQLKQSGPGLVQPSQSLSTCTVSGFSLTSYGVHWVRQSPGK GLEWLGVIWSSGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQ ANDTAIYYCAKYRYDSFAYWGQGTLLTVSA
6	3G1 clone light chain	DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYMYWYQQ KPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEE DAATYYCQHSRELPTYFGGGKLEIK
7	3A6 clone heavy chain	QVQLKESGPGLVAPSQSLSTCTVSGFSLISYGVHWVRQPPGK GLEWLGVIWAGGSTNYNSALMSRLSINKDNSKSQVFLKINSL QTDDTAMYYCGRDYGILLIDYWGQGTLLTVSS
8	3A6 clone light chain	ENVLTQSPAISASPGEKVTMTCSASSSVSYMHWYQQKSSTS PKLWIHDTSKLASGVPGRFSGSGSGNSYSLTVSSMEAEDVAT YYCFQSGSGYPFTFGSGTKLEIK
9	3B3 clone heavy chain	DVQLQESGPGLVKPSQSLSTCTVTGYSITSDYAWIWRQFPG NKLEWMAYISYSGTTSYNPSLKSRLSITRDTSKNQFFLQLNSVT TEDTATYYCAGRRGAYYGNYEEDYWGRGTSVTVSS
10	3B3 clone light chain	QIVLTQSPAISASPGEKVTMTCSASSSVNYMYWYQQKPGSSP RLLIYDTSTLASGVPRFSGSGSGTSYSLTISRMEAEDAATYY CQQWSSYPPTYFGGGKLEIK

11	3D7 clone heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWVKQRP EQGLEWIGRIDPANGNSKYDPKFQGKATITADTSSNTAYLQLS SLTSEDTAVYYCARSYYDYDGGGCFDYWGQGTTTLTVSS
12	3D7 clone light chain	RIVMTQTPKFLPVSAGDRVTITCKASQSVSNDAWYQQKPGQ SPKPLIYYASNRYTGVPDRFTGSGYGTDFTFITSTVQTEDLAIY FCQQGYSSPLTFGAGTKLELK
13	3E5 clone heavy chain	EVQLQQSGPELVKPGASVKISCKASGYSFTGYFMNWVKQSH GKSLEWIGRINPYNGDTFYNQKFKGKATLTVDKSSNTAHMEL LSLTSEDSAVYYCGLRITYWGQGTLLTVSA
14	3E5 clone light chain	DILMTQSPSSMSVSLGDTVNITCHASQDINNIGWLQQRPGKS FKGLIYHGTNLEDGVPSRFSGSGSGTDYSLTSSLESEDFADYY CVQSVQFPYTFGGGKLEIK
15	3B4 clone heavy chain	EVKLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRP EQGLEWIGRIDPANGDTKYDPNFQVKATITADTSSNTASLQFS SLTSEDTAVYYCARSYYYGTTSWFASWGQGTLLTVSA
16	3B4 clone light chain	DIQMTQSSSSFSVSLGDRVTITCKASEDIYNHLAWYQQKPGNA PRLISGAPSLETGVPSRFSGSGSGKDYTLSTSLQTEDVATYY CQQYWSTPYTFGGGKLEIK
17	3D1 clone heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWVKQRP PEQGLEWIGRIDPANGNTKYDPKFQGKATIAADTSSNTAYM QLSSLTSEDTAVYYCARWDFGNYVDYAMDYWGQGTSTVTV SS
18	3D1 clone light chain	DIKMIQSPSSMYVSLGERVTITCKASQDLYSFLSWFQQKPGKS PKTLIYRANRLVDGVPSRFSGSGSGQDYSLTSSLEYEDMGIY YCLQYDAFPWTFGGGKLEIK
19	3A2 clone heavy chain	QVTLKESGPGILQPSQTLSTCSFSGFSLSTSGMGVSWIRQPSG KGLEWLAHIYWDDDKRYNPSLKSRLTISKDTSSSQVFLKITSV DTVDTATYYCARRGPLITTDGTFDVWGAGTTVTVSS
20	3A2 clone light chain	DIVMTQSRKFMSTSVGDRVSITCKASQDVGTSVAWYQQKPG QSPKLLIYWASTRHTGVDPDRFTGSGSGTDFTLTINSVQSEDLA DYFCQQYSSYPYTFGGGKLEMK
21	3C6 clone heavy chain	QVTLKESGPGILQPSQTLSTCSFSGFSLSTSGMGVSWIRQPSG KGLEWLAHIYWDDDKRYNPSLKSRLTISKDTSSNQVFLKIISV DTVDTATYYCARRGPLITTDGTFDVWGAGTTVTVSS

22	3C6 clone light chain	DIVMTQSHKFLSTSVGDRVITITCKASQDVTAVAWYQQKPG QSPKLLIYWASIRHTGVPDRFTGSGSGTDFTLTISNVQSDDLA DYFCQQYSSYPYTFGGGKLEIE
23	3H5 clone heavy chain	EVQLQQSGPELVKPGASVKISCKASGYSTGYFMNWVKQSH GKSLEWIGRINPYNGDTFYNQKFKGKATLTVDKSSSTAHMEL LSLTSEDSAVYYCGRGLYGKGYYYAMDYWGQGTSTVTVSS
4	3H5 clone light chain	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWY QQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVK AEDLAVYYCQQYSSYPWTFFGGGKLEIK
24	3G7 clone heavy chain	QVQLQQPGTELVRPGASVKLSCKASGYSTSYWMNWVKQRP GQGLEWIGMIHPDSETRLNQKFKDKATLTVDKSSSTAYMQL SSPTSEDSAVYYCARSSGYDWYFDVWGAGTTVTVSS
25	3G7 clone light chain	DIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWHQQ KPGQPPKLLIYRASNLESGIPARFSGSGSRTDFTLTINPVEADD VATYYCQQSNEDPWTFGGGKLEIK
19	3A7 clone heavy chain	QVTLKESGPGILQPSQTLSTCSFSGFSLSTSGMGVSWIRQPSG KGLEWLAHIYWDDDKRYNPSLKSRLTISKDTSSSQVFLKITSV DTVDTATYYCARRGPLITTDGTFDVWGAGTTVTVSS
20	3A7 clone light chain	DIVMTQSRKFMSTSVGDRVSITCKASQDVGTSAVWYQQKPG QSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTINSVQSEDLA DYFCQQYSSYPYTFGGGKLEMK
26	3C2 clone heavy chain	QVQLQQSGAELARPGASVKLSCKASGYAFTSYWMEWVKQR PGQGLEWIGSIYPGDGATRYTQKFKGKATLTADKSSSTAYME FSSLASEDSAVYFCARSGGYDWYFDVWGAGTTVTVSS
27	3C2 clone light chain	DIVLTQSPASLAVSLGQRATISCRASETVDSYGNSFMHWHYQQ KPGQPPKLLIYRASNLESGIPVRFSGSGSRTDFTLTINPVEADD VATYYCQQSNEDPWTFGGGKLEIK
28	3D5 clone heavy chain	EVQLQQSGAELVKPGASVTLSTASGFNIKDTYMHVVKQRP EQGLEWIGRIDPANGNTKYDPKFQGKATITADTSSNTAYLQLS SLTSEDTAVYYCTRYYYGSSGFFDVWGAGTTVTVSS
29	3D5 clone light chain	SIVMTQTPKFLVLSAGDRVITITCKASQSVSNDAVWYQQKPGQ SPKVLIYYASNRYTGVPDRFTGSGYGTDFTFITSTVQAEDLAV YFCQQDYSSPTFGAGTKLELK
30	3F6 clone heavy chain	EVQLQQSGAELVKSGASVKLSCTASGFNIKDTYMHVVKQRP EQGLEWIGRIDPASGNTKYDPKFQGKATITSDTSSNTAYLQLS SLTSEDTAVYFCARSYYTYDGFFDVWGAGTTATVSS

31	3F6 clone light chain		NIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFITISTVQAEDLAVYFCQQDYSSPTFGGGTKLEIK
32	9F2 clone heavy chain		EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWVKQRP EQGLEWIGMIDPANGNTKYDPKFQGKATITADTSSNTAYLQL RSLTSED TAVYYCARTYYYGSSYEAMDYWGQGTSTVTVSS
33	9F2 clone light chain		SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFITISTVQAEDLAVYFCQQDYSSPTFGGGTKLEIK
34	1-3A1	heavy chain	DVQLQESGPD LVKPSQSPSLTCTVTGY SITGDYSWHWIRQFPGNKLE WMGYIHYSGSADYNPSLKSRI SITRDT SKNQFFLQLNSVTTEDTATYF CARWNGKNAMDYWGQGTSTVTVSS
35	1-3A1	light chain	DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQKSPQLLVYNTKTLAEGVPSRFSGSGSDTQFSLKINSLQPEDFGYYYCQHHYGS PPTFGGGTKLEIK
36	1-3B1	heavy chain	DVQLQESGPGLVKPSQSLSLTCSVTGY SITSGYYWNWIRQFPGNKLE WMGYIHDGTNNYNPSLKNRISITRDT SKNQFFLKLNSVTSED TATY YCARVDYDVGHWFAYWGQGTSTVTVSA
37	1-3B1	light chain	ENVLTQSPAISASLGEKVTMSCRASSSVNYMYWYQQKSDASPKLWIYYTSNLAPGVPARFSGSGSGNSYSLTISSMEGEDAATYYCHQFTTSPWTFGGGTKLEIK
9	1-3B3	heavy chain	DVQLQESGPGLVKPSQSLSLTCTVTGY SITSDYAWIWIRQFPGNKLE WMAYISYSGTTSYNPSLKSRI SITRDT SKNQFFLQLNSVTTEDTATYY CAGRRGAYYGNYEEDYWGRGTSTVTVSS
10	1-3B3	light chain	QIVLTQSPAISASPGKVTMTCSSSSIVNYMYWYQQKPGSSPRLLIYDTSTLASGV PVRFSGSGSGTSYSLTISRMEAEDAATYYCQWSSYPYTFGGGTKLEIK
30	1-3B6	heavy chain	EVQLQQSGAELVKSGASVKLSCTASGFNIKDTYMHWVKQRPEQGLEWIGRIDPASGNTKYDPKFQGKATITSDTSSNTAYLQLSSLTSED TAVY FCARSYYTYDGFDDVWGAGTTATVSS
31	1-3B6	light chain	NIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFITISTVQAEDLAVYFCQQDYSSPTFGGGTKLEIK
38	1-3C3	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWVKQSHGKSLEWIGGINPNNGGTSYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSA VYYCARRQDGNWFAYWGQGTSTVTVSA
39	1-3C3	light chain	EIVLTQSPPTMAASPGKITITCSASSISSNYLHWYQQKPGFSPKLLIYRTSNLASGV PPARFSGSGSGTSYSLTIGTMEAEDVATYYCQQGSSIPRITFGAGTKLELK

40	1-3D2	heavy chain	QVQLQQSGAELAKPGASVKMSCKASGYTFSTYWIHWVQQRPGQGL EWIGYINPYTDYTEYNQKFQDKATLTADKSSNTAYMQLSSLTSEDS AVYYCARRYGNDAWFTYWGQGTLLTVSA
41	1-3D2	light chain	DIVMTQSHKFMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPK VLIYWASTRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYR TFGGGTKLEIK
32	1-3D3	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWVKQRPEQGLE WIGMIDPANGNTKYDPKFQGKATITADTSSNTAYLQLRSLTSED TAV YYCARTYYYGSSYEAMDYWGQGTSTVTVSS
33	1-3D3	light chain	SIVMTQTPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKL LIYYASNRYTGVPDRFTGSGYGTDFTFITISTVQAEDLAVYFCQQDYS SPTFGGGTKLEIK
32	1-3E1	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWVKQRPEQGLE WIGMIDPANGNTKYDPKFQGKATITADTSSNTAYLQLRSLTSED TAV YYCARTYYYGSSYEAMDYWGQGTSTVTVSS
33	1-3E1	light chain	SIVMTQTPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKL LIYYASNRYTGVPDRFTGSGYGTDFTFITISTVQAEDLAVYFCQQDYS SPTFGGGTKLEIK
42	1-3F1	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRPEQGLE WIGRIDPANGNTKYDPKFQDKAIITADTSSNTAYLQFSSLTSED TAVY YCVSGYYYYGSPYGAMDYWGQGTSTVTVSS
43	1-3F1	light chain	QIVLTQSPAIMASAPGEKVTISCSATSSVSYMYWYQQKPGSSPKWIY HTSNLASGVPARFSGSGSGTSYSLTISNMEAEDAATYYCQQYHGYPL TFGAGTKLELK
44	1-3F2	heavy chain	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYSLHWVKQTPGQGL EWIGAVYPGNDTTSYNQNFQKATLTADKSSNTAYMQLSSLTSEDS AVYYCARDGYFAMDYWGQGTSTVTVSS
45	1-3F2	light chain	DIVMSQSPSSLA VSGEKTVMSCSSQSLLYSTNQKNYLAWYQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKAEDLAVYY CHQYYSPWTFGGGTKLEIK
46	1-3F4	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRPEQGLE WIGRIDPANDNTKYDPKFQGKATITADTSSNTAYLQLTSLTSED TAV YYCTRYDYVYAMDYWGQGTSTVTVSS
47	1-3F4	light chain	DIQMTQSPASQSASLGESVTITCLASQTIGTWLAWYQQKPGKSPQLLI YAATSLADGVPSRFSGSGSGTKFSFRISLQAEDFVSYCQQLYSTPL TFGGGTKLEIK
19	1-3F5	heavy chain	QVTLKESGPGLQPSQTLSTLCSFSGFSLSTSGMGVSWIRQPSGKGLE WLAHIYWDDDKRYNP SLK SRLTISKDTSSSQVFLKITSVDTVDTATY YCARRGPLITTDGTFDVWGAGTTVTVSS

