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(71) Applicant: FRED HUTCHINSON CANCER CENTER

[US/US]; 1100 Fairview Avenue North, Seattle, Washington 98109 (US).

(72) Inventors: OVERBAUGH, Julie; c/o Fred Hutchinson

Cancer Center, 1100 Fairview Avenue North, Seattle, Washington 98109 (US). GUENTHOER, Jamie; c/o Fred

Hutchinson Cancer Center, 1100 Fairview Avenue North, Seattle, Washington 98109 (US).

(74) Agent: BAILEY, Mark A. et al.; Christensen O'Connor

Johnson Kindness PLLC, 1201 Third Avenue Suite 3600, Seattle, Washington 98101-3029 (US).

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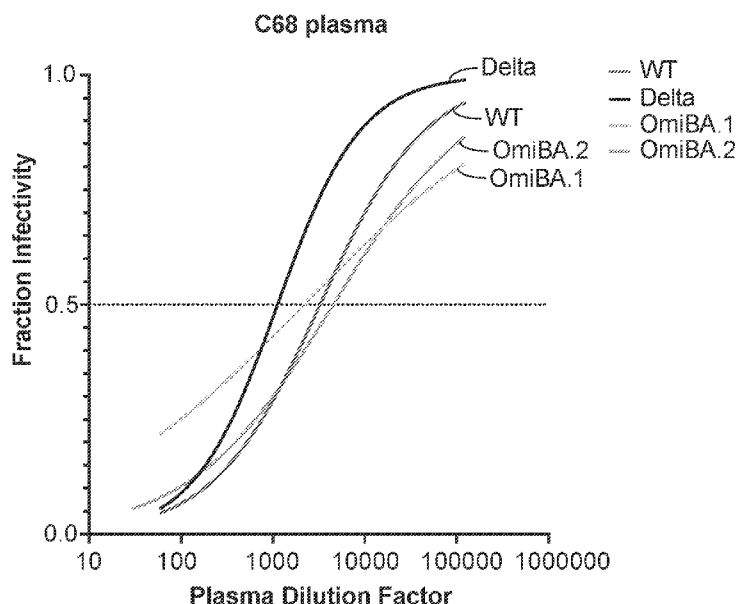


Fig. 1A

(57) Abstract: Provided herein are monoclonal antibodies or antigen-binding fragment thereof that bind to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Also provided herein are using such molecules for detecting and treating SARS-CoV-2.

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ANTIBODIES AGAINST SARS-COV-2

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/369,102, filed July 22, 2022; and U.S. Provisional Application No. 63/369,101, filed July 22, 2022; each of which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing XML associated with this application is provided in XML format and is hereby incorporated by reference into the specification. The name of the XML file containing the sequence listing is SWIS_007_01WO_SeqList_ST26.xml. The XML file is 36,338 bytes; was created on June 29, 2023, which is hereby incorporated by reference in its entirety; and is being submitted via Patent Center with the filing of the specification.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

This invention was made with government support under AI138709 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

The present disclosure generally relates to antibodies against the viral envelope spike (S) protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and derivatives thereof. The present disclosure also generally relates to use of such molecules for detecting and treating SARS-CoV-2.

BACKGROUND

Developing effective antibody-based approaches to limit viral infection and disease is a perennial challenge. This challenge has come to the fore in recent months with the rapid emergence of new SARS-CoV-2 variants of concern (VOCs) that have escaped antibodies directed to the original, ancestral pandemic strain (Wuhan-Hu-1; B.1),

which is the basis for currently administered vaccines. Current SARS-CoV-2 vaccines are based on the original Wuhan strain from early in the pandemic, but the virus has rapidly evolved since the start of the pandemic. Because SARS-CoV-2 is rapidly evolving, there has been a continual emergence of new VOCs, presenting new challenges for both vaccines and treatments. In particular, immune-based approaches to control SARS-CoV-2 infection and disease must be effective against diverse VOCs.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

The ability of antibodies to neutralize SARS-CoV-2 has been the foundation for developing vaccine approaches and therapeutics to treat COVID cases. The protective antiviral benefit of antibodies to SARS-CoV-2 is compromised by viral escape. Thus, effective antibodies must be both broad and potent to counter diverse evolving strains. The discovery of such antibodies is of critical importance for SARS-CoV-2 as the global emergence of new variants of concern (VOCs) has eroded vaccine efficacy and compromised the efficacy of therapeutic antibodies. For example, while antibody recognition of the Delta VOC (B.1.617) was only modestly reduced compared to the ancestral (B.1) vaccine strain, antibody recognition of the Omicron VOC (B.1.1.529) and its subvariants was much more significantly impacted, so much so that most therapeutic antibodies are not likely to be effective. Likewise, vaccine efficacy in preventing infection with Omicron has been compromised.

The major target of neutralizing antibodies is the Spike protein, which is the viral entry protein. Thus, therapeutic antibodies have been developed that bind to the Spike protein and prevent viral entry, leading to viral neutralization. These antibodies can target different regions of the Spike protein, but those currently licensed for therapeutic use all target the receptor binding domain (RBD), which is a critical domain for mediating viral entry. The antibodies that target the RBD are further divided into classes, depending on where within the RBD they bind. Mutations within RBD are common in VOCs. Generally, a single mutation can result in impaired activity of one or two classes of RBD antibodies, but not all classes. Omicron is an example of a variant that has evolved

multiple mutations enabling antibody evasion, 32 mutations alone in the Spike protein versus the ancestral strain and at least 20 more mutations compared to any of the prior VOCs. Subvariants of Omicron have additional mutations, including new mutations in the RBD. Because there are multiple mutations within the RBD of VOCs such as Omicron, the combination of mutations has eroded efficacy of multiple classes of antibodies.

In view of the limitation of the present art, a need remains for effective antibodies that are both broad and potent to counter diverse evolving strains of SARS-CoV-2. The present disclosure addresses these and related needs.

The present invention is directed to monoclonal antibodies or antigen-binding fragment thereof that specifically bind to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

In one aspect, provided herein is a monoclonal antibody or antigen-binding fragment thereof that specifically binds to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), wherein the monoclonal antibody or antigen-binding fragment thereof comprises: 1) a variable heavy chain CDR1 (HCDR1) comprising an amino acid sequence of SEQ ID No: 1, 7, 13, or 19; 2) a variable heavy chain CDR2 (HCDR2) comprising an amino acid sequence of SEQ ID No: 2, 8, 14, or 20; 3) a variable heavy chain CDR3 (HCDR3) comprising an amino acid sequence of SEQ ID No: 3, 9, 15, or 21; 4) a variable light chain CDR1 (LCDR1) comprising an amino acid sequence of SEQ ID No: 4, 10, 16, or 22; 5) a variable light chain CDR2 (LCDR2) comprising an amino acid sequence of SEQ ID No: 5, 11, 17, or 23; and 6) a variable light chain CDR3 (LCDR3) comprising an amino acid sequence of SEQ ID No: 6, 12, 18, or 24.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 1, a HCDR2 comprising an amino acid sequence of SEQ ID No: 2, a HCDR3 comprising an amino acid sequence of SEQ ID No: 3, a LCDR1 comprising an amino acid sequence of SEQ ID No: 4, a LCDR2 comprising an amino acid sequence of SEQ ID No: 5, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 6. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 7, a HCDR2 comprising an amino acid sequence of SEQ ID No: 8, a HCDR3 comprising an amino acid sequence of SEQ

ID No: 9, a LCDR1 comprising an amino acid sequence of SEQ ID No: 10, a LCDR2 comprising an amino acid sequence of SEQ ID No: 11, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 12. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 13, a HCDR2 comprising an amino acid sequence of SEQ ID No: 14, a HCDR3 comprising an amino acid sequence of SEQ ID No: 15, a LCDR1 comprising an amino acid sequence of SEQ ID No: 16, a LCDR2 comprising an amino acid sequence of SEQ ID No: 17, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 18. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 19, a HCDR2 comprising an amino acid sequence of SEQ ID No: 20, a HCDR3 comprising an amino acid sequence of SEQ ID No: 21, a LCDR1 comprising an amino acid sequence of SEQ ID No: 22, a LCDR2 comprising an amino acid sequence of SEQ ID No: 23, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 24.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 25, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 26. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 27, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 28. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 29, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 30. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 31, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 32.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid

sequence of SEQ ID No: 25, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 26. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 27, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 28. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 29, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 30. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 31, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 32.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof is a fully human antibody or antigen-binding fragment thereof.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof specifically binds to an epitope within the receptor binding domain (RBD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof specifically binds to an epitope outside of the receptor binding domain (RBD) of the viral envelope spike protein.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof neutralizes SARS-CoV-2. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof inhibits the fusion of SARS-CoV-2 and host cell membrane. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof is cytotoxic to a SARS-CoV-2 infected host cell.

In another aspect, provided herein is a polynucleotide encoding the monoclonal antibody or antigen-binding fragment thereof disclosed herein.

In another aspect, provided herein is a vector, comprising the polynucleotide disclosed herein.

In another aspect, provided herein is a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutical acceptable carrier. In some embodiments, the pharmaceutical composition is for use in treating a SARS-CoV-2 infection in a subject.

In another aspect, provided herein is a pharmaceutical composition comprising at least two components, wherein each component comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutical acceptable carrier. In some embodiments, the pharmaceutical composition is for use in treating a SARS-CoV-2 infection in a subject.

In another aspect, provided herein is a method of treating a SARS-CoV-2 infection in a subject, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition disclosed herein. In some embodiments, the method further comprises administering an anti-viral drug, a viral entry inhibitor, or a viral attachment inhibitor to the subject. In some embodiments, the pharmaceutical composition is administered to the subject prior to the exposure to SARS-CoV-2. In some embodiments, the pharmaceutical composition is administered to the subject after the exposure to SARS-CoV-2.

In another aspect, provided herein is a kit for detecting a SARS-CoV-2 infection in a subject, comprising the monoclonal antibody or antigen-binding fragment thereof disclosed herein.

In another aspect, provided herein is a method of detecting the presence of a SARS-CoV-2 in a sample, comprising (1) contacting the sample with the monoclonal antibody or antigen-binding fragment thereof disclosed herein, and (2) detecting the presence of an antibody-antigen complex, wherein the presence of the antibody-antigen complex indicates the presence of SARS-CoV-2. In some embodiments, the sample is a blood sample, a nasal swab, or a throat swab.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

Figs. 1A through 1B. Neutralization activity of 30-day post infection plasma against four SARS-CoV-2 strains. Neutralization was measured using a Spike pseudotyped lentivirus assay. **Fig. 1A.** Neutralization curves for C68 plasma against Wuhan-Hu-1 (WT, blue); Delta (red); Omicron BA.1 (OmiBA1, green); Omicron BA.2

(OmiBA2, purple) pseudoviruses are shown. Graphs are from a single, representative experiment with technical replicates. The dashed line indicates 0.5 fraction infectivity. **Fig. 1B.** IC₅₀ values for each experiment are plotted. Each combination was assayed at least 2-3 times. The means $\pm$ SD are plotted.

Figs. 2A through 2C. Binding affinity (EC₅₀) determined by the direct ELISA method. **Fig. 2A.** Binding affinity is measured by absorbance at 450 nm for the C68 monoclonal antibodies (mabs) to Wuhan-Hu-1 (WT), Delta, Omicron BA.1, and BA.2 Spike proteins (SinoBiological). FI6V3 was run in parallel as a negative control. Results are the average of 2-4 independent experiments. The mean $\pm$ SD are plotted. **Fig. 2B.** Log EC₅₀ values (ng/ml) represent binding to each Spike protein and are plotted for C68.3 (blue), C68.13 (red), C68.59 (green), C68.61 (purple). **Fig. 2C.** Lists the calculated EC₅₀ values (ng/mL) for each mab and the corresponding fold changes relative to WT Spike. FC>1 denotes weaker binding compared to WT Spike. Lower EC₅₀ values correspond to higher binding affinity and higher EC₅₀ values indicate lower binding affinity. Binding affinity was generally similar across different Spike proteins, except for C68.13, which showed lower binding affinity to the Delta Spike protein.

Fig 3. Binding by the direct ELISA method comparing RBD-specific monoclonal antibodies (mab) C68.61 to C68.59. Fig. 3 illustrates binding (absorbance at 450nm) of C68.61 and C68C.59 to WT Spike protein (black), RBD subdomain (red), NTD subdomain (blue), S2 subunit (purple) at different concentrations of mab. Specifically, C68.61 bound to Spike and RBD subunits at the 2 μ g concentration. C68.59 also bound to Spike at the 2 μ g concentration but did not bind to RBD, NTD, or S2, even at the 50 μ g concentration.

Figs. 4A through 4E. Neutralization activity of four monoclonal antibodies (mabs). Neutralization was measured using a Spike pseudotyped lentivirus assay. Neutralization curves for (**Fig. 4A**) C68.3, (**Fig. 4B**) C68.13, (**Fig. 4C**) C68.59, and (**Fig. 4D**) C68.61 against Wuhan-Hu-1 (WT, blue); Delta (red); Omicron BA.1 (OmiBA1, green); Omicron BA.2 (OmiBA2, purple) pseudoviruses are shown. Graphs are from a single, representative experiment with technical replicates. The dashed line indicates 0.5 fraction infectivity. **Fig. 4E.** Neutralization activity of plasma and monoclonal antibodies (mabs) in a pseudovirus assay. VOCs tested are indicated in the first row. The C68 plasma tested corresponded to the time of monoclonal antibody isolation (30 days post symptom onset). Neutralization of the four C68 antibodies and five authorized therapeutic

antibodies were tested in parallel. A dilution series of plasma or mabs was preincubated with Spike pseudotyped lentiviruses followed by infection of 293T-ACE2 cells for two days and measurement of infectivity by a luciferase-based assay. The calculated NT50 represents the reciprocal dilution of plasma that results in 50% neutralization of virus. Calculated IC₅₀ (ng/mL) for each mab represents the concentration of mab that results in 50% neutralization of virus. Darker blue denotes lower IC₅₀ and higher neutralization potency. Fold changes (FC) relative to WT were calculated with arrows to indicate the direction of the change.

Fig. 5. Neutralization activity of monoclonal antibodies (mabs) in a live virus assay. Neutralization of the four C68 antibodies and two authorized therapeutic antibodies tested in parallel was measured against the viruses indicated in the top row (e.g., WT + D614G; Alpha; Delta; and Omicron BA.1). For these experiments, a dilution series of each mab was preincubated with authentic virus followed by infection of Vero E6-TMPRSS2 cells at 600 TCID₅₀ per well. After one hour, the cells are washed and incubated for 2 days. Virus was measured based on nucleocapsid levels. The table shows the calculated IC₅₀ values (ng/mL) for each mab, indicated to the left, against each VOC. Darker blue indicates a lower IC₅₀ value and higher neutralization potency. Fold changes (FC) relative to WT are shown with arrows to indicate the direction of the change. Results are representative of three independent experiments.

Figs. 6A through 6D. Classification of RBD-specific epitopes using competition binding assays. Binding to Wuhan-Hu-1 Spike was performed with each biotinylated commercial mabs in the presence of unlabeled competitive C68 mab. To confirm competition, all assays were run in reverse with a biotinylated C68 mab competed with an unlabeled commercial mab. Results of the competition ELISAs are shown (**Fig. 6A**) with the magnitude of competition denoted by the intensity of the shading and symbols as described in the legend. Mab C68.3 competed most strongly with the Class 2 mab, and mab C68.13 competed with both Class 2 and 3 mabs. C68.61 did not compete with any of the commercial mabs. Bar graphs showing the percent competition of (**Fig. 6B**) C68.3, (**Fig. 6C**) C68.13, (**Fig. 6D**) C68.61 to all other mabs. For each mab, competition against self was set to 100%. HIV-1 VRC01 is included as negative control. The mean and $\pm$ SD are plotted.

DETAILED DESCRIPTION

The present disclosure provides monoclonal antibodies and antigen-binding fragments thereof that bind to the spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), compositions thereof, and methods of use in the treatment and/or prevention of SARS-CoV-2 infection.

Definitions

Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa unless the content clearly dictates otherwise. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

The terms “a”, “an”, and “the”, as used herein, include plural references unless the context clearly dictates otherwise.

The term “about”, as used herein, in reference to a number or range of numbers, is understood to mean the stated number and numbers +/- 10% thereof, or 10% below the lower listed limit and 10% above the higher listed limit for the values listed for a range.

The term “between”, as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B.

The terms “or” and “and/or”, as used herein, include any, and all, combinations of one or more of the associated listed items.

The terms “including”, “includes”, “included”, and other forms, as used herein, are not limiting.

The terms “comprise” and its grammatical equivalents, as used herein, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

The terms “administer”, “administration”, or “administering”, as used herein refers to the act of injecting or otherwise physically delivering a substance (e.g., a pharmaceutical composition provided herein) to a subject, such as by oral, mucosal, topical, intradermal, parenteral, intravenous, intravitreal, intraarticular, subretinal,

intramuscular, intrathecal delivery and/or any other method of physical delivery described herein or known in the art. The delivery can be systemic or to a specific tissue.

The term “antibody,” “immunoglobulin,” or “Ig” is used interchangeably herein, and is used in the broadest sense and specifically covers, for example, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length or intact monoclonal antibodies), antibody compositions with polyepitopic or monoepitopic specificity, polyclonal or monovalent antibodies, multivalent antibodies, and multispecific antibodies (e.g., bispecific antibodies so long as they exhibit the desired biological activity). A conventional antibody is composed of two identical pairs of polypeptide chains, wherein each pair has one heavy chain (about 50-70 kDa) and one light chain (about 25 kDa), each amino-terminal portion of each chain includes a variable region of about 100 to about 130 or more amino acids, and each carboxy-terminal portion of each chain includes a constant region. See, e.g., Antibody Engineering (Borrebæck, ed., 2d ed. 1995); and Kubly, Immunology (3d ed. 1997). An antibody can be human, humanized, chimeric and/or affinity matured, as well as an antibody from other species, for example, mouse and rabbit, etc. Antibodies also include, but are not limited to, synthetic antibodies, recombinantly produced antibodies, camelized antibodies or their humanized variants, and intrabodies. An antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4 (e.g., variants of IgG4 and IgG4 nullbody). An antibody can comprise kappa or lambda light chain constant sequences.

The term “antigen” refers to a molecule or a portion of a molecule capable of being bound by an antibody or an antigen-binding fragment thereof and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may be a polypeptide, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound.

The term “binds” or “binding”, as used herein, refers to a covalent or non-covalent interaction between molecules (e.g., forming a complex by interactions). Exemplary non-covalent interactions include hydrogen bonds, ionic bonds, hydrophobic interactions, and/or van der Waals interactions. As used herein, the term “specifically binds” refers to binding of an antibody or an antigen binding fragment thereof to an antigen with a dissociation constant (K_D) $\leq 10^{-7}$ M. The term “ K_D ” is intended to refer to the dissociation equilibrium constant of a particular antibody-antigen interaction. The

ratio of dissociation rate (k_{off}) to association rate (k_{on}) of an antibody to a monovalent antigen ($k_{\text{off}}/k_{\text{on}}$) is the dissociation constant K_D , which is inversely related to affinity. The lower the K_D value, the higher the affinity of the antibody. The value of K_D varies for different complexes of antibody and antigen and depends on both k_{on} and k_{off} . The dissociation constant K_D for an antibody provided herein can be determined using any method provided herein or any other method well known to those skilled in the art. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity.

The term “binding affinity”, as used herein, refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., a binding protein such as an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a binding molecule X for its binding partner Y can generally be represented by the dissociation constant (K_D). Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present disclosure.

The term “coding sequence” or a polynucleotide which “encodes” a polypeptide, as used herein, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A transcription termination sequence may be located 3' to the coding sequence.

The term “constant region” or “constant domain”, as used herein, refers to a carboxy terminal portion of the light and heavy chain which is not directly involved in binding of the antibody to antigen but exhibits various effector function, such as interaction with the Fc receptor. This portion has a conserved amino acid sequence relative to the variable region. The constant region may contain the CH1, CH2, and CH3 regions of the heavy chain and the CL region of the light chain.

The term “effective amount” or “therapeutically effective amount”, as used herein, refers to an amount of a therapeutic (e.g., a pharmaceutical composition provided herein) which is sufficient to treat, diagnose, prevent, delay the onset of, reduce and/or ameliorate the severity and/or duration of a given condition, disorder or disease and/or a symptom related thereto. The term also encompasses an amount necessary for the reduction, slowing, or amelioration of the advancement or progression of a given disease, reduction, slowing, or amelioration of the recurrence, development or onset of a given disease, and/or to improve or enhance the prophylactic or therapeutic effect (s) of another therapy or to serve as a bridge to another therapy.

The term “epitope”, as used herein, refers to a localized region of an antigen to which an antibody can bind. In the case of a polypeptide antigen, for example, an epitope can be contiguous amino acids of the polypeptide (a “linear” epitope) or an epitope can comprise amino acids from two or more non-contiguous regions of the polypeptide (a “conformational,” “non-linear” or “discontinuous” epitope). It will be appreciated by one of skill in the art that, in general, a linear epitope may or may not be dependent on secondary, tertiary, or quaternary structure. In some embodiments, an antibody binds to a group of amino acids regardless of whether they are folded in a natural three dimensional protein structure. In some embodiments, an antibody requires amino acid residues making up the epitope to exhibit a particular conformation (e.g., bend, twist, turn or fold) in order to recognize and bind the epitope.

The term “Fab” or “Fab region”, as used herein, refers to an antibody region that binds to antigens. A conventional IgG usually comprises two Fab regions, each residing on one of the two arms of the Y-shaped IgG structure. Each Fab region is typically composed of one variable region and one constant region of each of the heavy and the light chains. More specifically, the variable region and the constant region of the heavy chain in a Fab region are VH and CH1 regions, and the variable region and the constant region of the light chain in a Fab region are VL and CL regions. The VH, CH1, VL, and CL in a Fab region can be arranged in various ways to confer an antigen binding capability. For example, VH and CH1 regions can be on one polypeptide, and VL and CL regions can be on a separate polypeptide, similarly to a Fab region of a conventional IgG. Alternatively, VH, CH1, VL and CL regions can all be on the same polypeptide and oriented in different orders.

The term “Fc region”, as used herein, refers to a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include Clq binding; CDC; Fc receptor binding; ADCC; phagocytosis; downregulation of cell surface receptors (e.g., B cell receptor), etc. Such effector functions generally require the Fc region to be combined with a binding region or binding domain (e.g., an antibody variable region or domain) and can be assessed using various assays known to those skilled in the art.

The term “fragment”, as used herein, refers to a portion of a polypeptide or polynucleotide molecule containing less than the entire polypeptide or polynucleotide sequence. In some embodiments, a fragment of a polypeptide or polynucleotide comprises at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% of the entire length of the reference polypeptide or polynucleotide. In some embodiments, a fragment of a polypeptide or polynucleotide comprises about 10%-99%, about 20%-99%, about 30%-99%, about 40%-99%, about 50%-99%, about 60%-99%, about 70%-99%, about 80%-99%, about 90%-99%, about 95%-99%, about 96%-99%, about 97%-99%, or about 98%-99%, of the entire length of the reference polypeptide or polynucleotide. In some embodiments, a polypeptide or polynucleotide fragment may contain about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, or more nucleotides or amino acids.

The term “heavy chain”, when used in reference to an antibody, refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy -terminal portion includes a constant region. The constant region can be one of five distinct types, (e.g., isotypes) referred to as alpha, delta, epsilon, gamma, and mu, based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size: alpha, delta, and gamma contain approximately 450 amino acids, while epsilon and mu contain approximately 550 amino acids. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes (e.g., isotypes) of antibodies, IgA, IgD, IgE, IgG, and IgM, respectively, including four subclasses of IgG, namely IgG1, IgG2, IgG3, and IgG4.

The term “host”, as used herein, refers to an animal, such as a mammal (e.g., a human).

The term “host cell”, as used herein, refers to a particular subject cell into which an exogenous nucleic acid molecule may be introduced and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell comprising the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

The term “isolated nucleic acid”, as used herein, refers to a nucleic acid, for example, an RNA, DNA, or a mixed nucleic acids, substantially separated from other genomic DNA sequences as well as proteins or complexes such as ribosomes and polymerases that naturally accompany a native sequence, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

The term “light chain”, when used in reference to an antibody, refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy -terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa or lambda based on the amino acid sequence of the constant domains.

The term “monoclonal antibody,” as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, e.g., the individual antibodies

comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts, and each monoclonal antibody will typically recognize a single epitope on the antigen.

The term “neutralize,” when used in relation to an antibody, refers to the antibody’s ability to block the corresponding antigen’s function by binding to the antigen.

The term “operatively linked” and similar phrases (e.g., operably linked, genetically fused), as used herein, refer to the operational linkage of nucleic acid sequences or amino acid sequences placed in functional relationships with each other. For example, a promoter operatively linked to a polynucleotide encoding a polypeptide result in the transcription of the polynucleotide and ultimately the expression of the polypeptide. As another example, an operatively linked peptide is one in which the functional domains are placed with appropriate distance from each other to impart the intended function of each domain.

The term “pharmaceutically acceptable excipient, carrier or diluent”, as used herein, refers to any substance formulated alongside the active ingredient of a pharmaceutical composition that allows the active ingredient to retain biological activity and is non-reactive with the subject’s immune system. Such a substance can be included for the purpose of long-term stabilization, bulking up solid formulations that contain potent active ingredients in small amounts, or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating absorption, reducing viscosity, or enhancing solubility. The selection of appropriate substance can depend upon the route of administration and the dosage form, as well as the active ingredient and other factors. Compositions having such substances can be formulated by well-known conventional methods (see, e.g., Remington, *The Science and Practice of Pharmacy*, 23rd edition, A. Adejare, ed., Academic Press, 2020).

The term “pharmaceutical composition” or “therapeutic composition”, as used here, refers to a composition capable of being administered to a subject for the treatment of a particular disease or disorder.

The term “polynucleotide” or “nucleic acid”, as used herein, refers to deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and DNA/RNA hybrids. Polynucleotides may be single-stranded or double-stranded and either recombinant, synthetic, or isolated. Polynucleotides include, but are not limited to: pre-messenger RNA (pre-mRNA), messenger RNA (mRNA), RNA, genomic DNA (gDNA), PCR amplified

DNA, complementary DNA (cDNA), synthetic DNA, or recombinant DNA. Polynucleotides can comprise modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. Unless specified otherwise, the left-hand end of any single-stranded polynucleotide sequence disclosed herein is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction.

The terms "polypeptide" and "peptide" and "protein", as used herein, refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art.

The term "prevent", as used herein, refers to a pharmaceutical or other intervention regimen for reducing the likelihood of the onset (or recurrence) of a disease, disorder, condition, or associated symptom(s). Preventing includes delaying, preventing, or eliminating the appearance of a disease or condition, delaying, or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof.

The term "sequence identity", as used herein, refers to the percentage of bases or amino acids between two polynucleotide or polypeptide sequences that are the same, and in the same relative position. As such one polynucleotide or polypeptide sequence has a certain percentage of sequence identity compared to another polynucleotide or polypeptide sequence. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. The term "reference sequence" refers to a molecule to which a test sequence is compared. Methods of sequence alignment for comparison and determination of percent sequence identity and percent complementarity are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the homology alignment algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson

and Lipman, (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, (2003) Current Protocols in Molecular Biology), by use of algorithms known in the art including the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, (1977) Nuc. Acids Res. 25:3389-3402; and Altschul *et al.*, (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score 100, word length-2 to obtain nucleotide sequences homologous to a nucleic acid molecule described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, word length-3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, Nucleic Acids Res., 1997, 25:3389-402. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI BLAST programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The term “subject”, as used herein, refers to an “animal” and in particular a “mammal” such as a non-primate (*e.g.*, mice, rats, bovines, horses, household cats, tigers and other large cats, dogs, pigs, rabbits, goats, deer, sheep, ferrets, gerbils, guinea pigs, hamsters, bats, and birds (*e.g.*, chickens, turkeys, and ducks)) or a primate (*e.g.*, monkeys, baboons, chimpanzees, and human). The term may be used interchangeably with the term “patient” or “individual”. In some embodiments, the subject is a mammal, *e.g.*, a human,

diagnosed with a disease or disorder provided herein. In some embodiments, the subject is a mammal, e.g., a human, at risk of developing a disease or disorder provided herein.

The terms “treatment” and “treating”, as used herein, refer to a pharmaceutical or other intervention regimen for obtaining beneficial or desired results in the recipient. Treating may refer to eradication or amelioration of symptoms or of an underlying disorder being treated. Treating may also be achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder.

The term “variable region”, “variable domain”, “V region”, or “V domain”, as used herein, refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable region of the heavy chain may be referred to as “VH.” The variable region of the light chain may be referred to as “VL.” The term “variable” refers to the fact that certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of less variable (e.g., relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (e.g., extreme variability) called “hypervariable regions” or “complementarity determining regions” that are each about 9-12 amino acids long. The variable regions of heavy and light chains each comprise four FRs, largely adopting a β sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases form part of, the β sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, e.g., Kabat et al, Sequences of Proteins of Immunological Interest (5th ed. 1991)).

The complementarity determining regions (CDRs) have been defined by well-known numbering systems. For example, the Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (see,

e.g., Kabat, et al., supra). Chothia refers instead to the location of the structural loops (see, e.g., Chothia and Lesk, J. Mol. Biol., 1987, 196:901-17). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (see, e.g., Antibody Engineering Vol. 2 (Rontermann and Diibel, eds., 2d ed. 2010)). The "contact" CDRs are based on an analysis of the available complex crystal structures. Another universal numbering system that has been developed and widely adopted is ImMunoGeneTics (IMGT) Information System[®] (Lafranc, et al, Dev. Comp. Immunol., 2003, 27(1):55-77). IMGT is an integrated information system specializing in immunoglobulins (IG), T-cell receptors (TCR), and major histocompatibility complex (MHC) of human and other vertebrates. An additional numbering system (AHon) has been developed by Honegger and Pluckthun, J. Mol. Biol., 2001, 309: 657-70. Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT unique numbering system, is well known to one skilled in the art (see, e.g., Kabat, supra, Chothia and Lesk, supra; Martin, supra, Lefranc, et al., supra). The boundaries of a given CDR may vary depending on the scheme used for identification. Thus, unless otherwise specified, the CDRs of a given antibody or region thereof, such as a variable region, should be understood to encompass the complementary determining region as defined by any of the known schemes described herein. In some instances, the scheme for identification of a particular CDR or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, or Contact method. In other cases, the particular amino acid sequence of a CDR is given. As the "location" of the CDRs within the structure of the immunoglobulin variable domain is conserved between species and present in structures called loops, by using numbering systems that align variable domain sequences according to structural features, CDR and framework residues are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody.

The term “variant”, when used in relation to polypeptide, refers to a polypeptide comprising one or more (such as, for example, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) amino acid sequence substitutions, deletions, and/or additions as compared to a native or unmodified sequence. Variants may be naturally occurring, such as allelic or splice variants, or may be artificially constructed. Polypeptide variants may be prepared from the corresponding nucleic acid molecules encoding the variants.

The term “vector”, as used herein, refers to a substance that is used to carry or introduce a nucleic acid sequence (e.g., a nucleic acid sequence encoding an antibody as described herein) into a host cell. Vectors applicable for use include, for example, plasmids, phage vectors, viral vectors, episomes, and artificial chromosomes. A vector may include sequences that direct autonomous replication in a cell, or may include sequences sufficient to allow integration into host cell DNA. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like, which are well known in the art. When two or more nucleic acid molecules are to be co-expressed (e.g., both an antibody heavy and light chain or an antibody VH and VL), both nucleic acid molecules can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences. The introduction of nucleic acid molecules into a host cell can be confirmed using methods well known in the art. Such methods include, for example, nucleic acid analysis such as Northern blots or polymerase chain reaction (PCR) amplification of mRNA, immunoblotting for expression of gene products, or other suitable analytical methods to test the expression of an introduced nucleic acid sequence or its corresponding gene product. It is understood by those skilled in the art that the nucleic acid molecules are expressed in a sufficient amount to produce a desired product and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

General methods in molecular and cellular biochemistry can be found in such standard textbooks as *Molecular Cloning: A Laboratory Manual*, 3rd Ed. (Sambrook *et al.*, HaRBor Laboratory Press 2001); *Short Protocols in Molecular Biology*, 4th Ed. (Ausubel *et al.* eds., John Wiley & Sons 1999); *Protein Methods* (Bollag *et al.*, John Wiley & Sons 1996); *Nonviral Vectors for Gene Therapy* (Wagner *et al.* eds., Academic Press 1999); *Viral Vectors* (Kaplift & Loewy eds., Academic Press 1995); *Immunology Methods Manual* (I. Lefkovits ed., Academic Press 1997); and *Cell and Tissue Culture: Laboratory Procedures in Biotechnology* (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference.