20	1-3F5	light chain	DIVMTQSRKFMSTSVGDRVSITCKASQDVGTSVAWYQQKPGQSPKL LIYWASTRHTGVPDRFTGSGSGTDFTLTINSVQSEDLADYFCQQYSS YPYTFGGGKLEMK
28	1-3G2	heavy chain	EVQLQQSGAELVKPGASVTLSTASGFNIKDTYMHVWKQRPEQGLE WIGRIDPANGNTKYDPKFQGKATITADTSSNTAYLQLSSLTSEDYAV YYCTRYYYGSSGFFDVWGAGTTVTVSS
29	1-3G2	light chain	SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKV LIYYASNRYTGVPDRFTGSGYGTDFTFITSTVQAEDLAVYFCQQDYS SPTFGAGTKLELK
48	1-3G4	heavy chain	EVQLQQSGPELVKPGASVKMSCKASGYTFTDYYMKWVKQSHGKSL EWIGDINPNNGDTFYNQKFKGKATLTVDKSSSTAYMQLNSLTSEDS AVYYCARGGYRFAWYWGQGLTVTVSA
49	1-3G4	light chain	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKP GQPPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYY CQNDYSYPLTFGAGTKLELK
50	1-3G6	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTNMHWVKQRPEQGLE WIGRIDPANGDTKYDPKFQGKATITADTSSNTAYLQLSSLTSEDYAV YYCARLNYDGYDYAMDYWGQGTSTVTVSS
51	1-3G4	light chain	DIKMTQSPSSMYASLGERVTITCKASQDINSFLSWFQQKPGKSPKTLI YRANRLVDGVPSRFSGSGSGQDYSLTISSEYEDMGIYYCLQYDEFP FTFGSGTKLEIK
52	1-3H2	heavy chain	QVQLQQSGAELMKPGASVKISCKATGNTFSNYIEWVKQRPGHGL EWIGEILPGSDSTNYNEKFKGKATFTADTSSKAYMQLSSLTSEDSA VYYCARNRFYWFYFDVWGAGTTVTVSS
53	1-3H2	light chain	DVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPG QSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQ STHVPWTFGGGKLEIK
54	1-3H4	heavy chain	EVQLQQSGAELVKPGASIKLSCTASGFNIKDTYMYWVKQRPEQGLE WIGRIDPANGNSKYDPKFQGKATITADTSSNTAYLHLSSLTSEDYAV YYCARSYYDYDGGGCFDYWGQGTTLTVSS
55	1-3H4	light chain	RIVMTQTPKFLPVSVGDRVTITCKASQSVSNDVAWYQQKPGQSPKP LIYYASNRYTGVPDRFTGSGYGTDFTFITSTVQTEDLAIYFCQQGYSS PLTFGAGTKLELK
56	1-3H6	heavy chain	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGL EWVARIRSKSDNFAMYYADSVKDRFTISRDDSQSMYLYLQMSNLKTE DTAMYYCASYDGYRAWFAYWGQGLTVTVSA
57	1-3H6	light chain	DVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPG QSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS QSTHVPWTFGGGKLEIK

58	1-8C2	heavy chain	EVKLEESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQSPEKGL EWVAEIRLKSNNYATHYAESVKGRFTISRDDSSVYLQMNRLRAE DTGIYYCTSRAYWGQGTLLTVSA
59	1-8C2	light chain	DIVMTQSHKFMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKL LIYWASTRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYSS YPYTFGGGGTKLEIK
60	1-8D1	heavy chain	QVQLQQPGAELVKPGASVKLSCKASGYTFISYWMHWVKQRPGQGL EWIGEINPSNGRSNYNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSA VYYCARGDYDGSYWFYDVGAGTTVTVSS
61	1-8D1	light chain	DIVLTQSPASLA VSLGQRATISCRASESDNYGISFMNWFQQKPGQP PKLLIYAASNQSGVPARFSGSGSGTDFTSLNIHPMEEDDTAMYFCQQ SKEVPPTFGGGGTKLEIK
62	1-5A1	heavy chain	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNIHWVKQTPEQGLE WIGAIYPENGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAV YYCTRDGYDGGAHYGLDYWGQGTSTTVSS
63	1-5A1	light chain	DIVLTQSPASLA VSLGQRATISCRASKSVSTSGYSFIHWYQQKPRQP KFLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCHHTR ELPWTFGGGGTKLEIK
64	3-13A1	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTIYVWKQSHGKSLEW IGGINPNNGDTIYNQNFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARDGYGGIAYWGQGTSTTVSS
65	3-13A1	light chain	DIVMSQSPSSLA VSVGEKVTMSCKSSQSLLYSSNQKNYLAWFQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKAEDLAVYY CQQYYRYPLTFGGGGTKLEIK
66	5-1A5	heavy chain	EVKLVESGGGLVQPGGSLKLSAASGFTLSNYAMSWVRQTPEKRLE WVASISSGGSIYYLDSVKGRFTISRDNARNILYLQMSILRSGDTAMY YCVSGSGNYVDSPMDYWGQGTSTTVSS
67	5-1A5	light chain	QVVLTSQPAIMASLGEETLTCSASSSVSYMNWYQQKSGTSPKLLIY STSNLASGVPSRFSGSGSGTFYSLTISSVEAEDAADFCHQWSSWTF GGGGTKLEIK
68	5-1E5	heavy chain	EVQLQQSGAELVRSGASVKLSCTASGLNFKDYMHVWKQRPEQGL EWIGWIDPENGDTDYAPKFQKGKATMTADTSSNTAYLQLSSLTSED TAVYFCNAALGYWGQGTTLTVSS
69	5-1E5	light chain	DAVMTQTPLSLPVSLGDQASISCRSSQSLNSNGNTYLNWYLQKPG QSPQLLIYRVSNRFSGLDRFSGSGSGTDFTLKISRVEAEDLGVYFCF QVTHVPYTFGGGGTKLEIK
70	5-1F2	heavy chain	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSIHWVKQAPGKGLK WMGWINTETGEPTCADDKGRFAFSLETSASTAYLQINNKNEDTST YFCLRYWGIYVEWYFDLWGAGTTVTVSS

71	5-1F2	light chain	DVLMQTQPLSLPVRLGDQASISCRSSQSILHSNGNTYLEWYLQKPGQ FPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVESEDLGVYYCFQ GSHVPLTFGAGTKLELK
72	5-1F6	heavy chain	QVQLQQPGAELVRPGASVNLSCASGYTFTNYWINWVRQRPQGGL EWIGNIYPSDVYTNYNQKFKDKATLTVDKSSSTAYMQLSSPTSDDSA VYYCTRIYYISSYAGAMDYWGQGTSTVTVSS
73	5-1F6	light chain	NIVLTQSPRSMSSVGERVTLSCKASENVGTYSWYQQKPEQSPKL LIYGASNRYTGVPDRFTGSGSATDFSLTISSVQAEDLADYHCGQSFSY PLTFGAGTKLELK
74	5-1F8	heavy chain	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSMQWVKQAPGKGLK WMGWINTETGEPTYTDDFKGRFAFSLETSASTAYLQINILKNEDTAT YFCARPHYDYWYFDVWGAGTTVTVSS
75	5-1F8	light chain	DVLMTQIPLSLPVSLGNQASISCRSSQNIVHSDGNTYLEWYLQKPGQ SPKILYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQ GSHAPPTFGAGTKLELK
76	5-2B6	heavy chain	QVQLQQPGAELVKPGASVKMSCKASGYTFTSTWMHWVKQRPQGGL LEWIGTIDPSDSYASYNQKFKGKATLTVHTSSSTAYMQLSSLTSEDS AVYYCTANLDYWGQGTTLTVSS
77	5-2B6	light chain	DVVVTQTPSLSPVSFGDQVSISCRSSQSLANSYGNTYLSWYLHKPGQ SPQLLIYGISNRFSGVPDRFSGSGSGTDFTLKISTIKPEDLGMYYCLQG THQPYTFGGGTKLEIK
78	5-2D2	heavy chain	DVKLVESGGGLVKLGSLKLSAASGFTFSSYYMSWVRQTPEKRLE LVAAINSNGGSTYYPDTVKGRFTISRDNKNTLYLQMSSLKSEDTAL YYCARHPLGLPSWFAYWGQGTTLTVSA
79	5-2D2	light chain	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPK ALIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNS YPRTFGGGTKLEIK
80	5-3D2	heavy chain	QVQLQQSGPELVKPGASVRISCKASGYTFTSYIHWVKQRPQGGL WIGWIYPGNVNTKYNEKFKGKATLTADKSSSTAYMQLSSLTSEDSA VYFCARRGIYYRYDDYAMDYWGQGTSTVTVSS
81	5-3D2	light chain	NIVLTQSPASLA VSLGQRATISCRASEVDSYGNSFMHWYQQKPGQP PKLLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQN NEDPWTFGGGTKLEIK
82	5-3G1	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDAYMHWMKQRPQGL EWIGRIDPANGNTKYDPKFQKGATITADTSSNTAYLQLSSLTSEDTA VYYCARKGGYYLYYHAMDCWGQGTSTVTVSS
83	5-3G1	light chain	DIVLTQSPASLA VSLGQRATISCKASQSDYDGDSEFMNHWYQQKPGQ PPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQ SNEDPPTFGSGTKLEIK

84	5-5A2	heavy chain	QVQIQQSGAELARPGASVKLSCKASGYFTTIYWMQWVKQRPGQGL EWIGSIYPGDGNTRYTQKFKGKATLTADKSSSTAYMQLSSLTSDDSA VYYCARSVTYDWYFDVWGAGTTVTVSS
85	5-5A2	light chain	DIVVTHSPASLAVSLGQRATISCRASESVDSYGNFSFMHWYQQKPGQP PKLLIYRASNLGSGIPARFSGSGSRDFTLTINPVEADDVATYYCQQS NEVPWTFGGGTKLEIK
86	5-5B2	heavy chain	QVQLQQSGPELVKPGASVKISCKASGYAFSYSWMNWVKQRPGQGL EWIGRIYPGDGDTNYNGKFKGKATLTADKSSSTAYMQLSSLTSVDS AVYFCTRWDYDGYENTMDYWGQGTSTVTVSS
87	5-5B2	light chain	DIKMTQSPSSMYASLGERVTITCKASQDINSYLSWFQQKPGKSPKTLI YRANRLVDGVPSRFSGSGSGQDYSLTISSEYEDMGIYYCLQYDEFP YTFGGGTKLEIK
88	5-5D2	heavy chain	DVQLVASGGGLVQPGGSRKLSAASGFTFSNFGMHWRQAPEKGL EWVAYISSGSSTIYYDTLTKGRFTISRDNPKNLFLHMTSLTSEDAM YYCVRSAHGYGWFAYWGQGTSLTVSA
89	5-5D2	light chain	DIVMSQSPSSLA VSVGEKVTMSCMSSQSLLYSTDQKNYLAWYQQKP GQSPKLLIYWASTRESGVPRFTGSGSGDFTLTISSVKAEDLAVYY CQQYYSPWTFGGGTKLEIK
88	5-5F2	heavy chain	DVQLVASGGGLVQPGGSRKLSAASGFTFSNFGMHWRQAPEKGL EWVAYISSGSSTIYYDTLTKGRFTISRDNPKNLFLHMTSLTSEDAM YYCVRSAHGYGWFAYWGQGTSLTVSA
89	5-5F2	light chain	DIVMSQSPSSLA VSVGEKVTMSCMSSQSLLYSTDQKNYLAWYQQKP GQSPKLLIYWASTRESGVPRFTGSGSGDFTLTISSVKAEDLAVYY CQQYYSPWTFGGGTKLEIK
90	5-5H1	heavy chain	EVQLQQSGAELVKPGASVKLSCTASGFNIKDIYMHWVKQRPEQGLE WIGRIDPANGKTKYDPKFQGKATIIADTSSNTAYLQLSSLTSEDNAVY YCTRTPDYYGSYWGQGTSLTVSA
91	5-5H1	light chain	DIVLTQSPASLAVSLGQRATIFCKASQSVDDYDGSYMNWYQQKPGQ PPKLLIYAASNLESGIPARFSGSRSGDFTLTNIHPVEEDAATYYCQQS NEDPFTFGSGTKLEIR
92	6-1B1	heavy chain	EVQLQQSGPELVKPGSSVKISCKTSGYIFTEYTMWVKQSHGKSLE WIGGNNPNTGDTTYNQKFKGKATLTVDKSSGTAYLELRSLTSEDSA VYYCARDRYDRAMDYWGQGTSTVTVSS
93	6-1B1	light chain	DIQMTQSPSSLSASLGERVSLTCRASQEISGYLNWLQQKPDGTIKRLI YAASTLD SGVPKRFSRSGSDYSLTISSESEDFADYYCLQYASYPL TFGAGTKLELK
92	6-1F1	heavy chain	EVQLQQSGPELVKPGSSVKISCKTSGYIFTEYTMWVKQSHGKSLE WIGGNNPNTGDTTYNQKFKGKATLTVDKSSGTAYLELRSLTSEDSA VYYCARDRYDRAMDYWGQGTSTVTVSS

93	6-1F1	light chain	DIQMTQSPSSLSASLGERVSLTCRASQEISGYLNWLQQKPDGTIKRLI YAASTLD SGVPKRFSGRSGSDYSLTISSLESEDFADYYCLQYASYPL TFGAGTKLELK
94	6-3B1	heavy chain	QVTLKESGPGILQPSQTLSTCSFSGFSLSTSGMGVSWIRQPSGKGLE WLAHMYWDDDKRYNPSLKSRLTISKSTSSNQVFLKITSVDTADTAT YYCARREIHDFDGFAYWGQGLVTVSA
95	6-3B1	light chain	DIVMTQSQKFMSTSVGDRVSVTCKASQNVGSSVAWYQQKPGQSPK ALVYSASSRYSGVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYN NYPLTFGAGTKLELK
96	6-3C1	heavy chain	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGL EWIGYINRYNDGTEYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSA VYYCARSDYGYDYTM DYWGQTSVTVSS
97	6-3C1	light chain	DIVLTQSPASLA VSLGQRATISCRASESDNYGISFMNWFQQKPGQP PKLLIYTASNQSGV PARFSGSGSGTDFSLNIHPMEEDDAAMYFCQQ TKEVPYTFGGG TKLEIK
98	6-4B2	heavy chain	EVQLQQSGAELVRPGALVKLSCNASGFNIKDYFMNWKQRPEQGL EWIGWIDPENGNSIYDPKFQ GKASITADTSSNTAYLQLSSLTSEDTAV YYCATRDGGAWFAYWGQGLVTVSA
98	6-4B2	heavy chain	EVQLQQSGAELVRPGALVKLSCNASGFNIKDYFMNWKQRPEQGL EWIGWIDPENGNSIYDPKFQ GKASITADTSSNTAYLQLSSLTSEDTAV YYCATRDGGAWFAYWGQGLVTVSA
99	6-4B2	light chain	GIVMTQSQKFMSASVGDRVSVTCKASQNVVTNVAWYQQKPGQSPK ALIYSASYRYSGVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYNS YPLTFGSGTNLEIK
99	6-4B2	light chain	GIVMTQSQKFMSASVGDRVSVTCKASQNVVTNVAWYQQKPGQSPK ALIYSASYRYSGVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYNS YPLTFGSGTNLEIK
98	6-4C1	heavy chain	EVQLQQSGAELVRPGALVKLSCNASGFNIKDYFMNWKQRPEQGL EWIGWIDPENGNSIYDPKFQ GKASITADTSSNTAYLQLSSLTSEDTAV YYCATRDGGAWFAYWGQGLVTVSA
99	6-4C1	light chain	GIVMTQSQKFMSASVGDRVSVTCKASQNVVTNVAWYQQKPGQSPK ALIYSASYRYSGVPDRFTGSGSGTDFLTISNVQSEDLAEYFCQQYNS YPLTFGSGTNLEIK
100	7-1D1	heavy chain	QVQLQQPGAELVKPGASVKMSCKAS GYTFTSYNIHWVKQTPGQGLEWIGAIYPGNGDISYNQKFKGKATLT ADKSSSTAYMHLSSLTSEDSAVYYCARDGDYVRGFAYWGQGLVT VSA
101	7-1D1	light chain	NIVLTQSPASLA VSLGQRATISCRASESDSYGYSFMHWYLQKPGQP PKLLIYLASNLESGV PARFSGSGSRTDFLTIEPVEADDAATYYCQQN NEDPWTG GGT KLEIK