Antibodies and Antigen binding fragments

Provided herein are monoclonal antibodies or antigen-binding fragments thereof that binds to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). In some embodiments, the monoclonal antibodies, or antigen-binding fragments thereof, specifically bind to the viral envelope spike protein of a SARS-CoV-2, or a domain of the spike protein. In some embodiments, the monoclonal antibodies or antigen-binding fragments thereof bind to the receptor binding domain (RBD) of the Spike protein. In some embodiments, the monoclonal antibodies or antigen-binding fragments thereof bind to the N-terminal domain (NTD) of the Spike protein. In some embodiments, the monoclonal antibodies or antigen-binding fragments thereof bind to the S2 domain of the Spike protein. For example, the epitopes of two exemplary monoclonal antibodies (C68.3 and C68.13) overlap with those of known therapeutic antibodies against SARS-CoV-2 (*e.g.* LY-CoV555, REGEN10987). However, C68.3 and C68.13 retain breadth and potency across variants of concern. Another exemplary monoclonal antibody (C68.59) targets a novel epitope outside of the RBD. A fourth exemplary monoclonal antibody (C68.61) targets an epitope within the RBD that does not directly overlap with epitopes of known therapeutic antibodies against SARS-CoV-2. The monoclonal antibodies or antigen-binding fragment thereof disclosed herein can be used for detecting and treating SARS-CoV-2. For instance, they can be useful in a cocktail approach designed to minimize the chances of escape that can readily occur with single antibody approaches.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable heavy chain CDR1 (HCDR1) comprising an amino acid sequence of SEQ ID No: 1. In some embodiments, the monoclonal antibody or antigen-

binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No:7. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No:13. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No:19. In some embodiments, the HCDR1 comprises an amino acid sequence that is at least 75%, 80%, 87.5%, or 90% identical to one of SEQ ID NOs: 1, 7, 13, or 19. In some embodiments, the HCDR1 comprises an amino acid sequence that is 100% identical to one of SEQ ID NOs: 1, 7, 13, or 19 except for the substitution or deletion of one or two amino acids.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable heavy chain CDR2 HCDR2 comprising an amino acid sequence of SEQ ID No: 2. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR2 comprising an amino acid sequence of SEQ ID No: 8. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR2 comprising an amino acid sequence of SEQ ID No: 14. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR2 comprising an amino acid sequence of SEQ ID No: 20. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises an HCDR2 comprising an amino acid sequence that is at least 71%, at least 75%, at least 85%, or at least 87% identical to one of SEQ ID NOs: 2, 8, 14, or 20. In some embodiments, the HCDR2 comprises an amino acid sequence that is at least 100% identical to one of SEQ ID NOs: 2, 8, 14, or 20 except for the substitution or deletion of one or two amino acids.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable heavy chain CDR3 (HCDR3) comprising an amino acid sequence of SEQ ID No: 3. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR3 comprising an amino acid sequence of SEQ ID No: 9. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR3 comprising an amino acid sequence of SEQ ID No: 15. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR3 comprising an amino acid sequence of SEQ ID No: 21. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises an

HCDR3 comprises an amino acid sequence that is at least at least 75%, at least 81%, at least 85%, at least 87%, at least 90%, at least 92%, or at least 95% identical to one of SEQ ID NOs: 3, 9, 15, or 21. In some embodiments, the HCDR3 comprises an amino acid sequence that is at least 100% identical to one of SEQ ID NOs: 3, 9, 15, or 21 except for the substitution or deletion of one or two amino acids.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable light chain CDR1 (LCDR1) comprising an amino acid sequence of SEQ ID No: 4. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR1 comprising an amino acid sequence of SEQ ID No: 10. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR1 comprising an amino acid sequence of SEQ ID No: 16. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR1 comprising an amino acid sequence of SEQ ID No: 22. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises an LCDR1 comprising an amino acid sequence that is at least 66% or at least 83% identical to one of SEQ ID NOs: 4, 10, 16, or 22. In some embodiments, the LCDR1 comprises an amino acid sequence that is at least 100% identical to one of SEQ ID NOs: 4, 10, 16, or 22 except for the substitution or deletion of one or two amino acids.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable light chain CDR2 (LCDR2) comprising an amino acid sequence of SEQ ID No: 5. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR2 comprising an amino acid sequence of SEQ ID No: 11. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR2 comprising an amino acid sequence of SEQ ID No: 17. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR2 comprising an amino acid sequence of SEQ ID No: 23. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises an LCDR2 comprising an amino acid sequence that is at least 66% identical to one of SEQ ID NOs: 5, 11, 17, or 23. In some embodiments, the LCDR2 comprises an amino acid sequence that is at least 100% identical to one of SEQ ID NOs: 5, 11, 17, or 23 except for the substitution or deletion of one amino acid.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a variable light chain CDR3 (LCDR3) comprising an amino acid

sequence of SEQ ID No: 6. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR3 comprising an amino acid sequence of SEQ ID No: 12. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR3 comprising an amino acid sequence of SEQ ID No: 18. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a LCDR3 comprising an amino acid sequence of SEQ ID No: 24. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises an LCDR3 comprising an amino acid sequence that is at least 75%, at least 77%, at least 84%, at least 87%, at least 88%, or at least 92% identical to one of SEQ ID NOs: 6, 12, 18, or 24. In some embodiments, the LCDR3 comprises an amino acid sequence that is at least 100% identical to one of SEQ ID NOs: 6, 12, 18, or 24 except for the substitution or deletion of one or two amino acids.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises: 1) a HCDR1 comprising an amino acid sequence of SEQ ID No: 1, 7, 13, or 19; 2) a HCDR2 comprising an amino acid sequence of SEQ ID No: 2, 8, 14, or 20; 3) a HCDR3 comprising an amino acid sequence of SEQ ID No: 3, 9, 15, or 21; 4) a LCDR1 comprising an amino acid sequence of SEQ ID No: 4, 10, 16, or 22; 5) a LCDR2 comprising an amino acid sequence of SEQ ID No: 5, 11, 17, or 23; and 6) a LCDR3 comprising an amino acid sequence of SEQ ID No: 6, 12, 18, or 24.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 1, a HCDR2 comprising an amino acid sequence of SEQ ID No: 2, a HCDR3 comprising an amino acid sequence of SEQ ID No: 3, a LCDR1 comprising an amino acid sequence of SEQ ID No: 4, a LCDR2 comprising an amino acid sequence of SEQ ID No: 5, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 6. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 consisting of SEQ ID No: 1, a HCDR2 consisting of SEQ ID No: 2, a HCDR3 consisting of SEQ ID No: 3, a LCDR1 consisting of SEQ ID No: 4, a LCDR2 consisting of SEQ ID No: 5, and a LCDR3 consisting of SEQ ID No: 6.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 7, a HCDR2 comprising an amino acid sequence of SEQ ID No: 8, a HCDR3 comprising an amino acid sequence of SEQ ID No: 9, a LCDR1 comprising an amino acid sequence of

SEQ ID No: 10, a LCDR2 comprising an amino acid sequence of SEQ ID No: 11, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 12. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 consisting of SEQ ID No: 7, a HCDR2 consisting of SEQ ID No: 8, a HCDR3 consisting of SEQ ID No: 9, a LCDR1 consisting of SEQ ID No: 10, a LCDR2 consisting of SEQ ID No: 11, and a LCDR3 consisting of SEQ ID No: 12.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 13, a HCDR2 comprising an amino acid sequence of SEQ ID No: 14, a HCDR3 comprising an amino acid sequence of SEQ ID No: 15, a LCDR1 comprising an amino acid sequence of SEQ ID No: 16, a LCDR2 comprising an amino acid sequence of SEQ ID No: 17, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 18. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 consisting of SEQ ID No: 13, a HCDR2 consisting of SEQ ID No: 14, a HCDR3 consisting of SEQ ID No: 15, a LCDR1 consisting of SEQ ID No: 16, a LCDR2 consisting of SEQ ID No: 17, and a LCDR3 consisting of SEQ ID No: 18.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 19, a HCDR2 comprising an amino acid sequence of SEQ ID No: 20, a HCDR3 comprising an amino acid sequence of SEQ ID No: 21, a LCDR1 comprising an amino acid sequence of SEQ ID No: 22, a LCDR2 comprising an amino acid sequence of SEQ ID No: 23, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 24. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 consisting of SEQ ID No: 19, a HCDR2 consisting of SEQ ID No: 20, a HCDR3 consisting of SEQ ID No: 21, a LCDR1 consisting of SEQ ID No: 22, a LCDR2 consisting of SEQ ID No: 23, and a LCDR3 consisting of SEQ ID No: 24.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 25. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 25. In some

embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 25.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 27. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 27. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 27.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 29. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 29. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 29.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 31. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 31. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 31.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a light chain variable domain (VL) comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 26. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence

of SEQ ID No: 26. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL consisting of SEQ ID No: 26.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 28. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence of SEQ ID No: 28. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL consisting of SEQ ID No: 28.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 30. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence of SEQ ID No: 30. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL consisting of SEQ ID No: 30.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 32. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL comprising an amino acid sequence of SEQ ID No: 32. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VL consisting of SEQ ID No: 32.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 25, and a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 26.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 27, and a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 28.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 29, and a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 30.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 31, and a VL comprising an amino acid sequence that is at least about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID No: 32.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 25, and a VL comprising an amino acid sequence of SEQ ID No: 26. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 25, and a VL consisting of SEQ ID No: 26.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 27, and a VL comprising an amino acid sequence of SEQ ID No: 28. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 27, and a VL consisting of SEQ ID No: 28.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 29, and a VL

comprising an amino acid sequence of SEQ ID No: 30. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 29, and a VL consisting of SEQ ID No: 30.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH comprising an amino acid sequence of SEQ ID No: 31, and a VL comprising an amino acid sequence of SEQ ID No: 32. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof comprises a VH consisting of SEQ ID No: 31, and a VL consisting of SEQ ID No: 32.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope within the N-terminal domain (NTD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope within the receptor binding domain (RBD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope within the subdomain (SD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope within the S2 subunit of the viral envelope spike protein.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope outside of the N-terminal domain (NTD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope outside of the receptor binding domain (RBD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope outside of the subdomain (SD) of the viral envelope spike protein. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof binds to an epitope outside of the S2 subunit of the viral envelope spike protein.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof is a neutralizing antibody or antigen-binding fragment thereof. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof neutralizes SARS-CoV-2. In some embodiments, the monoclonal antibody or antigen-binding fragment thereof inhibits the fusion of SARS-CoV-2 and host cell membrane.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof is a non-neutralizing antibody or antigen-binding fragment thereof. In some

embodiments, the monoclonal antibody or antigen-binding fragment thereof is cytotoxic to a SARS-CoV-2 infected host cell.

In some embodiments, the monoclonal antibodies, or antigen binding fragments thereof, described herein are an IgG1, IgG2, IgG3, or IgG4 isotype. In some embodiments, the monoclonal antibodies, or antigen binding fragments thereof, described herein are IgG1 isotypes.

Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan et al. (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos et al. 2002 J Mol Biol 320:415-428).

CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

The fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the antibody or antibody fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the antibody or antibody fragment must possess a bioactive property, such as specific binding to its cognate antigen. Functional or active regions of the antibody or antibody fragment may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can

include site-specific mutagenesis of the nucleic acid encoding the antibody or antibody fragment. (Zoller, M.J. *Curr. Opin. Biotechnol.* 3:348-354, 1992).

As discussed herein there are numerous variants and derivatives of the antibodies, CDRs, VH, and VL proteins disclosed herein. Protein variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. As used herein, "insertions" refer to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the parent, often the naturally occurring, molecule. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions, or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such

substitutions generally are made in accordance with the following Table B and are referred to as conservative substitutions.

Table A: Amino Acid Abbreviations

Amino Acid	Abbreviations	
Alanine	Ala	A
allosoleucine	Alle	
Arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
Cysteine	Cys	C
glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
phenylalanine	Phe	F
proline	Pro	P
pyroglutamic acid	pGlu	
Serine	Ser	S
Threonine	Thr	T
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Valine	Val	V

Table B: Amino Acid Substitutions - Exemplary Conservative Substitutions

Original AA	Conservative Substitution
Ala	Ser
Arg	Lys; Gln
Asn	Gln; His
Asp	Glu

Original AA	Conservative Substitution
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Pro
His	Asn;Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table B, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. Conservative amino acid substitutions include the ones in which the amino acid residue is replaced with an amino acid residue having similar structural or chemical properties. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cystine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

The replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Monoclonal Antibody Manufacturing

Monoclonal antibodies or functional fragments thereof (e.g., antigen-binding fragment thereof) may be made using, for example, the hybridoma method or the phage display method.

In the hybridoma method (see, e.g., described in Kohler, et al., *Nature*, 1975, 256:495-7), a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice* 59-103 (1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium, which, in some embodiments, contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for

the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which prevent the growth of HGPRT-deficient cells.

Exemplary parental myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Exemplary myeloma cell lines are murine myeloma lines, such as SP-2 and derivatives, for example, X63-Ag8-653 cells available from the American Type Culture Collection (Manassas, VA), and those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center (San Diego, CA). Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *Immunol.* 1984, 133:3001-05; and Brodeur, et al., *Monoclonal Antibody Production Techniques and Applications*, 1987, 51-63).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as RIA or ELISA. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al, *Anal. Biochem.*, 1980, 107:220-39.

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, DMEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal, for example, by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxyapatite chromatography, gel electrophoresis, dialysis, etc.

In the phage display method, synthetic antibody clones are selected by screening phage libraries containing phages that display various fragments of antibody variable region (Fv) fused to phage coat protein. Such phage libraries are screened against the desired antigen. Clones expressing Fv fragments capable of binding to the desired antigen

are adsorbed to the antigen and thus separated from the non-binding clones in the library. The binding clones are then eluted from the antigen and can be further enriched by additional cycles of antigen absorption/elution.

Variable domains can be displayed functionally on phage, either as single-chain Fv (scFv) fragments, in which VH and VL are covalently linked through a short, flexible peptide, or as Fab fragments, in which they are each fused to a constant domain and interact non-covalently, as described, for example, in Winter et al., 1994, *Ann. Rev. Immunol.* 12:433-55.

Repertoires of VH and VL genes can be separately cloned by PCR and recombined randomly in phage libraries, which can then be searched for antigen-binding clones as described in Winter et al, *supra*. Libraries from immunized sources provide high-affinity antibodies to the antigen without the requirement of constructing hybridomas. Alternatively, naive libraries can be cloned to provide a single source of human antibodies to a wide range of non-self and self antigens without any immunization as described by Griffiths et al, *EMBO J*, 1993, 12:725-34. Finally, naive libraries can also be made synthetically by cloning the unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro* as described, for example, by Hoogenboom and Winter, *J. Mol. Biol.*, 1992, 227:381-88.

Screening of the libraries can be accomplished by various techniques known in the art. For example, the antigen can be used to coat the wells of adsorption plates, expressed on host cells affixed to adsorption plates or used in cell sorting, conjugated to biotin for capture with streptavidin-coated beads, or used in any other method for panning display libraries. The selection of antibodies with slow dissociation kinetics (e.g., good binding affinities) can be promoted by use of long washes and monovalent phage display as described in Bass, et al, *Proteins*, 1990, 8:309-14 and WO 92/09690, and by use of a low coating density of antigen as described in Marks et al., *Biotechnol.*, 1992, 10:779-83.

Exemplary phage display methods that can be used herein include those disclosed in *Antibody Phage Display: Methods and Protocols* (O'Brien and Aitken, eds., 2002); Brinkman, et al., *J. Immunol. Methods*, 1995, 182:41-50; Ames, et al, *Immunol. Methods*, 1995, 184:177-86; Kettleborough, et al., *Eur. J. Immunol.*, 1994, 24:952-8; Persic, et al, *Gene*, 1997, 187:9-18; Burton et al., *Advances in Immunology*, 1994, 57:191-280; PCT Application No. PCT/GB91/01 134; International Publication Nos. WO

90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and W097/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108.

DNA encoding the monoclonal antibodies is readily isolated from the hybridoma cells and the screened libraries. Such DNA can be 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). Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells, such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra, et al, Curr. Opinion in Immunol., 1993, 5:256-62 and Pluckthun, Immunol. Revs., 1992, 130:151-88.

Once an antibody molecule provided herein has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies provided herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

Humanized, Human, and Recombinant Antibodies

The antibodies disclosed herein can comprise “chimeric” sequences 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, so long as they exhibit the desired biological activity (see U S. Pat. No. 4,816,567; and Morrison, et al., Proc. Natl. Acad. Sci. USA, 1984, 81:6851-55).

The antibodies disclosed herein can be humanized antibodies. A humanized antibody can comprise human framework region and human constant region sequences.

In some embodiments, one or more FR region residues of the human immunoglobulin are replaced by corresponding nonhuman residues. In some embodiments, humanized antibodies comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance.

Humanized antibodies can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, *Molecular Immunology*, 1991, 28(4/5):489-498; Studnicka, et al., *Protein Engineering*, 1994, 7(6):805-814; and Roguska, et al., *Proc. Natl. Acad. Sci. USA*, 1994, 91:969-73), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, e.g., U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 93/17105; Tan, et al., *J. Immunol.*, 2002, 169:1119-25; Caldas, et al, *Protein Eng.*, 2000, 13(5):353-60; Morea et al, *Methods*, 2000, 20(3):267-79, Baca, et al., *J. Biol. Chem.*, 1997, 272(16):10678-84; Roguska, et al., *Protein Eng.*, 1996, 9(10):895-904; Couto, et al., *Cancer Res.*, 1995, 55 (23 Supp):5973s-5977s; Couto, et al., *Cancer Res.*, 1995, 55(8): 1717-22; Sandhu, J.S., *Gene*, 1994, 150(2):409-10 and Pedersen, et al, *J. Mol. Biol.*, 1994, 235(3):959-73. See also U.S. Patent Pub. No. US 2005/0042664 A1 (Feb. 24, 2005), each of which is incorporated by reference herein in its entirety. In some embodiments, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of the six CDRs of the parent non-human antibody (e.g., rodent) are grafted onto a human antibody framework. For example, Padlan, et al., determined that only about one third of the residues in the CDRs actually contact the antigen, and termed these the “specificity determining residues,” or SDRs (Padlan, et al, *FASEB J.*, 1995, 9: 133-9). In the technique of SDR grafting, only the SDR residues are grafted onto the human antibody framework (see, e.g., Kashmiri, et al., *Methods*, 2005, 36:25-34).

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. For example, according to the so-called “best-fit” method, the sequence of the variable domain of a non-human (e.g., rodent) antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent may be selected as the human framework for the humanized antibody (Sims et al., *J. Immunol.*, 1993, 151:2296-308; and Chothia et al., *J. Mol. Biol.*, 1987, 196:901-17).

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 (Carter et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89:4285-89; and Presta et al., *J Immunol.*, 1993, 151:2623-32). In some embodiments, the framework is derived from the consensus sequences of the most abundant human subclasses, VL6 subgroup I (VL6I) and VH subgroup III (VHIII).

In an alternative method based on comparison of CDRs, called superhumanization, FR homology is irrelevant. The method consists of comparison of the non-human sequence with the functional human germline gene repertoire. Those genes encoding the same or closely related canonical structures to the murine sequences are then selected. Next, within the genes sharing the canonical structures with the non-human antibody, those with highest homology within the CDRs are chosen as FR donors. Finally, the non-human CDRs are grafted onto these FRs (see, e.g., Tan et al., *J. Immunol.*, 2002, 169:1119-25).

Another method for antibody humanization is based on a metric of antibody humanness termed Human String Content (HSC). This method compares the mouse sequence with the repertoire of human germline genes, and the differences are scored as HSC. The target sequence is then humanized by maximizing its HSC rather than using a global identity measure to generate multiple diverse humanized variants (Lazar et al., *Mol. Immunol.*, 2007, 44:1986-98).

In addition to the methods described above, empirical methods may be used to generate and select humanized antibodies. These methods include those that are based upon the generation of large libraries of humanized variants and selection of the best clones using enrichment technologies or high throughput screening techniques. Antibody variants may be isolated from phage, ribosome, and yeast display libraries as well as by bacterial colony screening (see, e.g., Hoogenboom, *Nat. Biotechnol.*, 2005, 23:1105-16; Dufner, et al., *Trends Biotechnol.*, 2006, 24:523-9; Feldhaus, et al., *Nat. Biotechnol.*, 2003, 21:163-70; and Schlapschy et al, *Protein Eng. Des. Sel.*, 2004, 17:847-60).

In the FR library approach, a collection of residue variants is introduced at specific positions in the FR followed by screening of the library to select the FR that best supports the grafted CDR. The residues to be substituted may include some or all of the "Vernier" residues identified as potentially contributing to CDR structure (see, e.g., Foote

and Winter, J. Mol. Biol., 1992, 224:487-99), or from the more limited set of target residues identified by Baca, et al., J. Biol. Chem., 1997, 272:10678-84.

In FR shuffling, whole FRs are combined with the non-human CDRs instead of creating combinatorial libraries of selected residue variants (see, e.g., DalTAcqua et al., Methods, 2005, 36:43-60). The libraries may be screened for binding in a two-step process, first humanizing VL, followed by VH. Alternatively, a one-step FR shuffling process may be used. Such a process has been shown to be more efficient than the two-step screening, as the resulting antibodies exhibited improved biochemical and physicochemical properties including enhanced expression, increased affinity, and thermal stability (see, e.g., Damschroder, et al., Mol. Immunol., 2007, 44:3049-60).

The “humaneering” method is based on experimental identification of essential minimum specificity determinants (MSDs) and is based on sequential replacement of non-human fragments into libraries of human FRs and assessment of binding. It begins with regions of the CDR3 of non-human VH and VL chains and progressively replaces other regions of the non-human antibody into the human FRs, including the CDR1 and CDR2 of both VH and VL. This methodology typically results in epitope retention and identification of antibodies from multiple subclasses with distinct human V-segment CDRs. Humaneering allows for isolation of antibodies that are 91-96% homologous to human germline gene antibodies (see, e.g., Alfenito, Cambridge Healthtech Institute’s Third Annual PEGS, The Protein Engineering Summit, 2007).

The “human engineering” method involves altering a non-human antibody or antibody fragment, such as a mouse or chimeric antibody or antibody fragment, by making specific changes to the amino acid sequence of the antibody so as to produce a modified antibody with reduced immunogenicity in a human that nonetheless retains the desirable binding properties of the original non-human antibodies. Generally, the technique involves classifying amino acid residues of a non-human (e.g., mouse) antibody as “low risk,” “moderate risk,” or “high risk” residues. The classification is performed using a global risk/reward calculation that evaluates the predicted benefits of making particular substitution (e.g., for immunogenicity in humans) against the risk that the substitution will affect the resulting antibody’s folding. The particular human amino acid residue to be substituted at a given position (e.g., low or moderate risk) of a non-human (e.g., mouse) antibody sequence can be selected by aligning an amino acid sequence from the non-human antibody’s variable regions with the corresponding region

of a specific or consensus human antibody sequence. The amino acid residues at low or moderate risk positions in the non-human sequence can be substituted for the corresponding residues in the human antibody sequence according to the alignment. Techniques for making human engineered proteins are described in greater detail in Studnicka et al., *Protein Engineering*, 1994, 7:805-14; U.S. Pat. Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619; and PCT Publication WO 93/11794.

The antibodies disclosed herein can be composite human antibodies. A composite human antibody can be generated using, for example, Composite Human Antibody™ technology (Antitope Ltd., Cambridge, United Kingdom). To generate composite human antibodies, variable region sequences are designed from fragments of multiple human antibody variable region sequences in a manner that avoids T cell epitopes, thereby minimizing the immunogenicity of the resulting antibody. Such antibodies can comprise human constant region sequences, e.g., human light chain and/or heavy chain constant regions.

The antibodies disclosed herein can be deimmunized antibodies. A deimmunized antibody is an antibody in which T-cell epitopes have been removed. Methods for making deimmunized antibodies have been described (see, e.g., Jones, et al., *Methods Mol Biol.*, 2009, 525:405-23; and De Groot, et al., *Cell. Immunol.*, 2006, 244:148-153). Deimmunized antibodies comprise T-cell epitope-depleted variable regions and human constant regions. Briefly, VH and VL of an antibody are cloned and T-cell epitopes are subsequently identified by testing overlapping peptides derived from the VH and VL of the antibody in a T cell proliferation assay. T cell epitopes are identified via in silico methods to identify peptide binding to human MHC class II. Mutations are introduced in the VH and VL to abrogate binding to human MHC class II. Mutated VH and VL are then utilized to generate the deimmunized antibody.

It is further generally desirable that antibodies be humanized with retention of their affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are 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.

These include, for example, WAM (Whitelegg and Rees, *Protein Eng.*, 2000, 13:819-24), Modeller (Sali and Blundell, *J. Mol. Biol.*, 1993, 234:779-815), and Swiss PDB Viewer (Guex and Peitsch, *Electrophoresis*, 1997, 18:2714-23). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., 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 recipient and import sequences 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 antibodies disclosed herein can be fully human antibodies, which possess an amino acid sequence corresponding to that of an antibody produced by a human. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 1991, 227:381; Marks, et al., 1991, *J. Mol. Biol.*, 1991, 222:581) and yeast display libraries (Chao, et al, *Nature Protocols* , 2006, 1: 755-68). Also available for the preparation of human monoclonal antibodies are methods described in Cole, et al., *Monoclonal Antibodies and Cancer Therapy* 77 (1985); Boerner, et al., *J. Immunol.*, 1991, 147(1):86-95; and van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 2001, 5: 368-74. Human antibodies can also be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, e.g., mice (see, e.g., Jakobovits, *Curr. Opin. Biotechnol.*, 1995, 6(5):561-66; Bruggemann and Taussing, *Curr. Opin. Biotechnol.*, 1997, 8(4):455-58; and U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li, et al., *Proc. Natl. Acad. Sci. USA* , 2006, 103:3557-62, regarding human antibodies generated via a human B-cell hybridoma technology.

The antibodies disclosed herein can be recombinant human antibodies, which are human antibodies prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (e.g., a mouse or cow) that is transgenic and/or

transchromosomal for human immunoglobulin genes (see e.g., Taylor, L. D., et al., Nucl. Acids Res., 199220:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. In some embodiments, such recombinant human antibodies can have variable and constant regions derived from human germline immunoglobulin sequences (See Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In some embodiments, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

Modifications, Variations, and Derivatives

Amino acid sequence modification(s) of the monoclonal antibody or antigen-binding fragment thereof provided herein are contemplated. For example, it may be desirable to improve the binding affinity between the monoclonal antibody or antigen-binding fragment thereof and the viral envelope spike protein of a SARS-CoV-2; it may also be desirable to improve other biological properties of the monoclonal antibody or antigen-binding fragment thereof, including but not limited to specificity, thermostability, expression level, or solubility. Thus, in addition to the monoclonal antibody or antigen-binding fragment thereof described herein, it is contemplated that variants can be prepared.

In some embodiments, the monoclonal antibody or antigen-binding fragment thereof provided herein are chemically modified, for example, by the covalent attachment of any type of molecule to the monoclonal antibody or antigen-binding fragment thereof. Exemplary non-limiting modifications include glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Additionally, the monoclonal antibody or antigen-binding fragment thereof may contain one or more non-classical amino acids.

In some embodiments, variations may be a substitution, deletion, or insertion of one or more codons encoding the monoclonal antibody or antigen-binding fragment

thereof that results in a change in the amino acid sequence as compared with the original sequence. The variation allowed may be determined by systematically making insertions, deletions, or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, e.g., conservative amino acid replacements. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule provided herein, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

Substitutions may be in the range of about 1 to 100 amino acids. In some embodiments, the substitution includes fewer than about 100 amino acid substitutions, fewer than about 95 amino acid substitutions, fewer than about 90 amino acid substitutions, fewer than about 85 amino acid substitutions, fewer than about 80 amino

acid substitutions, fewer than about 75 amino acid substitutions, fewer than about 70 amino acid substitutions, fewer than about 65 amino acid substitutions, fewer than about 50 amino acid substitutions, fewer than about 45 amino acid substitutions, fewer than about 40 amino acid substitutions, fewer than about 35 amino acid substitutions, fewer than about 30 amino acid substitutions, fewer than about 25 amino acid substitutions, fewer than about 20 amino acid substitutions, fewer than about 15 amino acid substitutions, fewer than about 10 amino acid substitutions, fewer than about 5 amino acid substitutions, fewer than about 4 amino acid substitutions, fewer than about 3 amino acid substitutions, or fewer than about 2 amino acid substitutions relative to the original molecule.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from 1 residue to polypeptides containing 100 or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N or C terminus of the antibody to an enzyme (e.g., for antibody-directed enzyme prodrug therapy) or a polypeptide which increases the serum half-life of the antibody.

In some embodiments, the insertion is about 1 amino acid to about 100 amino acids. In some embodiments, the insertion is at least about 1 amino acid. In some embodiments, the insertion is at most about 100 amino acids. In some embodiments, the insertion is about 1 amino acid to about 5 amino acids, about 1 amino acid to about 10 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 30 amino acids, about 1 amino acid to about 40 amino acids, about 1 amino acid to about 50 amino acids, about 1 amino acid to about 60 amino acids, about 1 amino acid to about 70 amino acids, about 1 amino acid to about 80 amino acids, about 1 amino acid to about 90 amino acids, about 1 amino acid to about 100 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 30 amino acids, about 5 amino acids to about 40 amino acids, about 5 amino acids to about 50 amino acids, about 5 amino acids to about 60 amino acids, about 5 amino acids to about 70 amino acids, about 5 amino acids to about 80 amino acids, about 5 amino acids to about 90 amino acids, about 5 amino acids to about 100 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 50

amino acids, about 10 amino acids to about 60 amino acids, about 10 amino acids to about 70 amino acids, about 10 amino acids to about 80 amino acids, about 10 amino acids to about 90 amino acids, about 10 amino acids to about 100 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 70 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids to about 100 amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 60 amino acids, about 30 amino acids to about 70 amino acids, about 30 amino acids to about 80 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids to about 100 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about 100 amino acids, about 50 amino acids to about 60 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 100 amino acids, about 60 amino acids to about 70 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 100 amino acids, about 70 amino acids to about 80 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 100 amino acids, about 80 amino acids to about 90 amino acids, about 80 amino acids to about 100 amino acids, or about 90 amino acids to about 100 amino acids. In some embodiments, the insertion is about 1 amino acid, about 5 amino acids, about 10 amino acids, about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, or about 100 amino acids.

Amino acid sequence deletions include amino- and/or carboxyl-terminal deletions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence deletions of single or multiple amino acid residues.

In some embodiments, the deletion is about 1 amino acid to about 100 amino acids. In some embodiments, the deletion is at least about 1 amino acid. In some embodiments, the deletion is at most about 100 amino acids. In some embodiments, the

[illegible]

acids to about 90 amino acids, about 70 amino acids to about 100 amino acids, about 80 amino acids to about 90 amino acids, about 80 amino acids to about 100 amino acids, or about 90 amino acids to about 100 amino acids. In some embodiments, the deletion is about 1 amino acid, about 5 amino acids, about 10 amino acids, about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, or about 100 amino acids.

In some embodiments, a polypeptide variant comprising an amino acid sequence that is at least about 75 %, about 80 %, about 85 %, about 90 %, about 91 %, about 92 %, about 93 %, about 94 %, about 95 %, about 96 %, about 97 %, about 98%, or about 99 % identical to the amino acid sequence of a polypeptide disclosed herein.

Molecule derived from antibodies disclosed herein are contemplated. A “molecule derived from an antibody” refers to a functional antigen-binding fragment of an antibody. It is a portion of an antibody heavy and/or light chain polypeptide that retains some or all of the binding activity of the antibody from which the fragment was derived. Non-limiting examples of functional fragments include single-chain Fvs (scFv), Fab fragments, F(ab') fragments, F(ab)₂ fragments, F(ab')₂ fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fv fragments, diabody, triabody, tetrabody, and minibody. Such functional antigen-binding fragment can be found in, for example, Harlow and Lane, *Antibodies: A Laboratory Manual* (1989); Mol. Biology and Biotechnology: A Comprehensive Desk Reference (Myers, ed., 1995); Huston, et al, 1993, *Cell Biophysics* 22:189-224; Plickthun and Skerra, 1989, *Meth. Enzymol.* 178:497-515; and Day, *Advanced Immunochemistry* (2d ed. 1990).

Polynucleotides

Provided herein are polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof that binds to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) disclosed herein.

Polynucleotides disclosed herein can be at least about 5, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 40, at least about 50, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 1000, at least about 5000, at least about 10000, or at least about 15000 or more nucleotides in length, as well as all intermediate lengths. It will be readily understood that “intermediate lengths,” in this context, means any length between

the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.*

The present disclosure further relates to variants of the polynucleotides disclosed herein. The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both.