102	7-3C4	heavy chain	DVQLVESGGGLVKPGGSLKLSCAASGFAFSIYDMSWVRQIPEKRLE WVAYISSGGGSTDYLDTVKGRFTISRDNKNTLYLQMSSLKSEDTA MYYCARHNWDFDYWGQGTPLTVSS
103	7-3C4	light chain	DIVMTQSHKFMSTSVGDRVSITCKASQDVGSAAVWYQQKAGQSPK LLIYSASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYYCQQHS GTPLTFGAGTKLELK
104	7-3F5	heavy chain	QVQLQQSGPELVKPGASVKISCGASGYFTFTNYFIHWVRQRPQGGL WIGWIYPGDVNTQYNERFKDKAILTADKSSTTAYMQLTSLTSEDSA VYFCTRRQNFSGFFDYWGQGTTLTVSS
105	7-3F5	light chain	DIQMTQTSSSLASLGDRVTISCRASQAINNYLNWYQQKPDGTVKLL IYYTSKLHSGVPSRFSGSGSGTHYSLTISNLEQEDIATYFCQQGDTLP YTFGGGTNLEIK
106	7-3H3	heavy chain	QVQLEQSGPELVKPGASVKMSCKASGYAFTSYLIEWVKQRPQGGL WIGVINPGSGGKDKNEKFRGKATLTADKSSNTAFLQLSSLTSDDSAL YFCTRNLPGSGYFDVWGAGTTVTVSS
107	7-3H3	light chain	EILLTQSPAIIAASPGEKVTITCSASSSVSYMNWYQQKPGSSPKIWIYG ISNLASGVPARFSGSGSGTSFSFTINSMEAEDVATYYCQQRSGYPLTF GAGTKLELK
108	7-5A2	heavy chain	QVQLKQSGPGLVQPSQSLSTCTVSGFSLTSYGVHWVRQSPGKGL WLGVIWSSGGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQANDTAIY YCARGWFSYYLDYWGQGTTLTVSS
109	7-5A2	light chain	DIQMTQSPASLSVSVGETVTITCRASESIYSNLAWYQQKQKSPQLL VYAATNLADGVPSRFSGSGSGTQYSLKINSLSQSEDFGSYYCQHFWD NTWTFGGGTNLEIK
110	7-5A3	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTIYVWKQSHGKSLEW IGGINPNNGDTIYNQNFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARDYGGIAYWGQGTTLTVSA
111	7-5A3	light chain	DIVMSQSPSSLA VSVGEKVTMSCKSSQSLLYSSNQKNYLAWFQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKAEDLAVYY CQQYYRYPLTFGAGTKLELK
112	7-5A4	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTIHWVKQSHGKSLEW IGGINPNNGDTTYNQRFKGRATLTVDKSSSTAYMELRSLTSEDSAVY YCPREDYRNDEKIPFDYWGQGTTLTVSS
113	7-5A4	light chain	DIVMSQSPSSLT VSVGENITMSCKSSQSLLYSNNQKNYLAWYQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVKAEDLAVYY CQQYYSFPLTFGAGTKLDLK
114	7-5B1	heavy chain	EVQLQQSGPELVKPGASVKISCKASGYSFTDYFMTWVKQNHGKSLE WIGRINPNNGDTFYNQFKGKATLAVDKSSSTAHEMELSLTSEDSA VYYCGRFYRNGDFDVWGAGTTVTVSS

115	7-5B1	light chain	DIVMTQSHKFMSTSVGDRISITCKASQDVNI AVAWYQQKPGQSPEVL IYSASYRFTGVPDRFTGSGSGTDFTFTISSVQAEDLAVYHCQQHYSIP YTFGGGGTKLEIK
116	7-5B3	heavy chain	QVQLKESGPGLVAPSQSL SITCTVSGFSLTSYGVHWVRQPPGKGLEW LGAIWAGGSINYN SALMSRLSISKDNSKSQVFLKMNSLQTDDTAMY YCARGPRYDRAMDYWGQGTSTVTVSS
117	7-5B3	light chain	DILLTQSPAILSVSPGERVSFSCRASQSIGTSIHWYQQRNNGSPRLLIK YASESISGIPSRFSGSGTDFTLNINSVESEDIADYYCQQSNSWPTYT FGGGGTKLEIK
118	7-5C3	heavy chain	EVKLLES GGGLVQPEGSLKLSAASGFDFSRYWMSWVRQAPGKGL EWIGEINLDSSTINYAPSLEDKFIISRDNASTLYLQMSKVRSEDSALY YCAREFYGGSYLDYWGQGTTLTVSS
119	7-5C3	light chain	DIQMTQSPASLSVSVGETVTITCRASDNIYNNLAWYQQKQKSPQLL VYVATNLADGVPSRFSGSGGTQYSLKIDSLQSEDFGTYYCQHFHW TPWTFGGGGTKLEIK
110	7-5C4	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFEYTIYWKQSHGKSLEW IGGINPNNGDTIYNQNFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARD DYGGIAYWGQGTTLTVSA
111	7-5C4	light chain	DIVMSQSPSSLA VSVGEKVTMSCKSSQSLLYSSNQKNYLAWFQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYY CQYYRYPLTFGAGTKLELK
120	7-5D1	heavy chain	QVQLKQSGPGLVQPSQSL SITCTVSGFSLSSYGVHWVRQSPGKGLEW LGVIWSSGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQSNDAIYYC ARGWFSYYFDYWGQGSTLT VSS
121	7-5D1	light chain	DMQMTQSPASLSVSVGETVTITCRASENIYSNLAWYQQKQKSPQL LVYAATNLADGVPSRFSGSGGTQYSLKINSLQSEDFGSYYCQHFHW GNVWTFGGGGTKLEIK
122	7-5D4	heavy chain	QVQLQQSGAELVKPGASVKMSCKASGYTFSSYSVHWVKQTPGQGL EWIGAIYPNGDTSYNQNFKAATLTADESSSTAYMQLRSLTSEDSA VYFCARDGYPYALDYWGQGTSTVTVSS
123	7-5D4	light chain	DIVMTQSQKFMSTSVGDRVSVTCKASQNVDTNVAWYQQKPGQSPK PLIYSASYRYS GVPDRFTGGSGTDFTLTISNVQSEDLAEYFCQQYHR YPWTFGGGGTKLEIK
118	7-5E2	heavy chain	EVKLLES GGGLVQPEGSLKLSAASGFDFSRYWMSWVRQAPGKGL EWIGEINLDSSTINYAPSLEDKFIISRDNASTLYLQMSKVRSEDSALY YCAREFYGGSYLDYWGQGTTLTVSS
119	7-5E2	light chain	DIQMTQSPASLSVSVGETVTITCRASDNIYNNLAWYQQKQKSPQLL VYVATNLADGVPSRFSGSGGTQYSLKIDSLQSEDFGTYYCQHFHW TPWTFGGGGTKLEIK

110	7-5F1	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTIYWVKQSHGKSLEW IGGINPNNGDTIYNQNFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARDYGGIAYWGQGLVTVSA
111	7-5F1	light chain	DIVMSQSPSSLA VSVGEKVTMSCKSSQSLLYSSNQKNYLAWFQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYY CQQYYRYPLTFGAGTKLELK
124	7-5G1	heavy chain	QVQLQQSGPELVKPGALVKISCKASGYTFTSYDISWVKQRPGQGLE WIGWIYPGDGSTEYNEKFKAKATLTADKSSSTAYMQLSSLTSENSA VYFCARRATTPTGYALDYWGQGTSTVTVSS
125	7-5G1	light chain	DIQMTQSPASLSVSVGETVTITCRASENIYSNLAWYQQKHGKSPQLL VYGAINLADGVPSRFTGSGSDTQYSLKINSLSQSEDFGNYYCQHFWDI PWTFGGGKLESK
110	7-5H3	heavy chain	EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTIYWVKQSHGKSLEW IGGINPNNGDTIYNQNFKGKATLTVDKSSSTAYMELRSLTSEDSAVY YCARDYGGIAYWGQGLVTVSA
111	7-5H3	light chain	DIVMSQSPSSLA VSVGEKVTMSCKSSQSLLYSSNQKNYLAWFQQKP GQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYY CQQYYRYPLTFGAGTKLELK

This disclosure also includes the following CDR sequences:

mAb clone	heavy chain			light chain		
	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
3C4, 3E4	GFNIKDTY (SEQ ID NO:126)	IDPASGKT (SEQ ID NO:134)	CASGYDVNYELDYW (SEQ ID NO:146)	QNIN VW (SEQ ID NO:161)	KAS	CQQGQSYPTF (SEQ ID NO:174)
3G1	GFSLSYSG (SEQ ID NO:127)	IWSGGST (SEQ ID NO:135)	CAKYRYDSFAYW (SEQ ID NO:147)	KSVSTSGYSY (SEQ ID NO:162)	LAS	CQHSRELPYTF (SEQ ID NO:175)
3A6	GFSLSISYSG (SEQ ID NO:128)	IWAGGST (SEQ ID NO:136)	CGRDYGILLIDYW (SEQ ID NO:148)	SSVSY (SEQ ID NO:163)	DTS	CFQGGSGYPFTF (SEQ ID NO:176)
3B3	GYSITS DYA (SEQ ID NO:129)	ISYSGTT (SEQ ID NO:137)	CAGRRGAYYGNYEEDY W (SEQ ID NO:149)	SIVNY (SEQ ID NO:164)	DTS	CQQWSSYPYTF (SEQ ID NO:177)
3D7	GFNIKDTY (SEQ ID NO:126)	IDPANGNS (SEQ ID NO:138)	CARSYYDYDGGGCFDY W (SEQ ID NO:150)	QSVSND (SEQ ID NO:165)	YAS	CQQGYSSPLTF (SEQ ID NO:178)
3E5	GYSFTGYF (SEQ ID NO:130)	INPYNGDT (SEQ ID NO:139)	CGLRTYW (SEQ ID NO:151)	QDINN (SEQ ID NO:166)	HGT	CVQSVQFPYTF (SEQ ID NO:179)

3B4	GFNIKDTY (SEQ ID NO:126)	IDPANGDT (SEQ ID NO:140)	CARSYYYGTTSWFASW (SEQ ID NO:152)	EDIYNH (SEQ ID NO:167)	GAP	CQQYWSTPYTF (SEQ ID NO:180)
3D1	GFNIKDTY (SEQ ID NO:126)	IDPANGNT (SEQ ID NO:141)	CARWDFGNVVDYAMD YW (SEQ ID NO:153)	QDLYSF (SEQ ID NO:168)	RAN	CLQYDAFPWTF (SEQ ID NO:181)
3A2	GFSLSTSGMG (SEQ ID NO:131)	IYWDDDK (SEQ ID NO:142)	CARRGPLITTDGTFDVW (SEQ ID NO:154)	QDVGTS (SEQ ID NO:169)	WAS	CQQYSSYPYTF (SEQ ID NO:182)
3C6	GFSLSTSGMG (SEQ ID NO:131)	IYWDDDK (SEQ ID NO:142)	CARRGPLITTDGTFDVW (SEQ ID NO:154)	QDVDTA (SEQ ID NO:170)	WAS	CQQYSSYPYTF (SEQ ID NO:182)
3H5	GYSFTGYF (SEQ ID NO:130)	INPYNGDT (SEQ ID NO:139)	CGRGLYGKGYYYAMD YW (SEQ ID NO:155)	QSLLYSSNQK NY (SEQ ID NO:171)	WAS	CQQYSSYPWTF (SEQ ID NO:183)
3G7	GYSFTSYW (SEQ ID NO:132)	IHPDSET (SEQ ID NO:143)	CARSSGYDWYFDVW (SEQ ID NO:156)	ESVDSYGNSF (SEQ ID NO:172)	RAS	CQQSNEDPWTF (SEQ ID NO:184)
3A7	GFSLSTSGMG (SEQ ID NO:131)	IYWDDDK (SEQ ID NO:142)	CARRGPLITTDGTFDVW (SEQ ID NO:154)	QDVGTS (SEQ ID NO:169)	WAS	CQQYSSYPYTF (SEQ ID NO:182)
3C2	GYAFTSYW (SEQ ID NO:133)	IYPGDGAT (SEQ ID NO:144)	CARSGGYDWYFDVW (SEQ ID NO:157)	ETVDSYGNSF (SEQ ID NO:173)	RAS	CQQSNEDPWTF (SEQ ID NO:184)
3D5	GFNIKDTY (SEQ ID NO:126)	IDPANGNT (SEQ ID NO:141)	CTRYYYGSSGFFDVW (SEQ ID NO:158)	QSVSND (SEQ ID NO:165)	YAS	CQQDYSSPTF (SEQ ID NO:185)
3F6	GFNIKDTY (SEQ ID NO:126)	IDPASGNT (SEQ ID NO:145)	CARSYYTYDGFFDVW (SEQ ID NO:159)	QSVSND (SEQ ID NO:165)	YAS	CQQDYSSPTF (SEQ ID NO:186)
9F2	GFNIKDTY (SEQ ID NO:126)	IDPANGNT (SEQ ID NO:141)	CARTYYYGSSYEAMDY W (SEQ ID NO:160)	QSVSND (SEQ ID NO:165)	YAS	CQQDYSSPTF (SEQ ID NO:185)

Antibodies 3A2 and 3A7 (and probably others in this panel) bind an epitope located in the S1 RBD with high affinity. Antibodies 3G7, 3F1, and 3H6 likely neutralize viral infectivity based on targeting of a different epitope.

In vitro RBD/hACE2 binding enhancement, and the ability to stain virus in heat-treated histological samples but not heat-inactivated virus, as well as binning and sequence data tell us that mAbs 3D2 and 3D7 bind another epitope that may only be exposed after extreme degradation and that might serve as target to be avoided due to potential antibody-dependent enhancement of infectivity.

An important embodiment of this IP is the creation of “cooperative pairs” of antibodies, defined as those that can simultaneously bind the same SARS-CoV-2 spike protein with high affinity. These pairs are especially useful for diagnostic detection of the virus, because they will not interfere with each other in sandwich-type assays. An example of these pairs are 3A7 and 3D7; 3A7 and 3F2.

1. Human and Humanized Antibodies

In some embodiments, the antibodies are humanized antibodies. Many non-human antibodies (*e.g.*, those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies in the methods serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

Transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J(H)) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge.

Optionally, the antibodies are generated in other species and “humanized” for administration in humans. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also contain residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will contain substantially all of at least one, and typically two, variable domains, in which all or substantially all, of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody

optimally also will contain at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule. Humanization can be essentially performed by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, a humanized form of a nonhuman antibody (or a fragment thereof) is a chimeric antibody or fragment, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important in order to reduce antigenicity. According to the “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies.

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so

that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

The antibody can be bound to a substrate or labeled with a detectable moiety or both bound and labeled. The detectable moieties contemplated with the present compositions include fluorescent, enzymatic and radioactive markers.

2. Single-Chain Antibodies

In some embodiments, the antibodies are single-chain antibodies. Methods for the production of single-chain antibodies are well known to those of skill in the art. A single chain antibody is created by fusing together the variable domains of the heavy and light chains using a short peptide linker, thereby reconstituting an antigen binding site on a single molecule. Single-chain antibody variable fragments (scFvs) in which the C-terminus of one variable domain is tethered to the N-terminus of the other variable domain via a 15 to 25 amino acid peptide or linker have been developed without significantly disrupting antigen binding or specificity of the binding. The linker is chosen to permit the heavy chain and light chain to bind together in their proper conformational orientation. These Fvs lack the constant regions (Fc) present in the heavy and light chains of the native antibody.

3. Monovalent Antibodies

In some embodiments, the antibodies are monovalent antibodies. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment, called the $F(ab')_2$ fragment, that has two antigen combining sites and is still capable of cross-linking antigen.

The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain domain including one or more cysteines from the antibody hinge region. The $F(ab')_2$ fragment is a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Antibody fragments originally were

produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

4. Hybrid Antibodies

In some embodiments, the antibodies are hybrid antibodies. In hybrid antibodies, one heavy and light chain pair is homologous to that found in an antibody raised against one epitope, while the other heavy and light chain pair is homologous to a pair found in an antibody raised against another epitope. This results in the property of multi-functional valency, *i.e.*, ability to bind at least two different epitopes simultaneously. Such hybrids can be formed by fusion of hybridomas producing the respective component antibodies, or by recombinant techniques. Such hybrids may, of course, also be formed using chimeric chains.

5. Conjugates or Fusions of Antibody Fragments

In some embodiments, the antibodies are conjugates or fusions of antibody fragments. The targeting function of the antibody can be used therapeutically by coupling the antibody or a fragment thereof with a therapeutic agent. Such coupling of the antibody or fragment (*e.g.*, at least a portion of an immunoglobulin constant region (Fc)) with the therapeutic agent can be achieved by making an immunoconjugate or by making a fusion protein, comprising the antibody or antibody fragment and the therapeutic agent.

Such coupling of the antibody or fragment with the therapeutic agent can be achieved by making an immunoconjugate or by making a fusion protein, or by linking the antibody or fragment to a nucleic acid such as an siRNA, comprising the antibody or antibody fragment and the therapeutic agent.

In some embodiments, the antibody is modified to alter its half-life. In some embodiments, it is desirable to increase the half-life of the antibody so that it is present in the circulation or at the site of treatment for longer periods of time. For example, it may be desirable to maintain titers of the antibody in the circulation or in the location to be treated for extended periods of time. Antibodies can be engineered with Fc variants that extend half-life, *e.g.*, using Xtend™ antibody half-life prolongation technology (Xencor, Monrovia, CA). In other embodiments, the half-life of the anti-DNA antibody is decreased to reduce potential side effects. The conjugates disclosed can be used for modifying a given biological response. The drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

B. Pharmaceutical Compositions

Pharmaceutical compositions including the disclosed antibodies or antigen binding fragments are provided. Pharmaceutical compositions containing the antibodies or antigen binding fragments can be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

In some *in vivo* approaches, the compositions disclosed herein are administered to a subject in a therapeutically effective amount. As used herein the term “effective amount” or “therapeutically effective amount” means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (*e.g.*, age, immune system health, etc.), the disease, and the treatment being affected.

For the disclosed antibodies or antigen binding fragments, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age, and general health of the recipient, will be able to ascertain proper dosing. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. For the disclosed antibodies or antigen binding fragments, generally dosage levels of 0.001 to 20 mg/kg of body weight daily are administered to mammals. Generally, for intravenous injection or infusion, dosage may be lower.

In certain embodiments, the antibodies or antigen binding fragments is administered locally, for example by injection directly into a site to be treated. Typically, the injection causes an increased localized concentration of the antibodies or antigen binding fragments composition which is greater than that which can be achieved by systemic administration. The antibodies or antigen binding fragments compositions can be combined with a matrix as described above to assist in creating an increased localized concentration of the polypeptide compositions by reducing the passive diffusion of the polypeptides out of the site to be treated.

1. Formulations for Parenteral Administration

In some embodiments, compositions disclosed herein, including those containing peptides and polypeptides, are administered in an aqueous solution, by parenteral injection.

The formulation may also be in the form of a suspension or emulsion. In general, pharmaceutical compositions are provided including effective amounts of a peptide or polypeptide, and optionally include pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions optionally include one or more for the following: diluents, sterile water, buffered saline of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (*e.g.*, TWEEN 20 (polysorbate-20), TWEEN 80 (polysorbate-80)), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), and preservatives (*e.g.*, Thimersol, benzyl alcohol) and bulking substances (*e.g.*, lactose, mannitol). Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. The formulations may be lyophilized and redissolved/resuspended immediately before use. The formulation may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions.

2. Formulations for Topical Administration

The disclosed antibodies or antigen binding fragments can be applied topically. Topical administration does not work well for most peptide formulations, although it can be effective especially if applied to the lungs, nasal, oral (sublingual, buccal), vaginal, or rectal mucosa.

Compositions can be delivered to the lungs while inhaling and traverse across the lung epithelial lining to the blood stream when delivered either as an aerosol or spray dried particles having an aerodynamic diameter of less than about 5 microns.

A wide range of mechanical devices designed for pulmonary delivery of therapeutic products can be used, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). Nektar, Alkermes and Mannkind all have inhalable insulin powder preparations approved or in clinical trials where the technology could be applied to the formulations described herein.

Formulations for administration to the mucosa will typically be spray dried drug particles, which may be incorporated into a tablet, gel, capsule, suspension or emulsion. Standard pharmaceutical excipients are available from any formulator.

Transdermal formulations may also be prepared. These will typically be ointments, lotions, sprays, or patches, all of which can be prepared using standard technology. Transdermal formulations may require the inclusion of penetration enhancers.

3. Controlled Delivery Polymeric Matrices

The antibodies or antigen binding fragments disclosed herein can also be administered in controlled release formulations. Controlled release polymeric devices can be made for long term release systemically following implantation of a polymeric device (rod, cylinder, film, disk) or injection (microparticles). The matrix can be in the form of microparticles such as microspheres, where the agent is dispersed within a solid polymeric matrix or microcapsules, where the core is of a different material than the polymeric shell, and the peptide is dispersed or suspended in the core, which may be liquid or solid in nature. Unless specifically defined herein, microparticles, microspheres, and microcapsules are used interchangeably. Alternatively, the polymer may be cast as a thin slab or film, ranging from nanometers to four centimeters, a powder produced by grinding or other standard techniques, or even a gel such as a hydrogel.

Either non-biodegradable or biodegradable matrices can be used for delivery of fusion polypeptides or nucleic acids encoding the fusion polypeptides, although in some embodiments biodegradable matrices are preferred. These may be natural or synthetic polymers, although synthetic polymers are preferred in some embodiments due to the better characterization of degradation and release profiles. The polymer is selected based on the period over which release is desired. In some cases linear release may be most useful, although in others a pulse release or “bulk release” may provide more effective results. The polymer may be in the form of a hydrogel (typically in absorbing up to about 90% by weight of water), and can optionally be crosslinked with multivalent ions or polymers.

The matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art. Bioerodible microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, *J. Controlled Release*, 5:13-22 (1987); Mathiowitz, et al., *Reactive Polymers*, 6:275-283 (1987); and Mathiowitz, et al., *J. Appl. Polymer Sci.*, 35:755-774 (1988).

The devices can be formulated for local release to treat the area of implantation or injection – which will typically deliver a dosage that is much less than the dosage for treatment of an entire body – or systemic delivery. These can be implanted or injected subcutaneously, into the muscle, fat, or swallowed.

III. Methods of Manufacture

A. Methods of Making Antibodies

The disclosed antibodies can be generated in cell culture, in phage, or in various animals, including but not limited to cows, rabbits, goats, mice, rats, hamsters, guinea pigs, sheep, dogs, cats, monkeys, chimpanzees, and apes. Therefore, in one embodiment, an antibody is a mammalian antibody. Phage techniques can be used to isolate an initial antibody or to generate variants with altered specificity or avidity characteristics. Such techniques are routine and well known in the art. In one embodiment, the antibody is produced by recombinant means known in the art. For example, a recombinant antibody can be produced by transfecting a host cell with a vector comprising a DNA sequence encoding the antibody. One or more vectors can be used to transfect the DNA sequence expressing at least one VL and one VH region in the host cell. Exemplary descriptions of recombinant means of antibody generation and production include Delves, Antibody Production: Essential Techniques (Wiley, 1997); Shephard, et al., Monoclonal Antibodies (Oxford University Press, 2000); Goding, Monoclonal Antibodies: Principles And Practice (Academic Press, 1993); Current Protocols In Immunology (John Wiley & Sons, most recent edition).

The disclosed antibodies can be modified by recombinant means to increase greater efficacy of the antibody in mediating the desired function. Thus, it is within the scope of the invention that antibodies can be modified by substitutions using recombinant means. Typically, the substitutions will be conservative substitutions. For example, at least one amino acid in the constant region of the antibody can be replaced with a different residue. *See, e.g.*, U.S. Pat. No. 5,624,821, U.S. Pat. No. 6,194,551, Application No. WO 9958572; and Angal, et al., *Mol. Immunol.* 30:105-08 (1993). The modification in amino acids includes deletions, additions, and substitutions of amino acids. In some cases, such changes are made to reduce undesired activities, *e.g.*, complement-dependent cytotoxicity. Frequently, the antibodies are labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. These antibodies can be screened for binding to proteins, polypeptides, or fusion proteins of SARS-CoV-2. *See, e.g.*, Antibody Engineering: A Practical Approach (Oxford University Press, 1996).

For example, suitable antibodies with the desired biologic activities can be identified using *in vitro* assays including but not limited to: proliferation, migration, adhesion, soft agar growth, angiogenesis, cell-cell communication, apoptosis, transport, signal transduction, and *in vivo* assays such as the inhibition of tumor growth. The antibodies provided herein can also

be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can be screened for the ability to bind to the specific antigen without inhibiting the receptor-binding or biological activity of the antigen. As neutralizing antibodies, the antibodies can be useful in competitive binding assays.

Antibodies that can be used in the disclosed compositions and methods include whole immunoglobulin (*i.e.*, an intact antibody) of any class, fragments thereof, and synthetic proteins containing at least the antigen binding variable domain of an antibody. The variable domains differ in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not usually evenly distributed through the variable domains of antibodies. It is typically concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies.

Also disclosed are fragments of antibodies which have bioactivity. The fragments, whether attached to other sequences or not, include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment.

Techniques can also be adapted for the production of single-chain antibodies specific to an antigenic peptide. Methods for the production of single-chain antibodies are well known to those of skill in the art. A single chain antibody can be created by fusing together the variable domains of the heavy and light chains using a short peptide linker, thereby reconstituting an antigen binding site on a single molecule. Single-chain antibody variable fragments (scFvs) in which the C-terminus of one variable domain is tethered to the N-terminus of the other variable domain via a 15 to 25 amino acid peptide or linker have been developed without significantly disrupting antigen binding or specificity of the binding. The linker is chosen to permit the heavy chain and light chain to bind together in their proper conformational orientation.

Divalent single-chain variable fragments (di-scFvs) can be engineered by linking two scFvs. This can be done by producing a single peptide chain with two VH and two VL regions,

yielding tandem scFvs. ScFvs can also be designed with linker peptides that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Diabodies have been shown to have dissociation constants up to 40-fold lower than corresponding scFvs, meaning that they have a much higher affinity to their target. Still shorter linkers (one or two amino acids) lead to the formation of trimers (triabodies or tribodies). Tetrabodies have also been produced. They exhibit an even higher affinity to their targets than diabodies.

A monoclonal antibody is obtained from a substantially homogeneous population of antibodies, *i.e.*, the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules. Monoclonal antibodies include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, as long as they exhibit the desired antagonistic activity.

Monoclonal antibodies can be made using any procedure which produces monoclonal antibodies. In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

Antibodies may also be made by recombinant DNA methods. DNA encoding the disclosed antibodies can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques.

Methods of making antibodies using protein chemistry are also known in the art. One method of producing proteins comprising the antibodies is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (tert -butyloxycarbonyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the antibody, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved

from its synthesis resin whereas the other fragment of an antibody can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form an antibody, or fragment thereof. Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described above. Once isolated, these independent peptides or polypeptides may be linked to form an antibody or antigen binding fragment thereof via similar peptide condensation reactions.

For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains. Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two-step chemical reaction. The first step is the chemoselective reaction of an unprotected synthetic peptide-alpha-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site.

IV. Method of Use

The disclosed antibodies and antigen binding fragments bind to SARS-CoV-2 spike protein and are useful in the detection, diagnosis, and treatment during active COVID-19 infection. In one embodiment, the antibodies are SARS-CoV-2-specific and can be used to detect and diagnose infection with SARS-CoV-2.

In another embodiment, the antibodies are strongly reactive with both SARS-CoV-1 and SARS-CoV-2 and can be used to detect and diagnose infection with SARS-CoV-1 and SARS-CoV-2.

The antibodies can inhibit, reduce or prevent the binding of coronaviruses, including but not limited to SARS-CoV-2 spike protein to cell surface proteins in a subject. In some embodiments, the disclosed antibodies and antigen-binding fragments thereof reduce or inhibit the growth or proliferation of or induce the death of a cell infected with a coronavirus to which they bind.

A. Treating coronavirus

One embodiment provides a method for treating coronavirus infection in a subject in need thereof by administering to the subject any one of the disclosed antibody or antigen-

binding fragments thereof in an amount effective to treat the coronavirus infection. The infection can be SARS-CoV-1 or SARS-CoV-2.

The infection can be acute or chronic. An acute infection is typically an infection of short duration. During an acute infection, immune cells begin expressing immunomodulatory receptors. Accordingly, in some embodiments, the method includes increasing an immune stimulatory response against an acute infection.

Another embodiment provides a method for inhibiting the production of additional viral particles in a SARS-CoV-2-infected cell, wherein the growth of the SARS-CoV-2 infected cell is at least in part dependent upon the expression of a SARS-CoV-2 spike protein (wherein the SARS-CoV-2 spike protein may be expressed either within the infected cell itself or a cell that produces polypeptide(s) that have a growth potentiating effect on the infected cells), by contacting the SARS-CoV-2 spike protein with an antibody that binds to the SARS-CoV-2 spike protein, thereby antagonizing the growth-potentiating activity of the SARS-CoV-2 spike protein and, in turn, inhibiting the growth of the infected cell. Preferably the growth of the infected cell is completely inhibited. More preferably, binding of the antibody to the SARS-CoV-2 spike protein induces the death of the infected cell.