In some embodiments, a polynucleotide variant comprising a nucleotide sequence that is at least about 75 %, about 80 %, about 85 %, about 90 %, about 91 %, about 92 %, about 93 %, about 94 %, about 95 %, about 96 %, about 97 %, about 98%, or about 99 % identical to the nucleotide sequence of a polynucleotide disclosed herein.

In some embodiments, a polynucleotide variant contains substitutions, additions, or deletions that alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant contains silent substitutions, additions, or deletions that do not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

In some embodiments, polynucleotides are codon-optimized. As used herein, the term “codon-optimized” refers to substituting codons in a polynucleotide encoding a polypeptide in order to increase the expression, stability and/or activity of the polypeptide. Factors that influence codon optimization include, but are not limited to one or more of: (i) variation of codon biases between two or more organisms or genes or synthetically constructed bias tables, (ii) variation in the degree of codon bias within an organism, gene, or set of genes, (iii) systematic variation of codons including context, (iv) variation of codons according to their decoding tRNAs, (v) variation of codons according to GC %, either overall or in one position of the triplet, (vi) variation in degree of similarity to a reference sequence for example a naturally occurring sequence, (vii) variation in the codon frequency cutoff, (viii) structural properties of mRNAs transcribed from the DNA sequence, (ix) prior knowledge about the function of the DNA sequences

upon which design of the codon substitution set is to be based, (x) systematic variation of codon sets for each amino acid, (xi) isolated removal of spurious translation initiation sites and/or (xii) elimination of fortuitous polyadenylation sites otherwise leading to truncated RNA transcripts.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide, or fragment of variant thereof, as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated in particular embodiments, for example polynucleotides that are optimized for human and/or primate codon selection. Further, alleles of the genes comprising the polynucleotide sequences provided herein may also be used. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides.

The polynucleotides contemplated herein, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters and/or enhancers, untranslated regions (UTRs), signal sequences, Kozak sequences, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, internal ribosomal entry sites (IRES), recombinase recognition sites (*e.g.*, LoxP, FRT, and Att sites), termination codons, transcriptional termination signals, and polynucleotides encoding self-cleaving polypeptides, epitope tags, as disclosed elsewhere herein or as known in the art, such that their overall length may vary considerably. It is therefore contemplated that a polynucleotide fragment of almost any length may be employed in particular embodiments, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

Polynucleotides can be prepared, isolated, purified, manipulated, and/or expressed using any of a variety of well-established techniques known and available in the art.

Vectors

Provided herein are vectors comprising the polynucleotides or nucleic acid molecules disclosed herein.

In order to express the monoclonal antibody or antigen-binding fragment thereof described herein in a cell, an expression cassette encoding the monoclonal antibody or antigen-binding fragment thereof can be inserted into a nucleic acid vector. The

“expression cassette” contains the gene of interest. The cassette is positionally and sequentially oriented within the vector such that the nucleic acid in the cassette can be transcribed into RNA, and when necessary, translated into a protein or a polypeptide, undergo appropriate post-translational modifications required for activity in the host cell, and be translocated to the appropriate compartment for biological activity by targeting to appropriate intracellular compartments or secretion into extracellular compartments. Preferably, the cassette has its 3' and 5' ends adapted for ready insertion into a vector, e.g., it has restriction endonuclease sites at each end. The cassette can be removed and inserted into a plasmid or viral vector as a single unit.

The host cell may be co-transfected with two vectors provided herein, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature*, 1986, 322:52; and Kohler, *Proc. Natl. Acad. Sci. USA*, 1980, 77:2197-9).

In some embodiments, vectors include, without limitation, plasmids, phagemids, cosmids, transposons, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. In some embodiments, the coding sequences of the monoclonal antibody or antigen-binding fragment thereof disclosed herein can be ligated into such vectors for expression in mammalian cells.

In some embodiments, non-viral vectors are used to deliver one or more polynucleotides contemplated herein. In some embodiments, the recombinant vector comprising a polynucleotide encoding the monoclonal antibody or antigen-binding fragment thereof described herein is a plasmid. Numerous suitable plasmid expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other plasmid vector may be used so long as it is compatible with the host cell.

In some embodiments, viral vectors are used to deliver one or more polynucleotides contemplated herein. Suitable viral vectors include, but are not limited to, viral vectors based on adenovirus (see, e.g., Li et al., *Invest Ophthalmol Vis Sci* 35:2543-2549, 1994; Borrás et al., *Gene Ther* 6:515-524, 1999; Li and Davidson, *PNAS* 92:7700-7704, 1995; Sakamoto et al., *Hum Gene Ther* 5:1088-1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., U.S. Patent No. 7,078,387; Ali et al., *Hum Gene Ther* 9:81-86, 1998; Flannery et al., *PNAS* 94:6916-6921, 1997; Bennett et al., *Invest Ophthalmol Vis Sci* 38:2857-2863, 1997; Jomary et al., *Gene Ther* 4:683-690, 1997; Rolling et al., *Hum Gene Ther* 10:641-648, 1999; Ali et al., *Hum Mol Genet* 5:591-594, 1996; Srivastava in WO 93/09239; Samulski et al., *J. Vir.* (1989) 63:3822-3828; Mendelson et al., *Virol.* (1988) 166:154-165; and Flotte et al., *PNAS* (1993) 90:10613-10617); alphaviruses; arenaviruses; baculovirus; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., *PNAS* 94:10319-23, 1997; Takahashi et al., *J Virol* 73:7812-7816, 1999); poliovirus; poxvirus; retrovirus (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); SV40; vaccinia virus; and the like. Examples of vectors are pCIneo vectors (Promega) for expression in mammalian cells; pLenti4/V5-DEST™, pLenti6/V5-DEST™, and pLenti6.2/V5-GW/lacZ (Invitrogen) for lentivirus-mediated gene transfer and expression in mammalian cells.

In some embodiments, the vector is a non-integrating vector, including but not limited to, an episomal vector or a vector that is maintained extrachromosomally. As used herein, the term “episomal” refers to a vector that is able to replicate without integration into host’s chromosomal DNA and without gradual loss from a dividing host cell also meaning that said vector replicates extrachromosomally or episomally. The vector is engineered to harbor the sequence coding for the origin of DNA replication or “ori” from a lymphotropic herpes virus or a gamma herpesvirus, an adenovirus, SV40, a bovine papilloma virus, or a yeast, specifically a replication origin of a lymphotropic herpes virus or a gamma herpesvirus corresponding to oriP of EBV. In some embodiments, the lymphotropic herpes virus may be Epstein Barr virus (EBV), Kaposi’s sarcoma herpes virus (KSHV), Herpes virus saimiri (HS), or Marek’s disease virus (MDV). Epstein Barr virus (EBV) and Kaposi’s sarcoma herpes virus (KSHV) are also examples of a gamma

herpesvirus. A viral vector delivered by such viruses or viral particles may be referred to by the type of virus to deliver the viral vector (e.g., a lentiviral vector is a viral vector that is to be delivered by a lentivirus). A viral vector can contain viral elements (e.g., nucleotide sequences) necessary for packaging of the viral vector into the virus or viral particle, replicating the virus, or other desired viral activities. A virus containing a viral vector may be replication competent, replication deficient or replication defective.

In some embodiments, the vector is an integrating vector. In some embodiments, a polynucleotide is introduced into a target or host cell using a transposon vector system. In some embodiments, the transposon vector system comprises a vector comprising transposable elements and a polynucleotide contemplated herein; and a transposase. In some embodiments, the transposon vector system is a single transposase vector system, see, e.g., WO 2008/027384. Exemplary transposases include, but are not limited to: piggyBac, Sleeping Beauty, Mos1, Tc1/mariner, Tol2, mini-Tol2, Tc3, MuA, Himar I, Frog Prince, and derivatives thereof. The piggyBac transposon and transposase are described, for example, in U.S. Patent 6,962,810, which is incorporated herein by reference in its entirety. The Sleeping Beauty transposon and transposase are described, for example, in Izsvak et al., *J. Mol. Biol.* 302: 93-102 (2000), which is incorporated herein by reference in its entirety. The Tol2 transposon which was first isolated from the medaka fish *Oryzias latipes* and belongs to the hAT family of transposons is described in Kawakami et al. (2000). Mini-Tol2 is a variant of Tol2 and is described in Balciunas et al. (2006). The Tol2 and Mini-Tol2 transposons facilitate integration of a transgene into the genome of an organism when co-acting with the Tol2 transposase. The Frog Prince transposon and transposase are described, for example, in Miskey et al., *Nucleic Acids Res.* 31:6873-6881 (2003).

In some embodiments, a polynucleotide sequence encoding the monoclonal antibody or antigen-binding fragment thereof disclosed herein is operably linked to one or more control elements that allow expression of the polynucleotide in both prokaryotic and eukaryotic cells. "Control elements" refer to those non-translated regions of the vector which interact with host cellular proteins to carry out transcription and translation. Non-limiting examples of control elements include origin of replication, selection cassettes, constitutive and inducible promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, transcription terminators, 5' and 3' untranslated regions. See e.g., Bitter et al. (1987) *Methods in Enzymology*, 153:516-544)

Such elements may vary in their strength and specificity. The transcriptional control element may be functional in either a eukaryotic cell (e.g., a mammalian cell) or a prokaryotic cell (e.g., bacterial or archaeal cell).

In some embodiments, polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof described herein are operably linked to a promoter and/or an enhancer. The term “promoter” as used herein refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. An RNA polymerase initiates and transcribes polynucleotides operably linked to the promoter. In some embodiments, promoters operative in mammalian cells comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide. The term “enhancer” refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements.

Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, a viral simian virus 40 (SV40) (e.g., early and late SV40), a spleen focus forming virus (SFFV) promoter, long terminal repeats (LTRs) from retrovirus (e.g., a Moloney murine leukemia virus (MoMLV) LTR promoter or a Rous sarcoma virus (RSV) LTR), a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1- α (EF1 α) promoter, early growth response 1 (EGR1) promoter, a ferritin H (FerH) promoter, a ferritin L (FerL) promoter, a Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, a eukaryotic translation initiation factor 4A1 (EIF4A1) promoter, a heat shock 70kDa protein 5 (HSPA5) promoter, a heat shock protein 90kDa beta, member 1 (HSP90B1) promoter, a heat shock protein 70kDa (HSP70) promoter, a β -kinesin (β -KIN) promoter, the human ROSA 26 locus (Irions et al., Nature Biotechnology 25, 1477-1482 (2007)), a Ubiquitin C (UBC) promoter, a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β -actin (CAG) promoter, a β -actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter, and mouse

metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In some embodiments, a polynucleotide sequence encoding the monoclonal antibody or antigen-binding fragment thereof described herein is operably linked to a constitutive promoter. In such embodiments, the polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof described herein are constitutively and/or ubiquitously expressed in a cell.

In some embodiments, a polynucleotide sequence encoding the monoclonal antibody or antigen-binding fragment thereof described herein is operably linked to an inducible promoter. In such embodiments, polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof described herein are conditionally expressed. As used herein, “conditional expression” may refer to any type of conditional expression including, but not limited to, inducible expression; repressible expression; expression in cells or tissues having a particular physiological, biological, or disease state (e.g., cell type or tissue specific expression) etc. Illustrative examples of inducible promoters/systems include, but are not limited to, steroid-inducible promoters such as promoters for genes encoding glucocorticoid or estrogen receptors (inducible by treatment with the corresponding hormone), metallothionein promoter (inducible by treatment with various heavy metals), MX-1 promoter (inducible by interferon), the “GeneSwitch” mifepristone-regulatable system (Sirin et al., 2003, *Gene*, 323:67), the cumate inducible gene switch (WO 2002/088346), tetracycline-dependent regulatory systems, etc.

In some embodiments, the vectors described herein further comprise a transcription termination signal. Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increase heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In some embodiments, vectors comprise a polyadenylation sequence 3' of a polynucleotide encoding a polypeptide to be expressed. The term “polyA site” or “polyA sequence” as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3' end of the coding sequence and thus, contribute to increased translational efficiency. Cleavage and polyadenylation are directed by a poly(A) sequence in the RNA. The core

poly(A) sequence for mammalian pre-mRNAs has two recognition elements flanking a cleavage-polyadenylation site. Typically, an almost invariant AAUAAA hexamer lies 20-50 nucleotides upstream of a more variable element rich in U or GU residues. Cleavage of the nascent transcript occurs between these two elements and is coupled to the addition of up to 250 adenosines to the 5' cleavage product. In some embodiments, the core poly(A) sequence is an ideal polyA sequence (e.g., AATAAA, ATTAAA, AGTAAA). In some embodiments, the poly(A) sequence is an SV40 polyA sequence, a bovine growth hormone polyA sequence (BGHpA), a rabbit β -globin polyA sequence (r β gpA), variants thereof, or another suitable heterologous or endogenous polyA sequence known in the art.

The expression vector may also include nucleotide sequences encoding protein tags (e.g., 6xHis tag, hemagglutinin tag, green fluorescent protein, etc.) that are fused to the site-directed modifying polypeptide, thus resulting in a chimeric polypeptide.

Methods of introducing polynucleotides and recombinant vectors into a host cell are known in the art. Suitable methods include e.g., viral or bacteriophage infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro injection, nanoparticle-mediated nucleic acid delivery (see, e.g., Panyam et al., *Adv Drug Deliv Rev.* 2012 Sep 13. pii: S0169-409X(12)00283-9), microfluidics delivery methods (See e.g., International PCT Publication No. WO 2013/059343), and the like.

In some embodiments, delivery via electroporation comprises mixing the cells with the polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof in a cartridge, chamber, or cuvette and applying one or more electrical impulses of defined duration and amplitude. In some embodiments, cells are mixed with polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof in a vessel connected to a device (e.g., a pump) which feeds the mixture into a cartridge, chamber, or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel. Illustrative examples of polynucleotide delivery systems suitable for use in particular embodiments contemplated include, but are not limited to, those provided by Amaxa Biosystems, Maxcyte, Inc., BTX Molecular Delivery Systems, NeonTM Transfection Systems, and Copernicus Therapeutics Inc. Lipofection reagents are sold commercially (e.g.,

Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient lipofection of polynucleotides have been described in the literature. See e.g., Liu et al. (2003) *Gene Therapy*. 10:180–187; and Balazs et al. (2011) *Journal of Drug Delivery*. 2011:1-12.

In some embodiments, polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof described herein are introduced to a cell in a non-viral delivery vehicle, such as a transposon, a nanoparticle (e.g., a lipid nanoparticle), a liposome, an exosome, an attenuated bacterium, or a virus-like particle. In some embodiments, the vehicle is an attenuated bacterium (e.g., naturally or artificially engineered to be invasive but attenuated to prevent pathogenesis including *Listeria monocytogenes*, certain *Salmonella* strains, *Bifidobacterium longum*, and modified *Escherichia coli*), bacteria having nutritional and tissue-specific tropism to target specific cells, and bacteria having modified surface proteins to alter target cell specificity. In some embodiments, the vehicle is a genetically modified bacteriophage (e.g., engineered phages having large packaging capacity, less immunogenicity, containing mammalian plasmid maintenance sequences and having incorporated targeting ligands). In some embodiments, the vehicle is a biological liposome. For example, the biological liposome is a phospholipid-based particle derived from human cells (e.g., erythrocyte ghosts, which are red blood cells broken down into spherical structures derived from the subject and wherein tissue targeting can be achieved by attachment of various tissue or cell-specific ligands), secretory exosomes, or subject-derived membrane-bound nanovesicles (30 -100 nm) of endocytic origin (e.g., can be produced from various cell types and can therefore be taken up by cells without the need for targeting ligands).

In some embodiments, vectors comprising polynucleotides encoding the monoclonal antibody or antigen-binding fragment thereof described herein are introduced to cells by viral delivery methods, e.g., by viral transduction. A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. The heterologous nucleic acid can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to the engineered mammalian cell in vitro or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are

used. In some embodiments, self-inactivating lentiviral vectors are used. For example, self-inactivating lentiviral vectors carrying the immunomodulator (such as immune checkpoint inhibitor) coding sequence and/or self-inactivating lentiviral vectors carrying chimeric antigen receptors can be packaged with protocols known in the art. The resulting lentiviral vectors can be used to transduce a mammalian cell (such as primary human T cells) using methods known in the art. Vectors derived from retroviruses such as lentivirus are suitable tools to achieve long-term gene transfer, because they allow long-term, stable integration of a transgene and its propagation in progeny cells. Lentiviral vectors also have low immunogenicity, and can transduce nonproliferating cells.

In some embodiments, the vehicle is a mammalian virus-like particle. For example, modified viral particles can be generated (e.g., by purification of the “empty” particles followed by ex vivo assembly of the virus with the desired cargo).