In some embodiments, the disclosed antibodies are used to prevent coronavirus infection in subjects that are at a high risk for coronavirus infection. Exemplary at-risk subjects include but are not limited to immunocompromised individuals, children, the elderly, or subjects with diabetes, obesity, asthma, heart disease, lung disease, cancer, or coronary disease. In one embodiment, high-risk subjects are prophylactically administered the disclosed antibodies in an amount effective to prevent coronavirus infection should they be exposed.

B. Detecting and diagnosing coronavirus

Another embodiment provides a method of determining the presence of a SARS-CoV-2 spike protein in a sample suspected of containing the SARS-CoV-2 spike protein, by exposing the sample to an antibody that binds to the SARS-CoV-2 spike protein and determining binding of the antibody to the SARS-CoV-2 spike protein in the sample, wherein the presence of such binding is indicative of the presence of the SARS-CoV-2 spike protein in the sample. Optionally, the sample may contain cells (which may be fibroblasts, epithelial cells, mucosal cells, and the like) suspected of expressing the SARS-CoV-2 spike protein. The antibody employed in these methods may optionally be detectably labeled, attached to a solid support, or the like.

This disclosure is further directed to mAbs and related binding proteins that bind specifically to the spike protein of the SARS-CoV-2 virus and to the use of those mAbs and

related binding proteins in epitope blocking ELISAs. Thus, one embodiment provides methods for detecting SARS-CoV-2 virus spike protein in a biological sample by contacting the sample with an antigen which contains an epitope of a spike protein and determining whether an antibody in the sample binds to the epitope. Preferably the binding determination is made in an epitope blocking ELISA. These methods thereby provide highly sensitive and specific epitope blocking ELISAs (EB ELISA) for detecting SARS-CoV-2 spike subtypes.

A further embodiment provides a method of diagnosing the presence of a SARS-CoV-2 infection in a mammal, by detecting the level of expression of a gene encoding a SARS-CoV-2 spike protein in a test sample of tissue cells obtained from the mammal, wherein detection of expression of the SARS-CoV-2 spike protein in the test sample is indicative of the presence of SARS-CoV-2 infection in the mammal from which the test sample was obtained.

Another embodiment provides a method of binding an antibody to a cell that expresses a SARS-CoV-2 spike protein, by contacting a cell that expresses a SARS-CoV-2 spike protein with the antibody of this disclosure under conditions which are suitable for binding of the antibody to the SARS-CoV-2 spike protein and allowing binding therebetween. In preferred embodiments, the antibody is labeled with a molecule or compound that is useful for qualitatively and/or quantitatively determining the location and/or amount of binding of the antibody to the cell.

Other embodiments of this disclosure include the use of a SARS-CoV-2 spike protein, a nucleic acid encoding a SARS-CoV-2 spike protein, or a vector or host cell comprising that nucleic acid, or an anti-SARS-CoV-2 spike protein antibody in the preparation of a medicament useful for (i) the therapeutic treatment or diagnostic detection of a SARS-CoV-2 infection, or (ii) the therapeutic treatment or prevention of a SARS-CoV-2 infection-related disorder.

Yet another embodiment provides a method of therapeutically treating a viral infection in a mammal, wherein the infection is at least in part dependent upon the expression of a SARS-CoV-2 spike protein, by administering to the mammal a therapeutically effective amount of an antibody or antigen-binding fragment thereof that binds to the SARS-CoV-2 spike protein, thereby antagonizing the activity of the SARS-CoV-2 spike protein and resulting in the effective therapeutic treatment of the infection in the mammal. Optionally, the antibody is a monoclonal antibody, antibody fragment, chimeric antibody, humanized antibody, or single-chain antibody. Antibodies employed in these methods may optionally be conjugated to a growth inhibitory agent or cytotoxic agent such as a toxin, or the like. The antibodies employed in the methods of this disclosure may optionally be produced in CHO cells or bacterial cells.

One embodiment provides a bispecific antibody that binds to SARS-CoV-2 spike protein and a cell-surface protein of an immune cell. The immune cell can be a T cell, for example a cytotoxic T cell, a macrophage, a dendritic cell, or a neutrophil.

Immunoassays were developed using optimal mAb pairings with high sensitivity for spike protein, viral culture supernatant, and infected tissue samples. Thus, the mAbs of this disclosure are capable of recognizing spike protein in a variety of environments with potential uses in immunoassay development and as immunodiagnostic reagents for clinical sample and tissue confirmation of SARS-CoV-2.

In another embodiment, the disclosed antibodies and antigen-binding fragments thereof are detectably labeled, attached to a solid support, or the like, such as a lateral flow assay device which provides for point-of-care detection of SARS-CoV-2 and/or diagnosis.

V. Combination Therapies

The antibodies or antigen binding fragments disclosed herein can be administered to a subject in need thereof alone or in combination with one or more additional therapeutic agents. In some embodiments, the antibodies or antigen binding fragments and the additional therapeutic agent are administered separately, but simultaneously. The antibodies or antigen binding fragments and the additional therapeutic agent can also be administered as part of the same composition. In other embodiments, the antibodies or antigen binding fragments and the second therapeutic agent are administered separately and at different times, but as part of the same treatment regime.

The subject can be administered a first therapeutic agent 1, 2, 3, 4, 5, 6, or more hours, or 1, 2, 3, 4, 5, 6, 7, or more days before administration of a second therapeutic agent. In some embodiments, the subject can be administered one or more doses of the first agent every 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35, or 48 days prior to a first administration of second agent. The disclosed antibodies or antigen binding fragments can be the first or the second therapeutic agent.

The antibodies or antigen binding fragments and the additional therapeutic agent can be administered as part of a therapeutic regimen. For example, if a first therapeutic agent can be administered to a subject every fourth day, the second therapeutic agent can be administered on the first, second, third, or fourth day, or combinations thereof. The first therapeutic agent or second therapeutic agent may be repeatedly administered throughout the entire treatment regimen.

Exemplary molecules include, but are not limited to, cytokines, chemotherapeutic agents, radionuclides, other immunotherapeutic, enzymes, antibiotics, antivirals (especially protease inhibitors alone or in combination with nucleosides for treatment of HIV or Hepatitis B or C), anti-parasites (helminths, protozoans), growth factors, growth inhibitors, hormones, hormone antagonists, antibodies and bioactive fragments thereof (including humanized, single chain, and chimeric antibodies), antigen and vaccine formulations (including adjuvants), peptide drugs, anti-inflammatories, ligands that bind to Toll-Like Receptors (including but not limited to CpG oligonucleotides) to activate the innate immune system, molecules that mobilize and optimize the adaptive immune system, other molecules that activate or up-regulate the action of cytotoxic T lymphocytes, natural killer cells and helper T-cells, and other molecules that deactivate or down-regulate suppressor or regulatory T-cells.

The additional therapeutic agents are selected based on the condition, disorder or disease to be treated. For example, the disclosed antibodies or antigen binding fragments can be co-administered with one or more additional agents that function to enhance or promote an immune response or reduce or inhibit an immune response.

VI. Kits

The disclosed antibodies and antigen binding fragments can be packaged in a hermetically sealed container, such as an ampoule or sachet, indicating the quantity. The agent can be supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, e.g., with water or saline to the appropriate concentration for administration to a subject. For example, the agent can be supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, or at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized agent can be stored at between 2 and 8°C in their original container and are typically administered within 12 hours, or within 6 hours, or within 5 hours, or within 3 hours, or within 1 hour after being reconstituted.

In an alternative embodiment, agent can be supplied in liquid form in a hermetically sealed container indicating the quantity and concentration. In some embodiments, the liquid form of the agent supplied in a hermetically sealed container including at least 1 mg/ml, or at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/ml, at least 25 mg/ml, at least 50 mg/ml, at least 100 mg/ml, at least 150 mg/ml, at least 200 mg/ml of the agent.

Pharmaceutical packs and kits including one or more containers filled with agent are also provided. Additionally, one or more other prophylactic or therapeutic agents useful for

the treatment of a disease can also be included in the pharmaceutical pack or kit. The pharmaceutical pack or kit can also include one or more containers filled with one or more of the ingredients of the disclosed pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Kits designed for the above-described methods are also provided. Embodiments typically include one or more of the disclosed antibodies and antigen binding fragments . In particular embodiments, a kit also includes one or more other prophylactic or therapeutic agents useful for the treatment of cancer, in one or more containers. In other embodiments, a kit also includes one or more anti-inflammatory agents useful for the treatment inflammatory and autoimmune diseases, in one or more containers.

EXAMPLES

Example 1. *SARS-CoV-2 Spike protein subunit vaccine strategy and humoral immune response in mice*

In order to target the key S1 RBD of SARS-CoV-2, three polypeptide variations of this motif were employed: a commercially available S1 domain (residues 1-685) with C-terminal hexahistidine tag (Sino Biological, designated His-S1), a commercial RBD sequence (residues 319-541) (Wang, Q.H. et al. *Cell* 2020, 181, 894-904). fused at the C-terminus with a mouse IgG1 Fc domain (Sino Biological, designated mFc-RBD), and the noncovalent complex of mFc-RBD with the PP7 bacteriophage virus-like particle (VLP) engineered to express two sequential Fc-binding Z-domains at 120 places on the VLP exterior surface (Zhao, L., et al. *ACS Nano* 2019, 13, 4443-4454). The last species provides a large (Fig. 1E) polyvalent display of RBD domains which could productively engage immune cells and stimulate affinity maturation. The use of Fc-tagged antigens is well known to increase antigen uptake and processing, (Levin, D. et al. *Trends Biotechnol.* 2015, 33, 27-34) including many examples with viral antigens (Liu, C. et al. *J. Clin. Invest.* 1996, 98, 2001-2007; He, Y. et al. *Biochemical and Biophysical Research Communications* 2004, 324, 773-781; Lu, L., et al. *J. Virol.* 2011, 85, 10542-10553; Loureiro, S. et al. *Journal of Virology* 2011, 85, 3010-3014; Konduru, K. et al., *The Journal of Immunology* 2013, 190, 205.218-205.218). While the virus-like particles used here are regarded as self-adjuvanting, (Bachmann, M.F. et al. *Nat. Rev. Immunol.* 2010, 10, 787-796; Mohsen, M.O. et al. *Vaccines* **2018**, 6, 37) the recombinant S1 and RBD proteins were usually augmented by TiterMax Gold emulsion adjuvant.

Three vaccination procedures with Balb/c mice (Fig. 1D) were employed and characterized by the use of only the recombinant His-S1 domain (Series 1), a combination of all three immunogens beginning with mFc-RBD (Series 2), and the VLP-displayed mFc-RBD with a final focusing boost of His-S1 (Series 3). Six-week old female Balb/c mice (n = 3 per group) were immunized with primary antigen and adjuvant on day 0 followed by boosts on days 14 and 27. Blood was collected for ELISA on days 0, 14, 30. The timing of boost injections (days 14 and 27) and harvesting of splenocytes (day 30) was considerably compressed from normal practice, and individual mice or pairs of mice were chosen for maximum immune response, rather than larger cohorts as one would use to determine reproducibility and mechanistic trends. Representative serum antibody titers, shown in Fig. 1F-1G, were found to be in a range indicating robust antibody development, as often observed in more classical hybridoma vaccine strategies requiring 6 weeks or more.

Example 2. *Characterization of anti-SARS-CoV-2 hybridoma clones and monoclonal antibodies.*

Following proof of anti-spike seroreactivity, 5-20 million splenocytes were harvested from each mouse on day 30 and subjected to myeloma fusion. The resulting hybridomas were distributed in 3D culture and selected by robotic probe based on high IgG secretion as evidenced by FITC signal from anti-IgG secondary antibody distributed through the culture medium (Fig. 2A). Series 3 mice (immunized and boosted with VLP-displayed mFc-RBD) provided relatively few high-secreting hybridomas. Supernatants from all of the selected clones were then screened by ELISA against recombinant His-S1 polypeptide and a SARS-CoV-2 spike ectodomain protein which exists primarily as a noncovalent trimer, thought to better represent the native structure of the viral spike (Fig. 1A) (Walls, A.C. et al, *Cell* 2020, 181, 281-292.e286; Wrapp, D et al. *Science* 2020, 367, 1260-1263).

As shown in Figs. 2B-2D, Series 2 (starting with mFc-RBD immunization) produced a far greater proportion of spike-binding antibodies than the other protocols, consistent with an overall superior immune response to the target antigen. In addition, Series 2 clones featured a significantly higher fraction of antibodies (38%) exhibiting strong binding to the plated ectodomain alone, with the rest binding approximately as well to both His-S1 and the ectodomain trimer. Only one of these (0.6%) selectively bound the His-tagged S1 protein. As none of the Series 2 immunization or boost injections contained the S2 subunit, these results suggested strong recognition of virus-relevant conformations or exposed epitopes in S1. The 157 spike-binding hybridomas from Series 2 were chosen to move forward.

The quantitative binding avidities of each of these 157 mAbs (reported here as the inverse of the observed adsorption constant, $1/K_{ads}$ to immobilized spike ectodomain) were determined by biolayer interferometry (BLI). From this panel, three antibodies (3H6, 3A7, 3G7) exhibited apparent dissociation constants of approximately 10 pM due to unusually slow off-rates. Thirty antibodies in total showed avidities by this assay of 6 nanomolar or better (Fig. 2E) and were taken forward for further characterization and functional testing. The majority of these antibodies were found to be IgG1 (k light chain). Overall, from an initial pool of more than 55 million B cells, we ultimately arrived at 37 clones with highly potent anti- SARS-CoV-2 characteristics, which were then produced in larger quantities for further testing.

The vast majority (33 out of 37) of these high-affinity antibodies bound both recombinant S1 subunit and ectodomain trimers, as determined by ELISA using plated antigen; only 3H6, 3G7, 3C3, and 3B1 showed a distinct preference for the ectodomain. Limits of detection for recombinant ectodomain by ELISA were determined by dilution of the spike

protein concentration used to coat the microtiter well plate, followed by washing and exposure to a fixed concentration (1 $\mu\text{g/mL}$) of purified monoclonal antibody. The data fitted well in each case to a standard adsorption titration curve, providing a sensitivity estimate designated as AC_{50} (half-maximal adsorbent concentration). The results were remarkably consistent, in which AC_{50} varied between 90 and 210 ng/mL for all but two mAbs (3C3, 3H5).

Example 3. Functional utility of SARS-CoV-2 mAbs.