Compositions and Kits

Provided herein are pharmaceutical compositions comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein. Also provided herein are pharmaceutical compositions comprising at least two components, wherein each component comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein, wherein each component is different.

In some embodiments, the pharmaceutical composition comprises two components, wherein each component comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein, wherein each component is different. In some embodiments, the pharmaceutical compositions comprise three components, wherein each component comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein, wherein each component is different. In some embodiments, the pharmaceutical compositions comprise four components, wherein each component comprising the monoclonal antibody or antigen-binding fragment, the polynucleotide, or the vector disclosed herein, wherein each component is different.

In some embodiments, the present disclosure provides compositions comprising two or more monoclonal antibodies, or antigen binding fragments thereof, described herein. In some embodiments, the present disclosure provides compositions comprising 2, 3, or 4 monoclonal antibodies, or antigen binding fragments thereof, described herein. For

example, in some embodiments, the present disclosure provides a composition comprising the following combinations of monoclonal antibodies or antigen binding fragments thereof:

- a. C68.3 and C68.13;
- b. C68.3 and C68.59;
- c. C68.3 and C68.61;
- d. C68.13 and C68.59;
- e. C68.13 and C68.61;
- f. C68.59 and C68.61;
- g. C68.3, C68.13, C68.59;
- h. C68.3, C68.13, C68.61;
- i. C68.3, C68.59, and C68.61;
- j. C68.13, C68.59, and C68.61; or
- k. C68.3, C68.13, C68.59, and C68.61.

In some embodiments, the pharmaceutical compositions disclosed herein are for use in treating a SARS-CoV-2 infection in a subject. In some embodiments, the pharmaceutical compositions disclosed herein are for use in the prevention of a SARS-CoV-2 infection in a subject. Details related to treatment and prevention are disclosed in the “Therapeutic Methods and Applications” section below.

In some embodiments, the pharmaceutical composition further comprises a pharmaceutical acceptable carrier.

Pharmaceutically acceptable carrier, diluent or excipient includes, without limitation, any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter; waxes; animal and vegetable fats; paraffins; silicones; bentonites; silicic acid; zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols,

such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations. Except insofar as any conventional media and/or agent is incompatible with the agents of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

Pharmaceutically acceptable salt includes both acid and base addition salts. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pantoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-

dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine, tromethamine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Further guidance regarding formulations that are suitable for various types of administration can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, Pa., 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, Science 249:1527-1533 (1990).

Also provided herein are kits for detecting a SARS-CoV-2 infection in a subject. In some embodiments, the kit comprises the monoclonal antibody or antigen-binding fragment thereof disclosed herein. In some embodiments, the kit further comprises one or more dyes or labels. In some embodiments, the one or more dyes or labels are attached to the monoclonal antibody or antigen-binding fragment thereof disclosed herein, covalently or non-covalently. In some embodiments, the one or more dyes or labels are not attached to the monoclonal antibody or antigen-binding fragment thereof disclosed herein.

In some embodiments, the kit further comprises instructions for using the components of the kit to practice the methods of the present disclosure. The instructions for practicing the methods are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert or in the labeling of

the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging). In some embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g., CD-ROM, diskette, flash drive, etc. In some embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

Therapeutic Methods and Applications

Provided herein are methods of treating a SARS-CoV-2 infection in a subject, comprising administering to the subject a therapeutically effective amount of the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein. Also provided herein are methods of preventing a SARS-CoV-2 infection in a subject, comprising administering to the subject a therapeutically effective amount of the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein.

In some embodiments, the method described herein may be used in the treatment and/or prevention of SARS-CoV-2. Exemplary SARS-CoV-2 variants include WHO alpha variant, WHO beta variant, WHO gamma variant, WHO delta variant, WHO epsilon variant, WHO Eta variant, WHO iota variant, WHO kappa variant, WHO omicron variant, WHO zeta variant, WHO mu variant, and B.1.617.3.

In some embodiments, the subject may be a neonate, a juvenile, or an adult. In some embodiments, the subject is human. In some embodiments, the subject is non-human primates (*e.g.*, monkeys, baboons, and chimpanzees), mice, rats, bovines, horses, household cats, tigers and other large cats, dogs, pigs, rabbits, goats, deer, sheep, ferrets, gerbils, guinea pigs, hamsters, bats, and birds (*e.g.*, chickens, turkeys, and ducks). A number of these household pets and farm animals are capable of carrying and transmitting SARS-CoV-2 viruses without themselves getting substantially sick or dying, thereby transmitting the disease to humans. Thus, in some embodiments, these animals are treated not because they are suffering from disease, but rather, because they can transmit viruses to humans and cause human disease.

In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof are administered to a subject in order to prevent infection with a SARS-CoV-2 virus. In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein are administered to a subject prior to exposure to or infection with SARS-CoV-2 viruses. In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein are administered to a subject after the exposure to or infection with SARS-CoV-2 viruses.

In some embodiments, the present disclosure provides methods of preventing infection with a SARS-CoV-2 virus in an immunocompromised subject. Immunocompromised subjects include subjects that suffer from an immune deficiency (*e.g.*, a primary or acquired immune deficiency) or autoimmune disease, subjects that have undergone or are currently undergoing treatment with one or more immunosuppressive drugs (*e.g.*, chemotherapy, glucocorticoids, protease inhibitors, immune cell depleting monoclonal antibodies, etc.), subjects that have recently received an organ transplant or hematopoietic stem cell transplant, subjects that have received a CAR-T therapy, and subjects that have undergone or are currently undergoing radiation treatment. Immunosuppressive drugs are known to those in the art. See *e.g.*, Hussain Y, Khan H. Immunosuppressive Drugs. Encyclopedia of Infection and Immunity. 2022:726–40. Vaccines, including vaccines against SARS-CoV-2 infections, are less effective in immunocompromised subjects. Furthermore, some immunocompromised subjects may not be able to receive a SARS-CoV-2 vaccine. The antibodies and antigen-binding fragments thereof provided herein therefore provide a therapeutic option for subjects who cannot receive a SARS-CoV-2 vaccine or who demonstrate reduced efficacy of a SARS-CoV-2 vaccine.

In some embodiments, treating refers to the treatment of a disease in a mammal, *e.g.*, in a human, including (a) inhibiting the disease, *i.e.*, arresting disease development or preventing disease progression; (b) relieving the disease, *i.e.*, causing regression of the disease state or relieving one or more symptoms of the disease; and (c) curing the disease, *i.e.*, remission of one or more disease symptoms. In some embodiments, treatment results in an improvement or remediation of the symptoms of the disease. In some embodiments, treatment may refer to a short-term (*e.g.*, temporary and/or acute) and/or a long-term (*e.g.*, sustained) improvement or remediation in one or more disease symptoms. In some

embodiments, the improvement is an observable or measurable improvement. In some embodiments, the improvement is an improvement in the general feeling of well-being of the subject. In some embodiments, administration of the pharmaceutical compositions disclosed herein may reduce one or more symptoms of the SARS-COV-2 infection, including but not limited to, death, incidence of emphysema, incidence of pneumonia, shortness of breath, racing heart, fever, cough, sore throat, congestion, muscle or body aches, headaches, fatigue, vomiting, diarrhea, loss of taste or smell, cognitive issues like “brain fog”, memory or attention problems, and Postural Orthostatic Tachycardia Syndrome (POTS).

Administration of the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof can occur by infusion (*e.g.*, continuous or bolus), injection, irrigation, inhalation, consumption, electro-osmosis, hemodialysis, iontophoresis, and other methods known in the art.

In some embodiments administration route is intraarterial, intracranial, intradermal, intraduodenal, intramammary, intrameningeal, intraperitoneal, intrathecal, intratumoral, intravenous, intravitreal, ophthalmic, parenteral, spinal, subcutaneous, ureteral, urethral, vaginal, or intrauterine. In some embodiments, administration route is local or systemic.

The effective amount of the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof administered to a particular subject will depend on a variety of factors, several of which will differ from patient to patient including the disorder being treated and the severity of the disorder; activity of the specific agent(s) employed; the age, body weight, general health, sex and diet of the patient; the timing of administration, route of administration; the duration of the treatment; drugs used in combination; the judgment of the prescribing physician; and like factors known in the medical arts. Dosage amount and interval can be adjusted individually to provide plasma levels of the compound(s) which are sufficient to maintain therapeutic or prophylactic effect. In cases of local administration or selective uptake, such as local topical administration, the effective local concentration of active compound(s) cannot be related to plasma concentration. Skilled artisans will be able to optimize effective local dosages without undue experimentation.

Dosage amounts of the pharmaceutical compositions disclosed herein can typically be in the range of from about 0.0001 mg/kg/day to about 1000 mg/kg/day, but

can be higher or lower, depending upon, among other factors, the activity of the compound, its bioavailability, and various factors discussed above. In some embodiments, the dose is from about 0.0001 mg/kg to about 1000 mg/kg of body weight per day. In some embodiments, the dose is from about 0.001 mg/kg to about 1000 mg/kg of body weight per day. In some embodiments, the dose is from about 0.01 mg/kg to about 1000 mg/kg of body weight per day. In some embodiments, the dose is from about 0.1 mg/kg to about 100 mg/kg of body weight per day. In some embodiments, the dose is from about 0.5 mg/kg to about 50 mg/kg of body weight per day. In some embodiments, the dose is from about 1 mg/kg to about 25 mg/kg of body weight per day. In some embodiments, the dose is from about 5 mg/kg to about 15 mg/kg of body weight per day. In some embodiments, the dose is about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg, about 95 mg/kg, or about 100 mg/kg.

The number of administrations of treatment to a subject may vary. In some embodiments, introducing the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof into the subject may be a one-time event. In some embodiments, such treatment may require an on-going series of repeated treatments (e.g., once per day, once per week, or multiple times per day or week). In some embodiments, multiple administrations of the pharmaceutical compositions may be required before an effect is observed. The exact protocols depend upon the disease or condition, the stage of the disease and parameters of the individual subject being treated.

In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein are administered in combination with one or more additional therapeutic composition(s). In some embodiments, the additional therapeutic composition is an anti-viral drug. In some embodiments, the additional therapeutic composition is a viral entry inhibitor. In some embodiments, the additional therapeutic composition is a viral attachment inhibitor.

In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein and the additional therapeutic composition(s) are administered simultaneously. In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed

herein are administered before the additional therapeutic composition(s). In some embodiments, the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein are administered after the additional therapeutic composition(s).

In some embodiments, administration of the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein in combination with the additional therapeutic composition(s) results in an enhanced therapeutic effect in a subject infected with SARS-CoV-2 viruses than is observed by treatment with either the antibodies, antigen-binding fragments thereof, or pharmaceutical compositions thereof disclosed herein or the additional therapeutic composition(s) alone.

Also provided herein are methods of detecting the presence of a SARS-CoV-2 in a sample. In some embodiments, the method comprises (1) contacting the sample with the monoclonal antibody or antigen-binding fragment thereof disclosed herein, and (2) detecting the presence of an antibody-antigen complex. In some embodiments, the presence of the antibody-antigen complex indicates the presence of SARS-CoV-2.

In some embodiments, the sample is a blood sample. In some embodiments, the sample is a nasal swab. In some embodiments, the sample is a throat swab. In some embodiments, the sample is a biopsy, a cheek swab, a nasal aspiration, a sputum sample, a saliva sample, a urine sample, a feces sample, or a semen sample.

EMBODIMENTS

This invention provides the following non-limiting embodiments.

Embodiment 1. An antibody or derivative thereof that binds to an epitope in the viral envelope spike (S) protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), wherein the antibody or derivative thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and a light chain variable domain (VL) comprising an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 2. The antibody or derivative thereof of embodiment 1, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 3. The antibody or derivative thereof of embodiment 1, wherein the VH comprises an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 4. The antibody or derivative thereof of embodiment 1, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and the VL comprises an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 5. The antibody or derivative thereof of any one of embodiments 1-4, wherein the antibody is a monoclonal antibody or a derivative thereof.

Embodiment 6. The antibody or derivative thereof of any one of embodiments 1-5, wherein the antibody or derivative thereof binds to an epitope of the receptor binding domain (RBD) of SARS-CoV-2.

Embodiment 7. The antibody or derivative thereof of any one of embodiments 1-5, wherein the antibody or derivative thereof binds to an epitope outside of the RBD of SARS-CoV-2.

Embodiment 8. The antibody or derivative thereof of any one of embodiments 1-7, wherein the antibody or derivative thereof neutralizes SARS-CoV-2.

Embodiment 9. The antibody or derivative thereof of any one of embodiments 1-7, wherein the antibody or derivative thereof inhibits viral and cell membrane fusion.

Embodiment 10. The antibody or derivative thereof of any one of embodiments 1-7, wherein the antibody or derivative thereof has activity that is cytotoxic to SARS-CoV-2 infected cells.

Embodiment 11. The antibody or derivative thereof of any one of embodiments 1-10, wherein the antibody or derivative thereof is a fully human antibody.

Embodiment 12. The antibody or derivative thereof of any one of embodiments 1-11, wherein the antibody or derivative thereof comprises a VH comprising amino acid sequence SEQ ID NO: 1 and a VL comprising amino acid sequence SEQ ID NO: 2; a VH comprising amino acid sequence SEQ ID NO: 3 and a VL comprising amino acid sequence SEQ ID NO: 4; a VH comprising amino acid sequence SEQ ID NO: 5 and a VL comprising amino acid sequence SEQ ID NO: 6; and a VH comprising amino acid sequence SEQ ID NO: 7 and a VL comprising amino acid sequence SEQ ID NO: 8.

Embodiment 13. The antibody or derivative thereof of any one of embodiments 1-11, wherein the antibody or derivative thereof comprises a VH comprising amino acid sequence SEQ ID NO: 1 and a VL comprising amino acid sequence SEQ ID NO: 2.

Embodiment 14. The antibody or derivative thereof of any one of embodiments 1-11, wherein the antibody or derivative thereof comprises a VH comprising amino acid sequence SEQ ID NO: 3 and a VL comprising amino acid sequence SEQ ID NO: 4.

Embodiment 15. The antibody or derivative thereof of any one of embodiments 1-11, wherein the antibody or derivative thereof comprises a VH comprising amino acid sequence SEQ ID NO: 5 and a VL comprising amino acid sequence SEQ ID NO: 6.

Embodiment 16. The antibody or derivative thereof of any one of embodiments 1-11, wherein the antibody or derivative thereof comprises a VH comprising amino acid sequence SEQ ID NO: 7 and a VL comprising amino acid sequence SEQ ID NO: 8.

Embodiment 17. A composition comprising at least one antibody or derivative thereof of any one of embodiments 1-16 and a pharmaceutically acceptable carrier.

Embodiment 18. A composition in a unit dose form, the unit dose form comprising a first component comprising at least two antibodies or derivative thereof of any one of embodiments 1-16 and a second component comprising a pharmaceutically acceptable carrier.

Embodiment 19. A composition in a unit dose form, the unit dose form comprising a first component comprising at least three antibodies or derivative thereof of any one of embodiments 1-16 and a second component comprising a pharmaceutically acceptable carrier.

Embodiment 20. A composition in a unit dose form, the unit dose form comprising a first component comprising at least one antibody or derivative thereof of any one of embodiments 1-16 and a second antibody specific to SARS-CoV-2 and/or an infection accompanying SARS-CoV-2, and a second component comprising a pharmaceutically acceptable carrier.

Embodiment 21. The composition of any one of embodiments 18-20, wherein the unit dose form comprises the first component combined with the second component in a first container.

Embodiment 22. The composition of any one of embodiments 18-20, wherein the unit dose form comprises the first component in a first container and the second component in a second container.

Embodiment 23. The composition of embodiment 22, wherein the first component is administered simultaneously with the second component.

Embodiment 24. The composition of embodiment 22, wherein the first component is administered before the second component.

Embodiment 25. The composition of embodiment 22, wherein the first component is administered following the second component.

Embodiment 26. A method of preventing or treating a disease or disorder caused by a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, the method comprising administering to a subject in need thereof, a therapeutically effective amount of at least one antibody or derivative thereof of any one of embodiments 1-16.

Embodiment 27. A method of preventing or treating a disease or disorder caused by a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, the method comprising administering to a subject in need thereof, a therapeutically effective amount of a composition of any one of embodiments 17-25.

Embodiment 28. A method to inhibit entry of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) into cells in a subject in need thereof, the method comprising administering to the subject in need thereof, a therapeutically effective amount of at least one antibody or derivative thereof of any one of embodiments 1-16.