The ability of the selected mAbs to inhibit or increase the binding of the SARS-CoV-2 RBD to the human ACE2 receptor was assessed with a direct split-luciferase reporter of the RBD-ACE2 interaction. Antibody-promoted ACE2 binding is associated with the important phenomenon of antibody-dependent enhancement (ADE) of coronavirus infectivity (Yang, Z. et al, *Proc. Natl. Acad. Sci. U.S.A.* 2005, 102, 797-801; Walls, A.C. et al. *Nat. Struct. Mol. Biol.* 2016, 23, 899–905; Wan, Y. et al. *J. Virol.* 2020, 94, e2015-2019). This is thought to result either from direct stabilization or conformational advantage conferred upon the S1 RBD by a targeting antibody, or by mAb Fc domain interaction with Fc γ receptors that assist viral entry; the assay used here is relevant to the former mechanism. Ten of 32 mAbs tested were found to inhibit RBD-hACE2 binding in dose-dependent fashion (Fig. 3A-3B), seven of these (3A2, 3F2, 3H2, 3B3, 3F5, 3A7, and 5B1) defined as potent (>80% inhibition). Nine mAbs with varying levels of RBD/hACE2 binding enhancement (3B4, 3C4, 3E4, 3B6, 3F6, 3G6, 3D7, 9B1 and 9F2) were also identified, and 16 with little or no effect on this interaction. By switching the RBD-luciferase antigen from SARS2 to SARS1, mAb cross-reactivity between the two coronaviruses was assessed, and it was discovered that most of the antibodies inhibited ACE2 receptor engagement by both SARS-CoV variants.

The ability of a subset of these antibodies to neutralize SARS-CoV-2 infectivity was assessed *in vitro* by two assays in which infectious SARS-CoV-2 was pre-mixed with varying concentrations of antibody, followed by passage in cultured mammalian cells. Initial screening was performed with a GFP reporter variant of the virus and Vero E6 cells (two days viral passage). An early patient isolate (SARS-CoV-2-WA P#4) was used and passaged for five days in cell culture. The clinical isolate proved to be far more sensitive to antibody, but the trends in the two assays were very similar. Eight antibodies were found to be strongly neutralizing under these conditions (3F5, 3B3, 3A7, 3A2, 3C2, 3H2, 3D3, 3F2), with neutralization titers ranging from 24 to 98 ng/mL in the second assay (Figure 3C). Antibody 3F5 proved uniquely active, far outperforming the others with the GFP reporter strain and being the most potent

(with 3B3) against the WA strain. Seven of the top eight antibodies (excepting only 3D3) also comprised the cohort that most strongly inhibited RBD-ACE2 binding (Fig. 3A). Seven antibodies were found to bind to a detectable degree to a synthetic biotinylated 15-mer peptide of the receptor binding motif covering amino acids 486-501, which contains four out of five key RBD-hACE2 contact residues (Fig. 3D) (Shang, J. et al, *Nature* **2020**, 581, 221-224; Wan, Y.; et al. *J. Virol.* **2020**, 94, e00127-00120). These data therefore suggest that neutralization is associated with binding of the SARS-CoV-2 RBD domain, as for other neutralizing human antibodies recently reported. (Liu, L.; et al., *bioRxiv* 2020)

An important diagnostic application of mAbs is their use as immunohistochemical staining reagents for the analysis of potentially infected tissue. Most such histological samples are subjected to severe treatment involving fixation (protein crosslinking or embedding in a matrix) and heating just prior to analysis to expose antigen at the sample surface. The epitopes available in these materials may bear little relationship to those present on the active virus, and so independent testing of mAb candidates is required. Among the high-affinity mAb panel, two were identified, 3D2 and 3D7, that preferentially stained SARS-CoV-2 viral particles in lung tissue from infected patients (Fig. 3E). Interestingly, these clones appear to have functional characteristics opposite to those desired of a therapeutic molecule: they exhibited no detectable neutralization activity and enhanced binding of spike protein to the ACE2 receptor.

Example 4. Epitope binding patterns

The relative binding sites of a randomly chosen subset of 14 antibodies were assessed by competitive ELISA against plated spike ectodomain, summarized in Figure 4. In this experiment, low signal is presumed to result from competitive binding to the same or allosterically related epitopes, as illustrated by the uniformly low values on the diagonal. The results cluster into four major groups, which correlate to a significant degree with functional performance. Thus, the strong neutralizers tested here are found exclusively in groups 1 and 3. Group 1 also contains tighter binders overall, with average $1/K_{ads} = 0.36$ nM, compared to an average of 3.2 nM for the other groups. Antibodies in groups 2 and 4 were uniformly activators of spike-ACE2 binding (Fig. 3A), whereas groups 1 and 3 were almost exclusively (except for 3G7) inhibitors of that interaction. These unbiased correlations strongly support the assumption that binding site determines function.

Example 5. Amino acid sequence similarities of selected clones

RNA isolated from hybridomas producing most of the selected antibodies were subjected to reverse transcription and high-throughput DNA sequencing, with results shown in Figure 5, grouped by sequence similarity of heavy and light chain variable regions. Two pairs of antibodies (3A2 and 3A7, 3C4 and 3E4) have identical sequences, consistent with their very similar measured functions. Most striking is the clustering of group 1 antibodies (3A2/3A7, 3C6) and the other potent neutralizers and ACE2 binding inhibitors (3B3, 3A6, 3G1, 3C2, which were not subjected to the cross-reactive binning shown in Fig. 4), and the separate clustering of ACE2 binding enhancers.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

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

We claim:

1. An antibody or antigen-binding fragment thereof comprising a heavy chain variable region and a light chain variable region selected from the group consisting of:

a) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:1, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:2;

b) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:3, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:4;

c) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:5, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:6;

d) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:7, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:8;

e) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:9, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:10;

f) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:11, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:12;

g) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:13, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:14;

h) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:15, and a light chain variable region

having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:16;

i) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:17, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:18;

j) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:19, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:20;

k) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:21, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:22;

l) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:23, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:4;

m) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:24, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:25;

n) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:26, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:27;

o) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:28, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:29;

p) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:30, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:31;

q) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:32, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:33;

r) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:34, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:35;

s) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:36, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:37;

t) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:38, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:39;

u) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:40, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:41;

v) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:42, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:43;

w) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:44, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:45;

x) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:46, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:47;

y) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:48, and light chain variable region having

at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:49;

z) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:50, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:51;

aa) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:52, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:53;

bb) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:54, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:55;

cc) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:56, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:57;

dd) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:58, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:59;

ee) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:60, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:61;

ff) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:62, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:63;

gg) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:64, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:65;

hh) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:66, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:67;

ii) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:68, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:69;

jj) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:70, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:71;

kk) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:72, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:73;

ll) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:74, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:75;

mm) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:76, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:77;

nn) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:78, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:79;

oo) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:80, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:81;

pp) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:82, and light chain variable region having

at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:83;

qq) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:84, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:85;

rr) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:86, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:87;

ss) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:88, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:89;

tt) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:90, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:91;

uu) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:92, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:93;

vv) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:94, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:95;

ww) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:96, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:97;

xx) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:98, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:99;

yy) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:100, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:101;

zz) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:102, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:103;

aaa) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:104, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:105;

bbb) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:106, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:107;

ccc) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:108, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:109;

ddd) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:110, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:111;

eee) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:112, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:113;

fff) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:114, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:115;

ggg) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:116, and a light chain variable region

having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:117;

hhh) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:118, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:119;

iii) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:120, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:121;

jjj) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:122, and light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:123; and

kkk) heavy chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:124, and a light chain variable region having at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or more sequence identity to SEQ ID NO:125.

2. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof is a monoclonal antibody or antigen-binding fragment thereof or a humanized antibody or an antigen-binding fragment thereof.

3. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof is conjugated to a solid support or resin.

4. A pharmaceutical composition comprising:

the antibody or antigen-binding fragment thereof of claim 1 in an amount effected to inhibit or reduce SARS-COV-2 spike protein bind to angiotensin converting enzyme 2 (ACE2) receptors under physiological conditions.

5. The pharmaceutical composition of claim 4, further comprising an excipient.

6. An immunoassay comprising the antibody or antigen-binding fragments thereof of claim 1.

7. The antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof is labeled with a detectable label.

8. The antibody or antigen-binding fragment thereof of claim 7, wherein the detectable label is selected from the group consisting of a fluorophore, quantum dot, magnetic particle, a dye, an enzyme, or a combination thereof.
9. The antibody of claim 1, wherein the antibody or antigen binding fragment thereof specifically binds to a SARS-CoV-2 spike protein or fragment thereof.
10. A method for treating coronavirus infection in a subject in need thereof of comprising:
administering the antibody or antigen-binding fragment thereof of any one of claims 1 or 10 to the subject in an amount effective to treat the coronavirus infection.
11. The method of claim 10, wherein the coronavirus is SARS-COV-2.
12. A method for detecting SARS-COV-2 in a sample, comprising:
contacting the sample with an antibody or antigen-binding fragment thereof according to claim 1;
detecting bound antibody or antigen-binding fragments thereof in the sample, wherein the presence of bound antibody or antigen-binding fragments thereof in the sample indicates the sample contains SARS-COV-2.
13. A method for diagnosing a subject with a SARS-COV-2 infection, comprising:
performing the method of claim 12 on a sample obtained from the subject, wherein the detection of bound antibody or antigen-binding fragments thereof in the sample indicates the subject has a SARS-COV-2 infection.
14. The method of claim 13, wherein the method further includes the step of diagnosing the subject with a SARS-COV-2 infection if bound antibody or antigen-binding fragments thereof are detected in the sample.
15. A kit comprising:
a container containing the antibody or antigen-binding fragment thereof of claim 1, and optionally, instructions for administering the antibody or antigen-binding fragment thereof.
16. A method for treating coronavirus in a subject in need thereof comprising administering an effective amount of the antibody or antigen binding fragment according to any one of claim 1.
17. The method of claim 16, wherein the one or more antibodies are monoclonal antibodies.
18. The method of claim 16, wherein the one or more antibodies are recombinant antibodies.

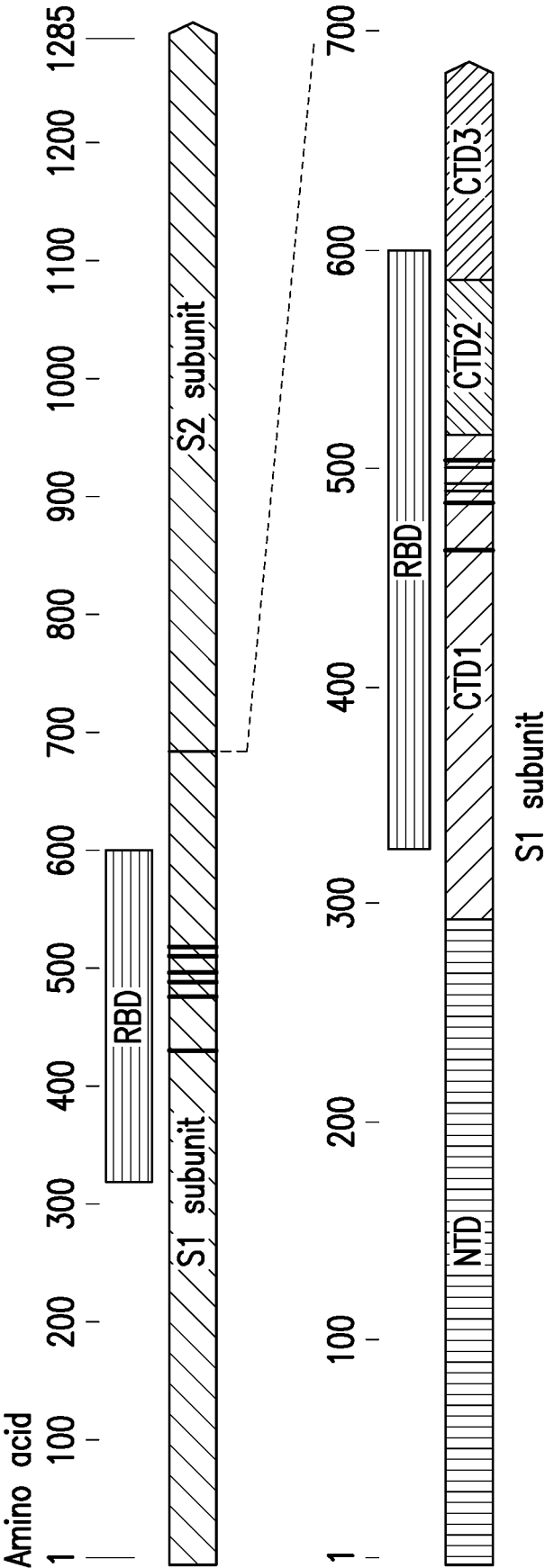
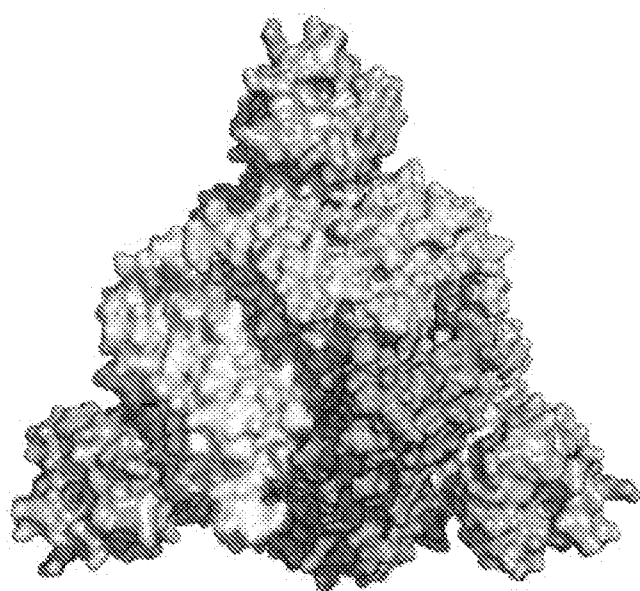


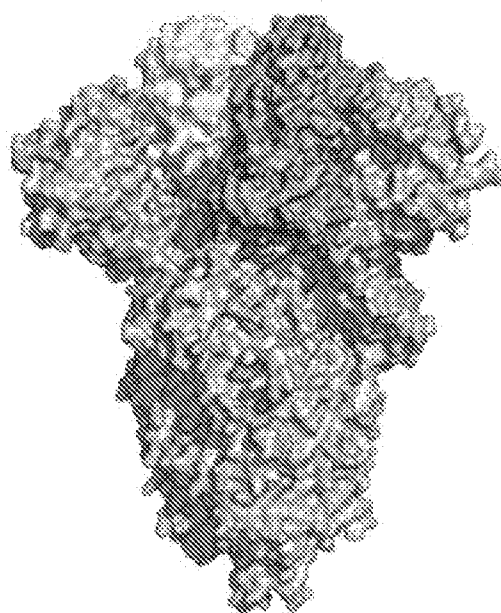
FIG.1A

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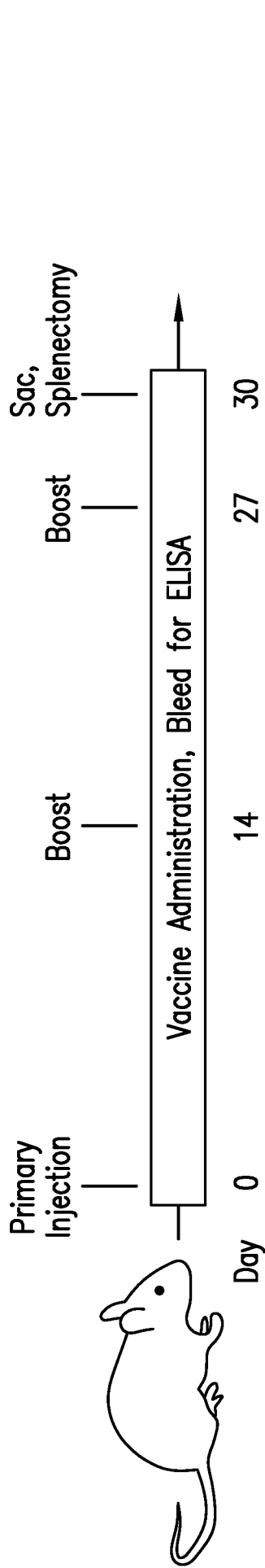
Top view

FIG.1B



Side view

FIG.1C



Series	Primary Immunization	First Boost	Final Boost
1	His-S1 + TMX (7.5 + ?? μ g)	His-S1 (15 μ g)	His-S1 + TMX (10 + ?? μ g)
2	mFc-RBD + TMX (7.5 + ?? μ g)	His-S1 (15 μ g)	mFc-RBD + PP7zz (10 + 10 μ g)
3	mFc-RBD + PP7zz (15 + 38 μ g)	mFc-RBD + PP7zz (15 + 15 μ g)	His-S1 + TMX (10 μ g + 10 μ g)

FIG.1D

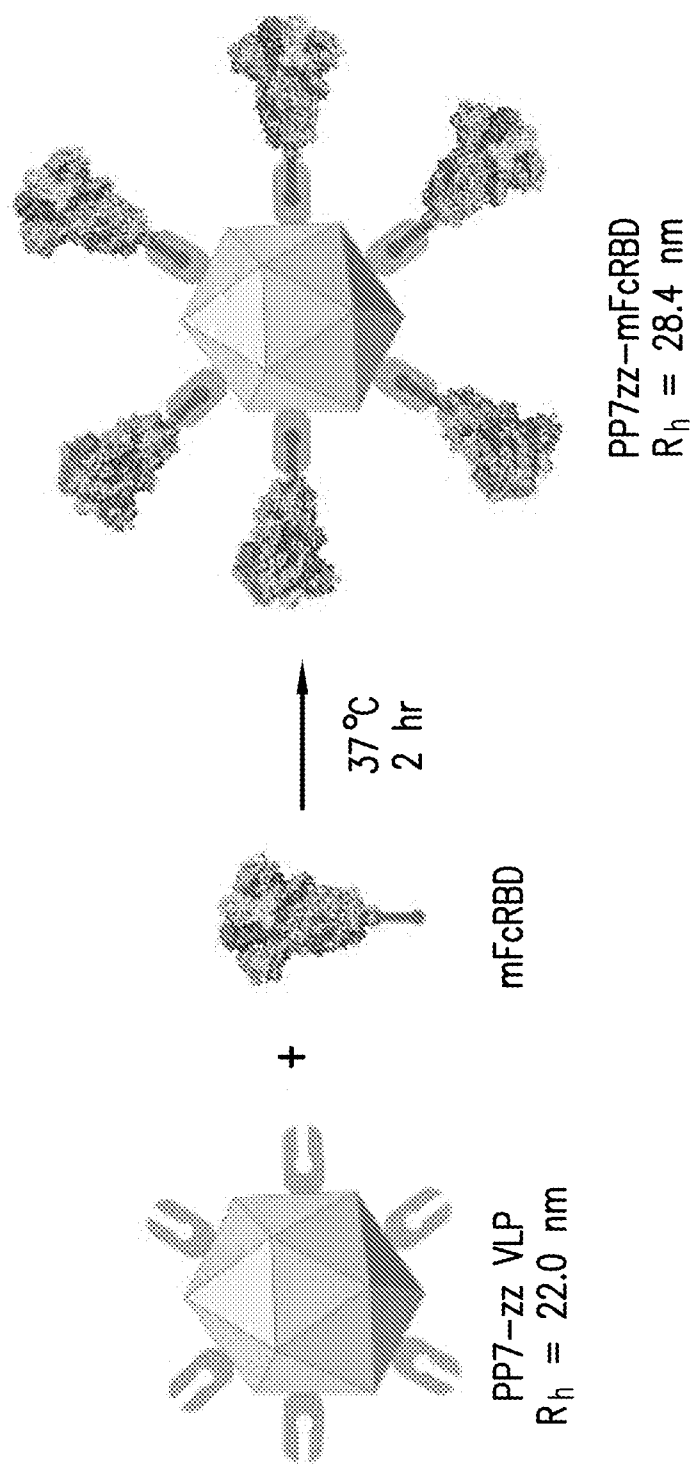


FIG.1E

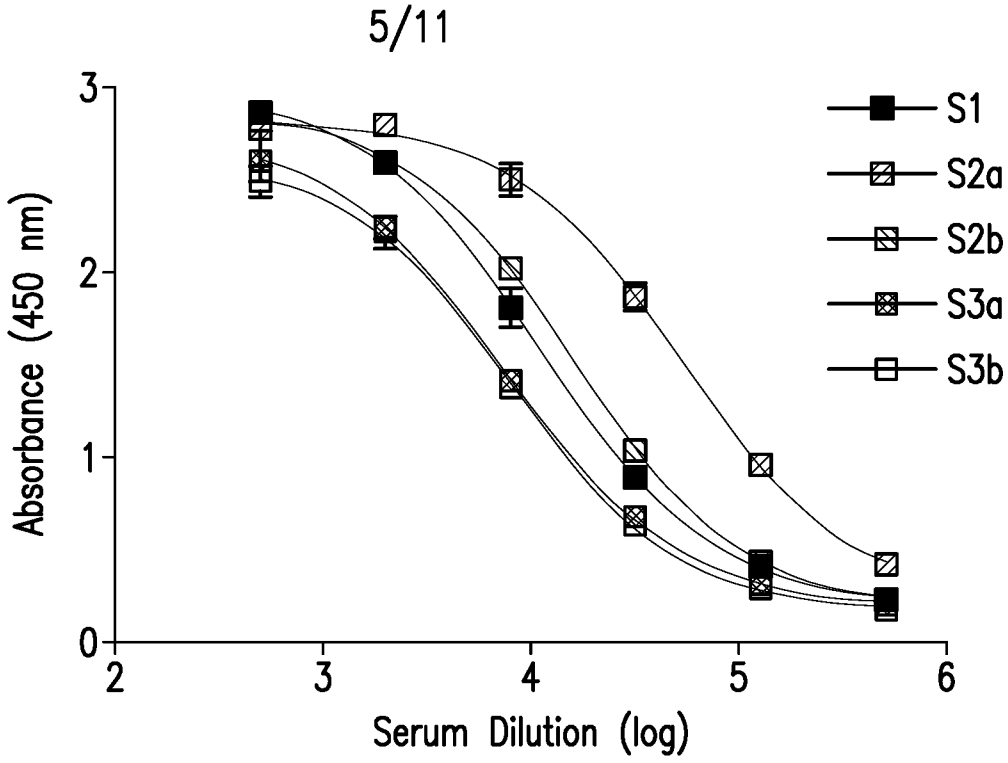


FIG.1 F

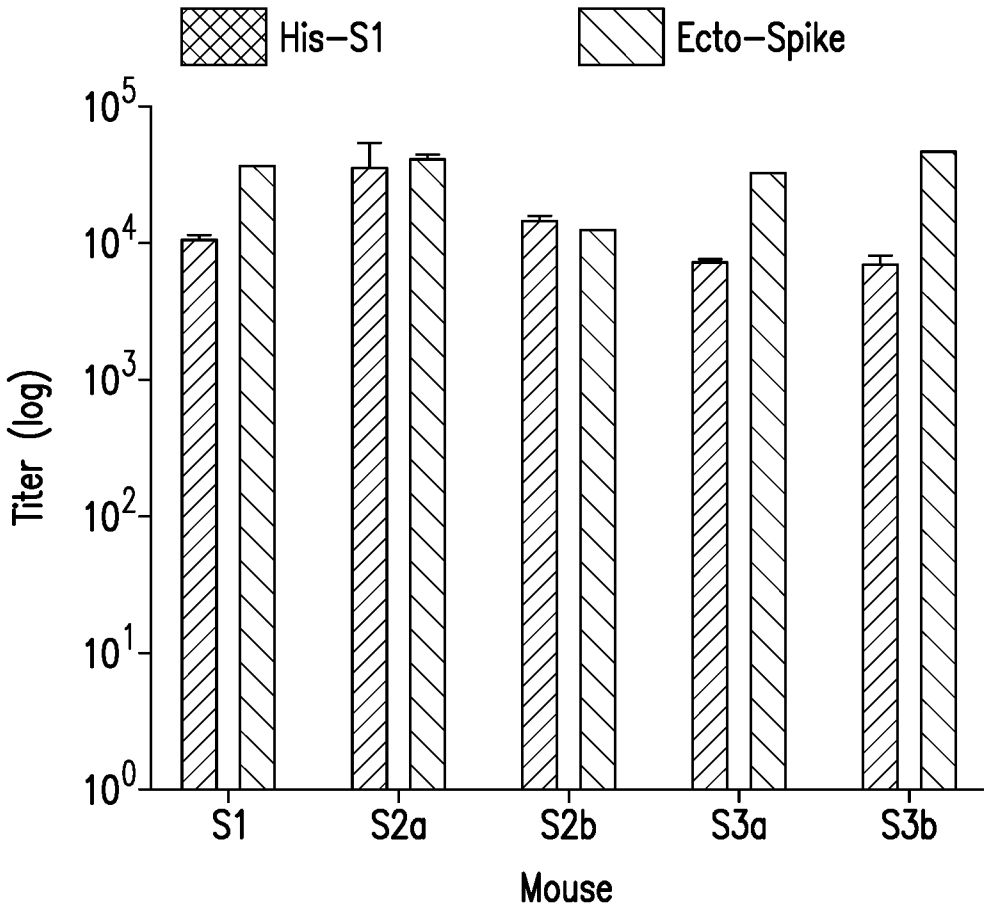


FIG.1 G

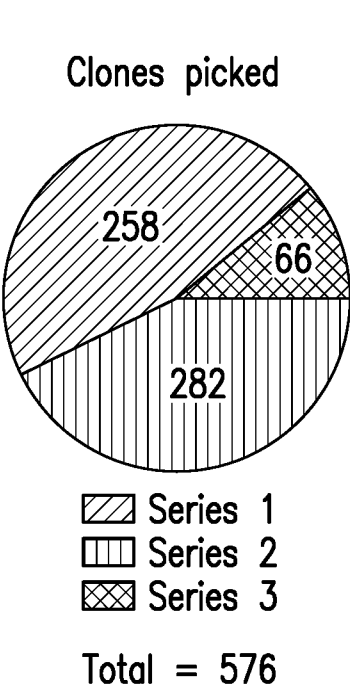


FIG.2A

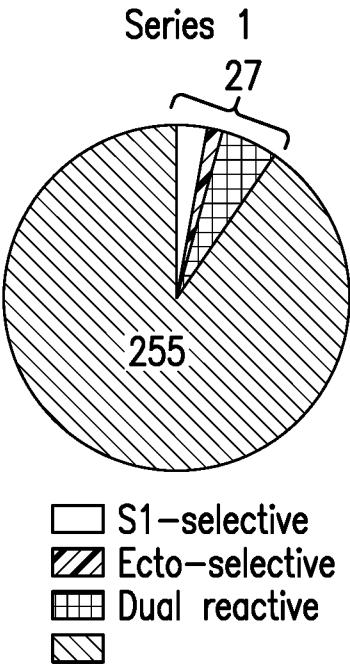


FIG.2B

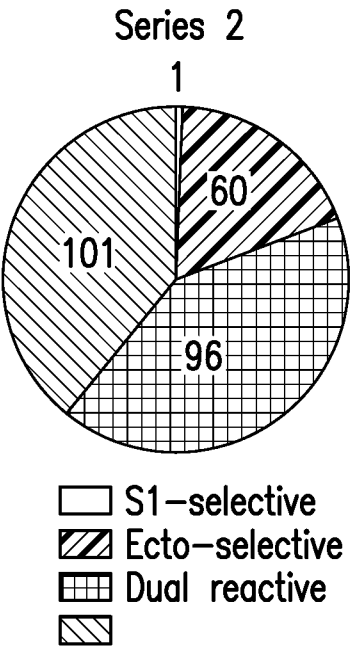


FIG.2C

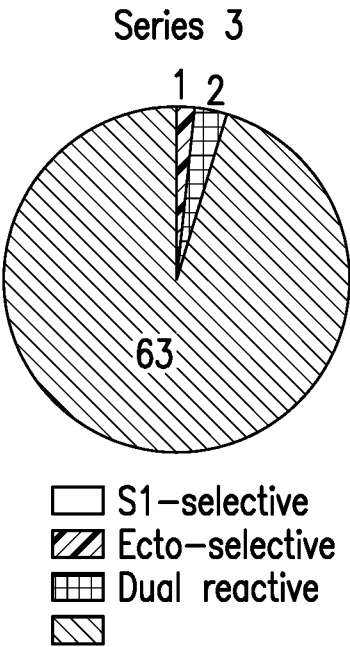


FIG.2D

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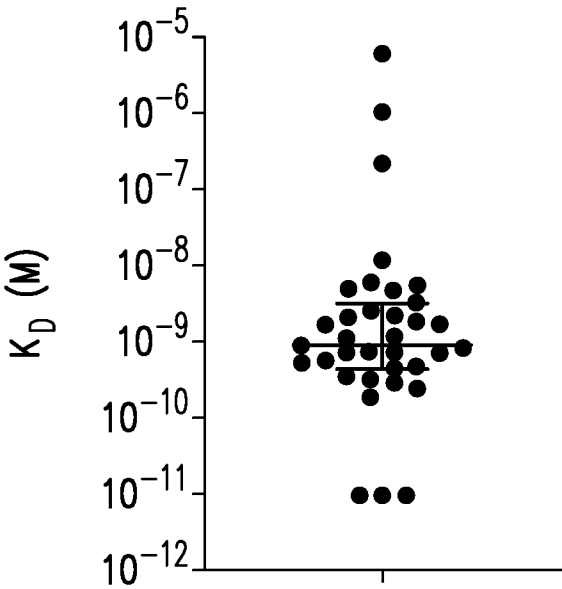