Embodiment 29. A method to inhibit entry of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) into cells in a subject in need thereof, the method comprising administering to the subject in need thereof, a therapeutically effective amount of a composition of any one of embodiments 17-25.

Embodiment 30. A method of treating one or more symptoms of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in a subject in need thereof, the method comprising administering to the subject in need thereof a therapeutically effective amount of at least one antibody or derivative thereof of any one of embodiments 1-16.

Embodiment 31. A method of treating one or more symptoms of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in a subject in need thereof, the method comprising administering to the subject in need thereof a therapeutically effective amount of a composition of any one of embodiments 17-25.

Embodiment 32. A method of delaying the onset of one or more symptoms of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in a subject in need thereof, the method comprising administering to the subject in need thereof a therapeutically effective amount of the antibody or derivative thereof of any one of embodiments 1-16.

Embodiment 33. A method of delaying the onset of one or more symptoms of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in a subject in need thereof, the method comprising administering to the subject in need thereof a therapeutically effective amount of a composition of any one of embodiments 17-25.

Embodiment 34. The method of any one of embodiments 26-33, wherein the method further comprises administering an anti-viral drug, a viral entry inhibitor, or a viral attachment inhibitor.

Embodiment 35. The method of any one of embodiments 26, 28, 30, and 32 wherein the antibody or derivative thereof is administered prior to or after exposure to SARS-CoV-2.

Embodiment 36. The method of any one of embodiments 27, 29, 31, and 33 wherein the composition is administered prior to or after exposure to SARS-CoV-2.

Embodiment 37. A method of detecting the presence of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a sample, the method comprising contacting the sample with an antibody or derivative thereof of any one of embodiments 1-16 and detecting the presence or absence of an antibody-antigen complex, thereby detecting the presence of SARS-CoV-2.

Embodiment 38. The method of embodiment 37, wherein the sample is obtained from a blood sample, a cheek scraping or a cheek swab, a nasal swab, a saliva sample, a biopsy, a urine sample, a feces sample, a sputum sample, a nasal aspiration, or a semen sample.

Embodiment 39. The method of embodiment 37 or embodiment 38, wherein the sample is obtained from a blood sample.

Embodiment 40. A nucleic acid comprising a sequence encoding an amino acid sequence of any one of SEQ ID NOs: 1-8.

Embodiment 41. An antigen-binding composition comprising an antibody or antigen-binding antibody fragment thereof that binds to an epitope in the viral envelope Spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2),

wherein the antibody or antigen-binding antibody fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and a light chain variable domain (VL) comprising an amino acid sequence with at least 75% sequence identity selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 42. The composition of embodiment 41, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, and 7 and the VL comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, and 8.

Embodiment 43. The composition of embodiment 41 or embodiment 42, wherein the antibody or antigen-binding antibody fragments thereof is a monoclonal antibody or a fragment thereof.

Embodiment 44. The composition of any one of embodiments 41-43, wherein the composition comprises two or more of the antibodies or antigen-binding antibody fragments thereof.

Embodiment 45. A diagnostic kit for detecting infection of a subject by a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), comprising at least one antibody or derivative thereof of any one of embodiments 1-16.

Embodiment 46. A diagnostic kit for detecting infection of a subject by a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), comprising the composition of any one of embodiments 17-25.

Embodiment 47. The diagnostic kit of embodiment 45 or embodiment 46, wherein the at least one antibody or antigen-binding antibody fragment thereof is bound to a detectable labelling group.

Embodiment 48. A diagnostic kit for detecting vaccination of a subject against a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), comprising at least one antibody or derivative thereof of any one of embodiments 1-16.

EXAMPLES

The following is a description of various methods and materials used in the studies. They are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent that the experiments below were performed and are all of the

experiments that may be performed. It is to be understood that exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate the data and the like associated with the teachings of the present invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, percentages, etc.), but some experimental errors and deviations should be accounted for.

EXAMPLE 1-ISOLATION OF NEUTRALIZING ANTIBODIES

This Example describes the isolation of neutralizing antibodies from a single individual who experienced a breakthrough infection with the Delta variant of concern (VOC). Of the isolated antibodies, four antibodies can neutralize the vaccine strain and the Delta VOC and also retain potency against the Omicron VOC. The four antibodies are from different lineages (Table 1) and target distinct epitopes on the Spike protein, including one novel epitope outside of the receptor binding domain (RBD).

Monoclonal antibodies were isolated from a subject who had a breakthrough infection with the Delta VOC two months after completion of a two-dose regimen of the Pfizer-BioNTech COVID-19 mRNA vaccine. See Table 1. Plasma from this subject was collected 30 days post infection. The subject's plasma showed considerable neutralization potency breadth when tested with the pseudovirus assay, including neutralization potency against the Omicron VOC, which had not yet begun circulating in the population at the time. B cells that recognized the SARS-CoV-2 Spike protein were identified using a pool of APC/PE-labeled Delta Spike and the Spike S2 peptide to enrich for those cells expressing the B cell receptor encoding Spike-specific antibodies. From these B cells, four monoclonal antibodies (mabs) were identified that bound to the full-length Wuhan-Hu-1 B.1 (wild-type, "WT") Spike by ELISA and also bound to the Delta and Omicron VOC Spike proteins (Figs. 2A-2C). One mab, 268C-59, only bound the full-length WT Spike protein and not to any of the Spike subdomain peptides that were tested (*e.g.*, RBD, N-terminal domain (NTC), Spike; see Fig. 3). This was true even when the concentration of mab C68.59 was increased (Fig. 3). This result suggests that 286C-59 can recognize a unique epitope.

Table 1. Inferred germline V/D/J gene information and percent difference for four C68 monoclonal antibodies.

	Chain	V-Gene	% Identity	% Difference	J-Gene	% Identity	% Difference	D-Gene
C68.3	Gamma	IGHV4-39*07 F	96.2	3.8	IGHJ4*02 F	87.5	12.5	IGHD5-12*01 F
	Lambda	IGLV3-21*03 F	98.2	1.8	IGLJ2*01 F or IGLJ3*01	92.1	7.9	
C68.13	Gamma	IGHV3-30*03 F or IGHV3-30*18 F or IGHV3-30-5*01 F	98.6	1.4	IGHJ4*02 F	93.8	6.3	IGHD3-22*01
	Kappa	IGKV1-5*01 F	98.2	1.8	IGKJ1*01 F	94.7	5.3	
C68.59	Gamma	IGHV3-74*01 F	94.8	5.2	IGHJ4*01 F or IGHJ4*03 F	85.4	14.6	IGHD2-15*01 F
	Lambda	IGLV3-1*01 F or IGLV3-1*02 F	95.3	4.7	IGLJ2*01 F or IGLJ3*01 F	97.3	2.7	
C68.61	Gamma	IGHV5-51*01F	97.2	2.8	IGHJ4*02 F	97.9	2.1	IGHD1-1*01 F
	Kappa	IGKV1-5*01F	97.5	2.5	IGKJ1*01 F	100.0	0.0	

The amino acid sequences of the CDRs, VH, and VL of each monoclonal antibody are listed in Table 2 below.

Table 2 The amino acid sequences of the CDRs, VH, and VL of each monoclonal antibody

SEQ NO	ID	Region	Amino Acid Sequence
1.		HCDR1 of C68.3	GASISTSSDY
2.		HCDR2 of C68.3	THYSGST

SEQ NO	ID	Region	Amino Acid Sequence
3.		HCDR3 of C68.3	ARGFEPEY
4.		LCDR1 of C168.3	NIGSKS
5.		LCDR2 of C68.3	NDS
6.		LCDR3 of C68.3	QVWDSTSDHPRVV
7.		HCDR1 of C68.13	GFTFSTYA
8.		HCDR2 of C68.13	ISFDGSNT
9.		HCDR3 of C68.13	AKDSYYSRSDYYWRSYGYFDL
10.		LCDR1 of C68.13	QSISSW
11.		LCDR2 of C68.13	DAS
12.		LCDR3 of C68.13	QQYRSYSGT
13.		HCDR1 of C68.59	GLTFTNYW
14.		HCDR2 of C68.59	INSDGSST
15.		HCDR3 of C68.59	GGTYCTGGSCGIVY
16.		LCDR1 of C68.59	KLGDKY
17.		LCDR2 of C68.59	QDS
18.		LCDR3 of C68.59	QAWDSSTLV
19.		HCDR1 of C68.61	AYIFTRYW
20.		HCDR2 of C68.61	IYPGDSDT
21.		HCDR3 of C68.61	ARSGTTNYFDY
22.		LCDR1 of C68.61	QSISSW
23.		LCDR2 of C68.61	DAS
24.		LCDR3 of C68.61	QQFNSYWT
25.		VH of C68.3	QVQLVESGPGVLKPSETLSLTCTVSGASISTSSDYWGWIRQPPGKG LEWIGSTHYSGSTHYNP SLKSRVTISVDTSKNQFSLKLSSVTAADTA VYYCARGFEPEYWGQGT LVT VSS
26.		VL of C68.3	SYELTQSPSVSVAPGKTARITCGGN NIGSKSVHWHYQQKPGQAPV LVVYNDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQV WDSTSDHPRVVFVGGGTKLTVP
27.		VH of C68.13	QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYAMHWVRQAPGK

SEQ NO	ID	Region	Amino Acid Sequence
			GLEWVAVISFDGSNTYYADSVKGRFTISRDNKNTLYLQMNSLRA EDTAVYYCAKDSYYSRSDYYWRSYGYFDLWGQGTLVTVSS
28.		VL of C68.13	DIQLTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPK LLIYDASSLESGVPSRFSGSGSGTEFTLTISLQPDLATYYCQQYRS YSGTFGQGTKVEIK
29.		VH of C68.59	EVQLVESGGGLIQPGGSLRLSCAASGLTFTNYWMLWVRQAPGK GLVWVSHINS DGSS TNNADSVKGRFTISRDNKNTLYLQMNSLR DEDTAVYYCGGTCTGGSCGIVYWGQGTLVTVSS
30.		VL of C68.59	SYELTQPPSVSVSPGQTASITCSGDKLGDKYVFWYQQKSGQSPAL VIYQDSL RPSGIPDRFSGSNAGNTATLTISGTPAVDEADYYCQAW DSSTLVFGGGTKLTVL
31.		VH of C68.61	EVQLVQSGAEVKKPGESLKISCKGSAYIFTRYWIGWVRQMPGKG LEWMGIIPGDS DTRYSPSFQGQVTISADKSISTAYLQWSSLKASD TAMYYCARSGTTNYFDYWGQGTLVTVSS
32.		VL of C68.61	DIQLTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPK LLIYDASSLD SGVPSRFSGSGSGTDFTLTISLQPD DFATYYCQQFN SYWTFGQGTKVEIK

The nucleotide sequences of the VH and VL of each monoclonal antibody are listed in Table 3 below.

Table 3: The nucleic acid sequences of the VH and VL of each monoclonal antibody

SEQ NO	ID	Region	Nucleotide Sequence
33.		VH of C68.3	caggtgcagctgggtggagtcgggcccaggactggtgaagccttcggagaccctgt ccctcacctgcactgtctctgggtgctccatcagcactagtagtgactactggggct ggatccgccagccccaggaaggggctggagtggttgggagtaccattatag tgggagcaccactacaacccgtcgctcaagagtcgagtcacccatcatcagtagac acgtccaagaaccagtctcctgaagctgagctctgtgaccgcccggaacacgg ccgtatattactgtgcgagaggcttcgaacctgagtagtggggccaggggaacctg

SEQ NO	ID	Region	Nucleotide Sequence
			gtcaccgtctcctcag
34.	VL of C68.3		tcctatgagctgactcagtcaccctcggtgtcagtggtggccccaggaaagacggcca ggattacctgtgggggaacaacattggaagtaaaagtgtgcactggtaccagca gaagccaggccaggccccgtgctggctgtctataatgatagcgaccggccctca gggatccctgagcgattctctggctccaactctggaacacggccaccctgacat cagcagggtcgaagccggggatgaggccgactattactgtcaggtgtgggatagt actagtgatcatcctcggtgggttttcggcggagggaaccaagctgaccgtcccag
35.	VH of C68.13		cagggtgcagctggtggagtctgggggaggcgtggtccagcctgggaggtccctga gactctcctgtgcagcctctggattcaccttcagtacatgcatgactgggtcc gccaggctccaggcaaggggctggagtgggtggcagttatatcattgatggaag taatacatactatgcagactccgtgaaggccgattcacatctccagagacaatt ccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggctgt gtattactgtgcgaaagattcgtattactctgatagaagtattactggaggctc gtacggctactttgacctctggggccagggaaccctggtcaccgtctcctcag
36.	VL of C68.13		gacatccagttgaccagctctcctccaccctgtctgcatctgtaggagacagagtc accatcacttgccgggccagtcagagtattagtagctggttggcctggtatcagca gaaaccagggaagcccctaagctcctgatctatgatgcctccagtttgaaagt gggtcccatcaaggttcagtggtgagtggtctgggacagaattcactctcaccatc agcagcctgcagcctgatgatcttgcaacttattactgccaacagtataggagtat tcggggacgttcggccaagggaaccaaggtggagatcaaac
37.	VH of C68.59		gagggtgcagctggtggagtccgggggaggcttaattcagcctggggggtccctga gactctcctgtgcagcctctggactcaccttcactaactactggatgctctgggtcc gccaagctccagggaaggggctggtgtgggtctcacatataaatagtgatgggag cagcacaacaacgcggactccgtgaaggccgattcacatctccagagacaa cgccaagaatacgtgtatctgcagatgaacagctgagagacgaggacacggct gtatattattgtggggggacgtattgtactggtggcagttgcgggatagtctactgg ggccaaggaaccctggtcaccgtctcctcag
38.	VL of C68.59		tcctatgagctgactcagccaccctcagtggtccgtgtccccaggacagacagccag catcacctgctctggagataaattgggggataaatatgttttctggtatcagcaga agtcaggccagtcctcctgcactgggtcatctatcaagatagtttcggccctcaggg

SEQ NO	ID	Region	Nucleotide Sequence
			atccctgaccgattctctggctccaacgctgggaacacagccactctgaccatcag cgggacccccggctgtggatgaggctgactactactgtcaggcgtgggacagcagc actttggatttcggcggagggaaccaagctgaccgtcctag
39.		VH of C68.61	gaggtgcagctgggtgcagctctggagcagaggtgaaaaagcccgaggagtctctg aagatctcctgtaagggttcgcataatatctttaccagatactggatcggctgggtg cgccagatgccccgggaaaggcctggagtggatggggatcatctatcctgtgact ctgataccagatacagccgtcctccaaggccaggtcaccatctcagccgacaa atccatcagtagccctacctgcaatggagcagcctgaaggcctcgacaccgcc atgtattactgtgcgagaagtggaacgacgaattactttgactactggggccaggg aacctgtgtcaccgtctcctcag
40.		VL of C68.61	gacatccagttgaccagtcctcctccaccctgtctgcatctgtaggagacagagtt accatcacttgccgggagcagtagtattagtagctggttgccctggatcagca gaaaccagggaagcccctaagctcctgatctatgatgcctcagtttgatagtg gggtcccgtcaagggtcagcggcagtggttctgggacagatttcactctcaccatc agcagcctgcagcctgatgattttgcaacttattactgccaacagtttaatagttatt ggacgttcggccaagggaaccaaggtggaaatcaaac

EXAMPLE 2-BINDING AFFINITY OF ISOLATED MONOCLONAL ANTIBODIES

This Example describes the binding affinity (EC_{50}) of the isolated antibodies that showed binding and neutralization breadth to Wuhan-Hu-1 (B.1; WT), B.1.617 (Delta), and Omicron BA.1 (BA.1) Spike proteins. Binding measured by absorbance at 450nm of C68 mabs to Wuhan-Hu-1 (WT), Delta, Omicron BA.1, and BA.2 Spike proteins (SinoBiological). FI6V3 was run in parallel as a negative control. Results are representative of at least three independent experiments. The mean $\pm$ SD are plotted.

As illustrated in Figs. 2A-2C, all isolated mabs demonstrated high binding affinity to WT Spike protein (EC_{50} range 8-10 ng/mL) and most of these mabs had a similar affinity (EC_{50} within 3-fold) for the Delta VOC Spike protein, except for C68.13. Although the subject had not been exposed to the Omicron VOC, all four of the isolated mabs (*e.g.*, C68-3, C68-13, C68-59, C68-61) bound the Omicron BA.1 and BA.2 subvariant Spike proteins with an EC_{50} value similar (EC_{50} within 4-fold) to the wild-type

strain. As illustrated in Fig. 2A, log EC₅₀ values (ng/ml) of binding to each Spike protein are plotted for C68.3 (blue), C68.13 (red), C68.59 (green), C68.61 (purple). The table in Fig. 2C illustrates calculated EC₅₀ (ng/mL) values for each isolated antibody. Calculated EC₅₀ (ng/mL) for each and fold changes relative to WT Spike are shown where FC>1 denotes weaker binding compared to WT Spike. Lower EC₅₀ values correspond to higher binding affinity and higher EC₅₀ values indicates lower binding affinity. Binding affinity was generally similar across different Spike proteins, except for C68.13, which showed lower binding affinity to the Delta Spike protein.