FIG.2E

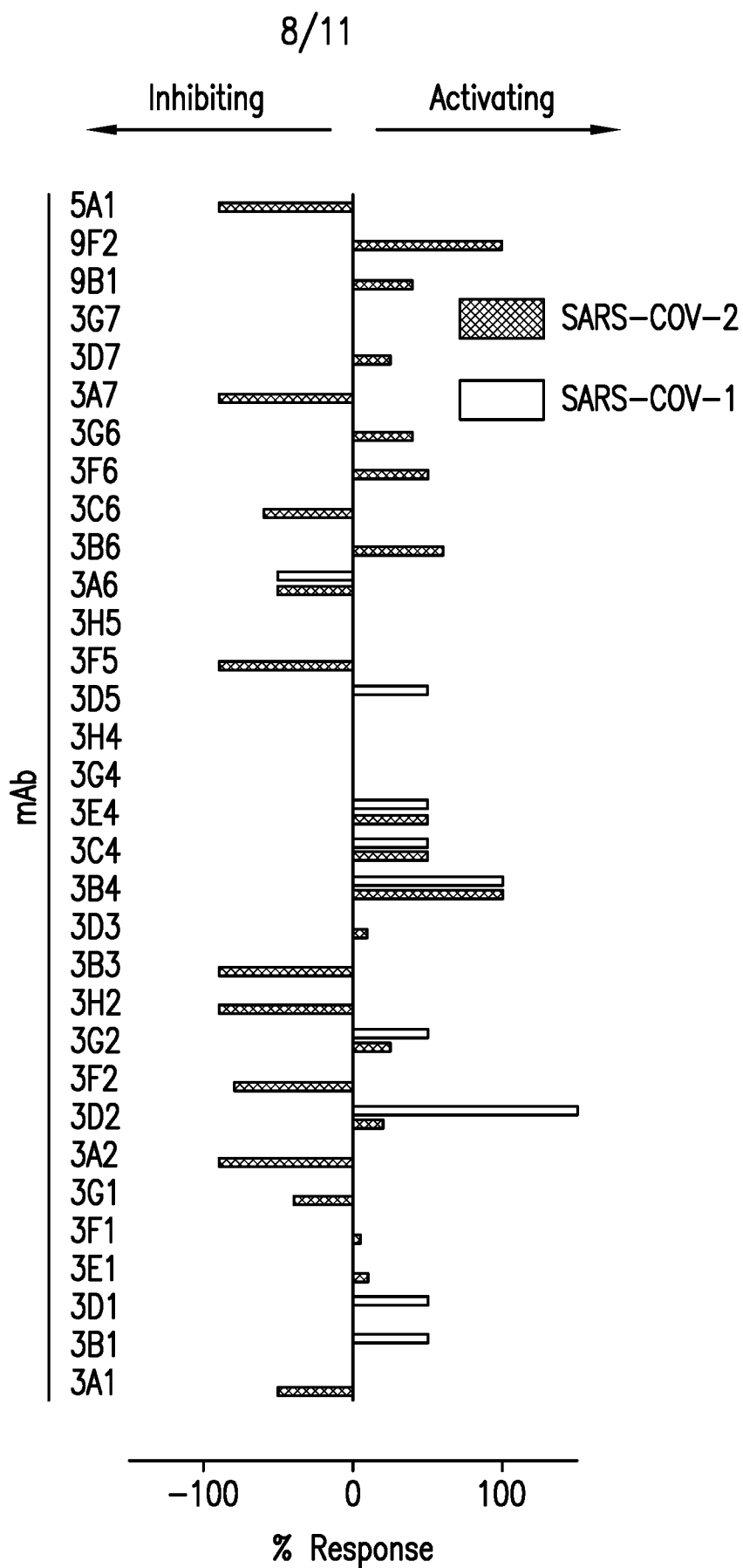


FIG.3A

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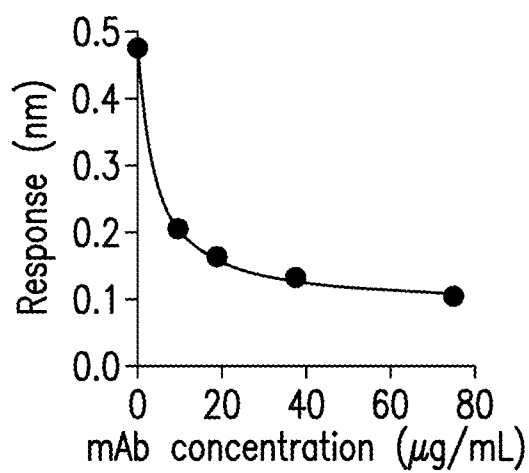


FIG.3B

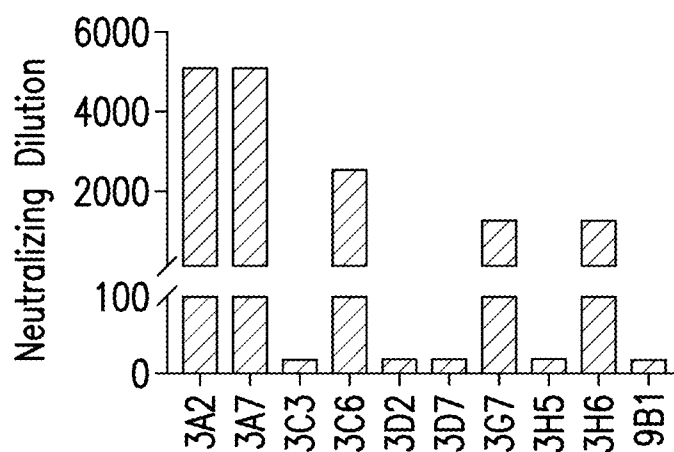


FIG.3C

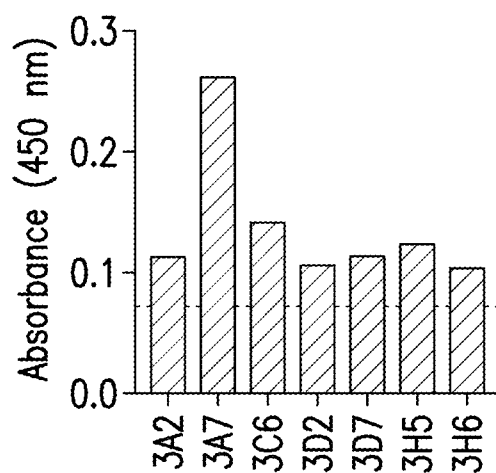


FIG.3D

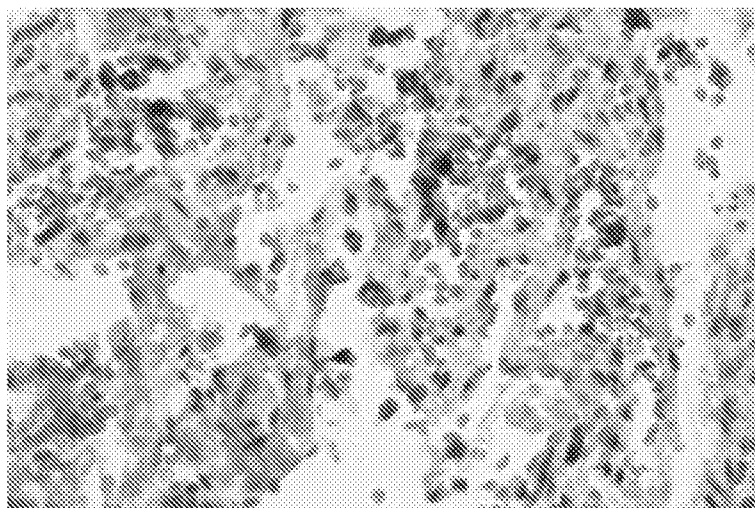


FIG.3E

	3A2	3A7	3C6	3F5	3G7	3H6	3D7	3F1	3D5	3H4	3F2	3H2	3D2	9B1
3A2	-0.001	-0.019	-0.008	-0.056	0.009	-0.006	0.303	0.257	0.318	0.344	0.367	0.274	0.176	0.079
3A7	-0.01	-0.02	-0.017	-0.05	0.0033	-0.021	0.272	0.268	0.282	0.275	0.359	0.27	0.164	0.058
3C6	-0.007	-0.013	-0.006	-0.044	0.024	0.018	0.27	0.256	0.277	0.185	0.307	0.216	0.177	0.091
3F5	-0.041	-0.053	-0.042	-0.044	-0.038	-0.054	0.282	0.255	0.294	0.276	0.316	0.242	0.159	0.06
3G7	-0.003	-0.016	-0.01	-0.026	0.008	0.256	0.265	0.314	0.291	0.238	0.302	0.201	0.198	0.059
3H6	0.036	0.026	0.029	-0.002	0.358	0.029	0.246	0.199	0.184	0.284	0.368	0.071	0.236	0.156
3D7	0.412	0.396	0.367	0.471	0.468	0.389	0.015	-0.036	-0.034	-0.025	0.343	0.372	0.149	0.121
3F1	0.31	0.244	0.242	0.29	0.325	0.203	-0.047	-0.04	-0.034	-0.043	0.324	0.287	0.186	0.101
3D5	0.376	0.304	0.325	0.347	0.37	0.189	-0.047	-0.044	-0.035	-0.034	0.361	0.261	0.194	0.049
3H4	0.346	0.314	0.307	0.346	0.374	0.205	-0.038	-0.025	-0.022	-0.019	0.336	0.278	0.106	0.084
3F2	0.315	0.245	0.26	0.223	0.369	0.338	0.325	0.315	0.312	0.245	-0.035	-0.065	0.249	0.066
3H2	0.21	0.195	0.162	0.271	0.425	-0.059	0.344	0.332	0.301	0.345	-0.019	-0.036	0.228	0.108
3D2	0.367	0.354	0.333	0.391	0.438	0.347	0.345	0.374	0.422	0.325	0.258	0.407	-0.002	0.098
9B1	0.316	0.323	0.292	0.291	0.409	0.332	0.367	0.372	0.261	0.312	0.275	0.309	0.239	0.005

Group 1a

Group 1b

Group 2

Group 3a

Group 3b

Group 4a

Group 4b

FIG.4

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mAb IGH % similarity heat map

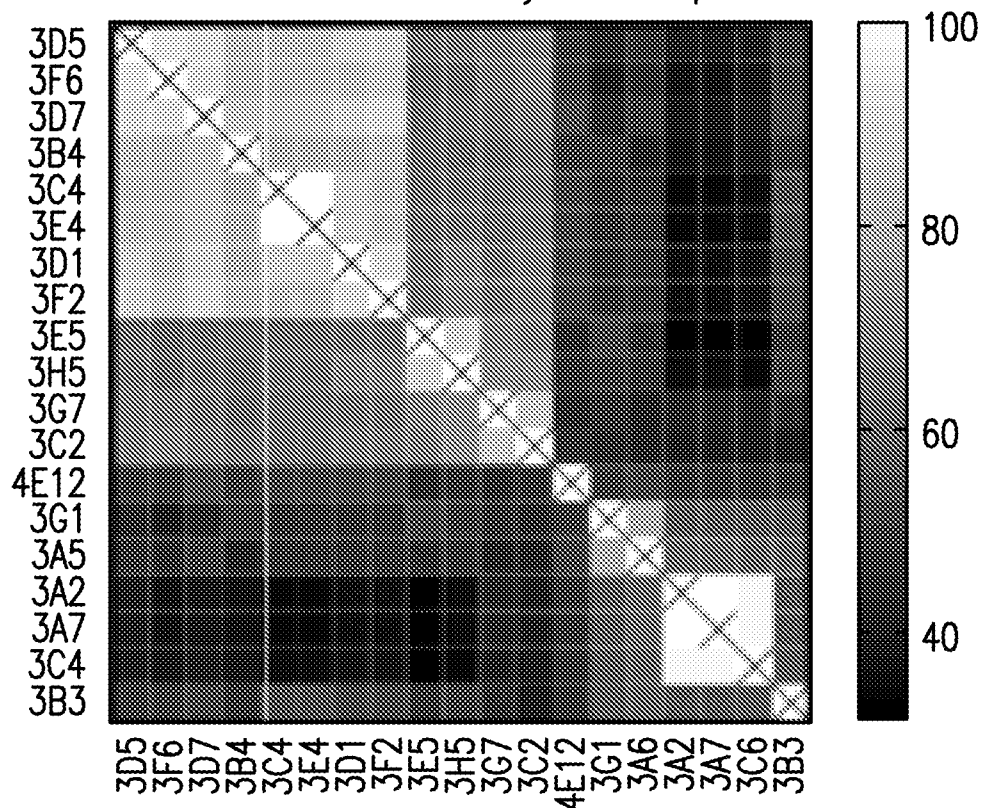


FIG.5A

mAb IGk % similarity heat map

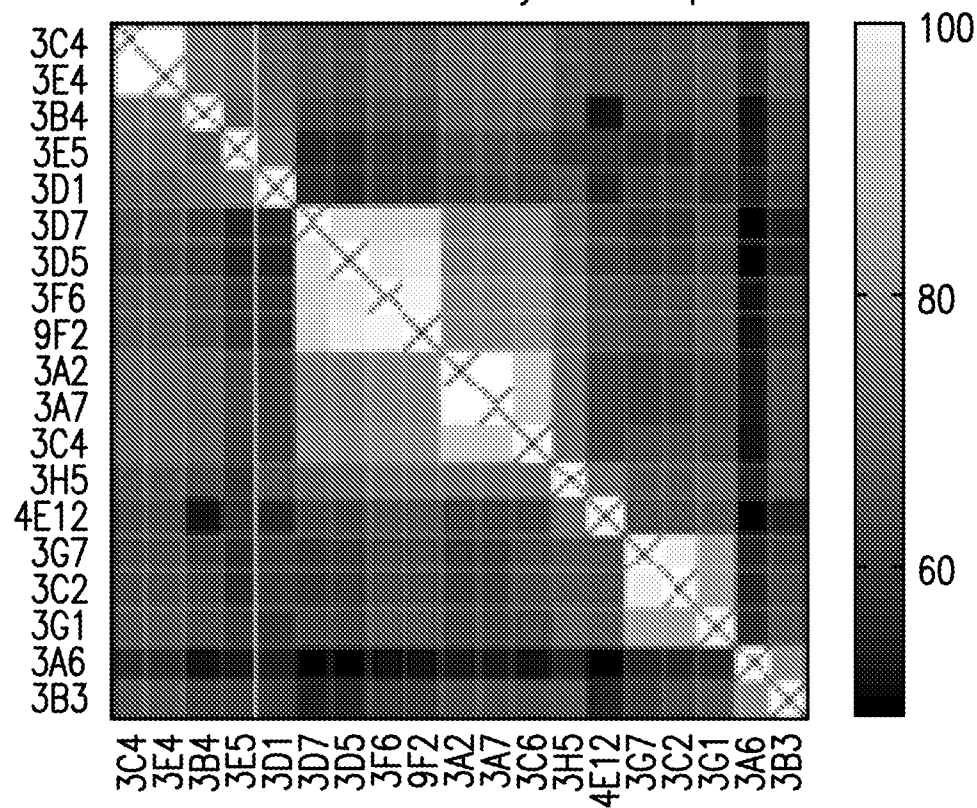


FIG.5B