Additionally, Fig. 3 illustrates binding assay results for C68.59. In Fig. 3, binding was determined by direct ELISA comparing RBD-specific mab C68.61 to C68.59. Binding (absorbance at 450nm) of C68.61 and C68C.59 to WT Spike protein (black), RBD subdomain (red), NTD subdomain (blue), S2 subunit (purple) at different concentrations of mab. C68.61 bound to Spike and RBD subunits at the 2 µg concentration. C68.59 also bound to Spike at the 2 µg concentration but did not bind to RBD, NTD, or Spike, even up to the 50 µg concentration.

EXAMPLE 3-NEUTRALIZATION ACTIVITY OF THE FOUR ISOLATED MONOCLONAL ANTIBODIES

In this Example, the neutralization activity of the isolated monoclonal antibodies was measured using a pseudovirus assay. Figs. 4A-4D illustrate neutralization curves for C68.3 (Fig. 4A), C68.13 (Fig. 4B), C68.59 (Fig. 4C), C68.61 (Fig. 4D) against Wuhan-Hu-1 (WT, blue); B.1.617 (Delta, red); Omicron BA.1 (BA.1, green); and Omicron BA.2 (BA.2, purple) pseudoviruses. To perform the neutralization experiments, a dilution series of each isolated mab was preincubated with Spike pseudotyped lentiviruses followed by infection of 293T-ACE2 cells for 2 days and measurement of infectivity by a luciferase-based assay. The dashed lines (Figs. 4A-4D) indicate 0.5 fraction infectivity. Results shown are from a single representative experiment in technical replicate. Fig. 4E lists the calculated IC₅₀ (ng/mL) for each mab, indicated to the left, against each SARS-CoV-2 variant, with the variant tested indicated across the top. Darker blue indicates lower IC₅₀ and higher neutralization potency. Fold changes (FC) relative to WT are shown for the variants with the direction of the change indicated with arrows. Results are an average of at least three biological replicate experiments each performed in technical replicate.

As demonstrated in Fig. 4, the four mabs showed breadth and potency across VOCs in the Spike-pseudotyped lentivirus neutralization assay. The mabs neutralized the

vaccine strain (WT) with varying potency ($IC_{50} = 8-178$ ng/mL) and showed generally similar neutralization potency against the Delta VOC ($IC_{50} = 14-156$ ng/mL) (Fig. 4E). Remarkably, most of these mabs had similar or slightly better (up to 5-fold) neutralization potency against the Omicron BA.1 and BA.2 subvariants ($IC_{50} = 12-125$ ng/ml). Several RBD-specific mabs that have emergency authorization for therapeutic use (*e.g.*, LY-CoV016, LY-CoV555, LY-CoV1404, REGN10987, S309) were tested in parallel to directly compare neutralization activities. The potency of the three most potent mabs (*e.g.*, C68.3, C68.13, C68.59) was similar to those of the therapeutic mabs against the WT ($IC_{50} = 8-50$ ng/mL) and Delta strains ($IC_{50} = 14-41$ ng/ml). However, while most of the therapeutic mabs lost potency against Omicron and were rendered ineffective for treatment of this VOC (IC_{50} values >2000 ng/ml), mabs C68.3, C68.13, C68.59 and C68.61 did not. These mabs had greater potency than the therapeutic mab S309, which is currently used in cases of Omicron infections; potency was up to ~160-fold higher against Omicron VOCs. Only one mab that recently received emergency authorization as a COVID-19 therapeutic against the Omicron variants (LY-CoV1404) showed greater potency than these novel mabs; it is 4-8-fold more potent than C68.3 and C68.59 against Omicron BA.1 and BA.2 VOCs.

Fig. 4E also lists the neutralization of the corresponding 30-day plasma. See Fig. 4E top line and also Fig. 1. In Fig. 1, the neutralization activity of 30-day post infection plasma against four SARS-CoV-2 strains. Neutralization was measured using a Spike pseudotyped lentivirus assay. (A) Neutralization curves for C68 plasma against Wuhan-Hu-1 (WT, blue); Delta (red); Omicron BA.1 (OmiBA1, green); Omicron BA.2 (OmiBA2, purple) pseudoviruses are shown. Graphs are from a single, representative experiment with technical replicates. The dashed line indicates 0.5 fraction infectivity. (B) Calculated IC_{50} s for each experiment are shown. Each combination was assayed at least 2-3 times. FC is the fold change relative to the wild type virus.

EXAMPLE 4-NEUTRALIZATION ACTIVITY OF THE FOUR ISOLATED MONOCLONAL ANTIBODIES AGAINST LIVE SARS-COV-2 VIRUS

Neutralization activity of the four mabs and therapeutic SARS-CoV-2 mabs against live SARS-CoV-2 variant viruses. Neutralization was measured against Wuhan-Hu-1 with D614G mutation (D614G), B.1.1.7 (Alpha), B.1.617 (Delta), Omicron BA.1 (BA.1) viruses. Fig. 5 shows the calculated IC_{50} value (ng/mL) for each mab, indicated to the left, against each SARS-CoV-2 variant, indicated at the top. Darker blue indicates

lower IC₅₀ and higher neutralization potency. Fold changes (FC) relative to WT are shown for the variants. For these experiments, a dilution series of each mab was preincubated with authentic SARS-CoV-2 D614G, Delta or BA.1 virus followed by infection of Vero E6-TMPRSS2 cells at 600 TCID₅₀ per well. After one hour, the cells are washed and incubated for 2 days. Virus was measured based on nucleocapsid levels. Results are representative of three independent experiments.

As illustrated in Fig. 5, the breadth and potency of the C68 mabs demonstrated in the live virus assay was similar to the breadth and potency demonstrated in the pseudovirus assay. For example, C68.3, C68.13, C68.59, and C69.61 all showed broad activity against the WT D614G strain, in addition to the Delta VOC, Omicron BA.1 VOC, and Alpha VOC. The potency was generally greater against the VOCs than WT, as seen in a lentivirus assay (2-15 fold; Fig. 4E). The IC₅₀ values against the SARS-CoV-2 strains were generally higher in the live virus assay compared to the pseudovirus assay, particularly against the WT virus and across all strains for C68.59. But in all cases, activity was retained against all VOCs, including the Alpha VOC. (See Fig. 5). For example, mabs C68.3 and C68.13 demonstrate notable potency, with IC₅₀ values in the range of 7-17 ng/ml against Delta VOC and Omicron BA.1 VOC, which is comparable to the IC₅₀ values for the therapeutic antibodies (*e.g.*, REGEN10933 and REGEN10987) against the WT D614G strain (5-13 ng/ml; Fig. 5). Given the efficacy of the therapeutic mabs for treatment of WT infections, these results indicate that the potency of these novel mabs in this assay are within the therapeutic range.

EXAMPLE 5-IDENTIFICATION OF ANTIBODY EPITOPES

In this Example, antibody epitopes were mapped relative to known receptor binding domain (RBD)-specific antibodies using competition binding assays. See Fig. 6. Binding of the antibody epitopes to Wuhan-Hu-1 Spike was performed with each biotinylated commercial mabs in the presence of unlabeled competitive C68 mab. To confirm competition, all assays were run in reverse with a biotinylated C68 mab competed with an unlabeled commercial mab. These commercial mabs were selected because these mabs represent different classes of RBD-specific mabs (Barnes et al., Nature, Dec 2020).

Three of these novel neutralizing mabs bound to the RBD (*e.g.*, C68.3, C68.13, C68.61), while C68.59 did not bind RBD in an ELISA (EC₅₀ > 50 mg/mL, Fig. 3). C68.59 does not bind RBD and the N terminal domain (NTD), which is upstream of the

RBD within the S1 subunit, and the S2 subunit (Fig. 3), suggesting this broad and potent mab targets a novel epitope.

To better define the epitopes of the mabs that bound to RBD, experiments to determine their classification compared to other RBD antibodies was performed. This classification system (Class 1-4) has been defined for RBD antibodies on the basis of structural analyses identifying the contact residues and RBD orientation associated with binding (Barnes CO, Jette CA, Abernathy ME, *et al.* SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* 2020;588(7839):682-687. DOI: 10.1038/s41586-020-2852-1). Competition ELISAs using several commercial mabs representing the classes (Class 1: LY-CoV016; Class 2: LY-CoV555; Class 3: S309, REGN10987, LY-CoV1404; Class 4: CR3022) indicate that C68.3 is a Class 2 mab, whereas C68.13 is a Class 3 mab, similar to the broadly potent antibody LY-CoV1404 (Westendorf K, Zentelis S, Wang L, *et al.* LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2 variants. *Cell Rep* 2022;39(7):110812. DOI: 10.1016/j.celrep.2022.110812) (Fig. 6). In the case of C68.61, no competition with any of the RBD class antibodies was observed despite the affinity and specificity C68.61 has for binding the RBD peptide.

Fig. 6A illustrates the results of the competition ELISAs, which demonstrate the magnitude of competition by the intensity of the shading and symbols as shown in the legend. For example, mab C68.3 competed most strongly with the Class 2 mab; mab C68.13 competed with Class 3 mabs. In contrast, C68.61 did not compete with any of the commercial mabs. Figs. 6B-6D illustrate bar graphs showing the percent competition of C68.3 (Fig. 6B), C68.13 (Fig. 6C), and C68.61 (Fig. 6D) compared to all other tested mabs and to competition against self. HIV-1 VRC01 is included as negative control. The mean and $\pm$ SD are plotted.

* * * * *

It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A monoclonal antibody or antigen-binding fragment thereof that binds to the viral envelope spike protein of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), wherein the monoclonal antibody or antigen-binding fragment thereof comprises:

a) a variable heavy chain CDR1 (HCDR1) comprising an amino acid sequence of SEQ ID No: 1, 7, 13, or 19;

b) a variable heavy chain CDR2 (HCDR2) comprising an amino acid sequence of SEQ ID No: 2, 8, 14, or 20;

c) a variable heavy chain CDR3 (HCDR3) comprising an amino acid sequence of SEQ ID No: 3, 9, 15, or 21;

d) a variable light chain CDR1 (LCDR1) comprising an amino acid sequence of SEQ ID No: 4, 10, 16, or 22;

e) a variable light chain CDR2 (LCDR2) comprising an amino acid sequence of SEQ ID No: 5, 11, 17, or 23; and

f) a variable light chain CDR3 (LCDR3) comprising an amino acid sequence of SEQ ID No: 6, 12, 18, or 24.

2. The monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the monoclonal antibody or antigen-binding fragment thereof comprises:

a) a HCDR1 comprising an amino acid sequence of SEQ ID No: 1, a HCDR2 comprising an amino acid sequence of SEQ ID No: 2, a HCDR3 comprising an amino acid sequence of SEQ ID No: 3, a LCDR1 comprising an amino acid sequence of SEQ ID No: 4, a LCDR2 comprising an amino acid sequence of SEQ ID No: 5, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 6;

b) a HCDR1 comprising an amino acid sequence of SEQ ID No: 7, a HCDR2 comprising an amino acid sequence of SEQ ID No: 8, a HCDR3 comprising an amino acid sequence of SEQ ID No: 9, a LCDR1 comprising an amino acid sequence of SEQ ID No: 10, a LCDR2 comprising an amino acid sequence of SEQ ID No: 11, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 12;

c) a HCDR1 comprising an amino acid sequence of SEQ ID No: 13, a HCDR2 comprising an amino acid sequence of SEQ ID No: 14, a HCDR3 comprising an amino acid sequence of SEQ ID No: 15, a LCDR1 comprising an amino acid sequence of SEQ ID No: 16, a LCDR2 comprising an amino acid sequence of SEQ ID No: 17, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 18; or

d) a HCDR1 comprising an amino acid sequence of SEQ ID No: 19, a HCDR2 comprising an amino acid sequence of SEQ ID No: 20, a HCDR3 comprising an amino acid sequence of SEQ ID No: 21, a LCDR1 comprising an amino acid sequence of SEQ ID No: 22, a LCDR2 comprising an amino acid sequence of SEQ ID No: 23, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 24.

3. The monoclonal antibody or antigen-binding fragment thereof of claim 1 or 2, wherein the monoclonal antibody or antigen-binding fragment thereof comprises a HCDR1 comprising an amino acid sequence of SEQ ID No: 13, a HCDR2 comprising an amino acid sequence of SEQ ID No: 14, a HCDR3 comprising an amino acid sequence of SEQ ID No: 15, a LCDR1 comprising an amino acid sequence of SEQ ID No: 16, a LCDR2 comprising an amino acid sequence of SEQ ID No: 17, and a LCDR3 comprising an amino acid sequence of SEQ ID No: 18.

4. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the monoclonal antibody or antigen-binding fragment thereof comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 25, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 26;

b) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 27, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 28;

c) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 29, and a light chain variable domain

(VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 30; or

d) a heavy chain variable domain (VH) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 31, and a light chain variable domain (VL) comprising an amino acid sequence that is at least about 85% identical to SEQ ID No: 32.

5. The monoclonal antibody or antigen-binding fragment thereof of claim 4, wherein the monoclonal antibody or antigen-binding fragment thereof comprises:

a) a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 25, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 26;

b) a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 27, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 28;

c) a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 29, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 30; or

d) a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 31, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 32.

6. The monoclonal antibody or antigen-binding fragment thereof of claim 5, wherein the monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable domain (VH) comprising an amino acid sequence of SEQ ID No: 29, and a light chain variable domain (VL) comprising an amino acid sequence of SEQ ID No: 30.

7. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the monoclonal antibody or antigen-binding fragment thereof is a fully human antibody or antigen-binding fragment thereof.

8. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the monoclonal antibody or antigen-binding fragment thereof binds

to an epitope within the receptor binding domain (RBD) of the viral envelope spike protein.

9. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the monoclonal antibody or antigen-binding fragment thereof binds to an epitope outside of the receptor binding domain (RBD) of the viral envelope spike protein.

10. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-9, wherein the monoclonal antibody or antigen-binding fragment thereof neutralizes SARS-CoV-2.

11. The monoclonal antibody or antigen-binding fragment thereof of claim 10, wherein the monoclonal antibody or antigen-binding fragment thereof inhibits the fusion of SARS-CoV-2 and host cell membrane.

12. The monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-9, wherein the monoclonal antibody or antigen-binding fragment thereof is cytotoxic to a SARS-CoV-2 infected host cell.

13. A polynucleotide encoding the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-12.

14. A vector, comprising the polynucleotide of claim 13.

15. A pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-12, the polynucleotide of claim 13, or the vector of claim 14.

16. A pharmaceutical composition comprising two or more of the monoclonal antibodies or antigen binding fragments thereof of any one of claims 1-12.

17. A pharmaceutical composition comprising at least two components, wherein each component comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-12, the polynucleotide of claim 13, or the vector of claim 14.

18. The pharmaceutical composition of any one of claims 15-17, wherein the pharmaceutical composition further comprises a pharmaceutical acceptable carrier.

19. The pharmaceutical composition of any one of claims 15-18, for use in treating a SARS-CoV-2 infection in a subject.

20. A method of treating a SARS-CoV-2 infection in a subject, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 15-18.

21. The method of claim 20, wherein the method further comprises administering an anti-viral drug, a viral entry inhibitor, or a viral attachment inhibitor to the subject.

22. The method of claim 20 or 21, wherein the pharmaceutical composition is administered to the subject after the exposure to SARS-CoV-2.

23. The pharmaceutical composition of any one of claims 15-17, for use in preventing a SARS-CoV-2 infection in a subject.

24. A method of preventing a SARS-CoV-2 infection in a subject, comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of any one of claims 15-17.

25. The method of claim 24, wherein the subject is immunocompromised.

26. A kit for detecting a SARS-CoV-2 infection in a subject, comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-12.

27. A method of detecting the presence of a SARS-CoV-2 in a sample, comprising (1) contacting the sample with the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-12, and (2) detecting the presence of an antibody-antigen complex, wherein the presence of the antibody-antigen complex indicates the presence of SARS-CoV-2.

28. The method of claim 27, wherein the sample is a blood sample, a nasal swab, or a throat swab.

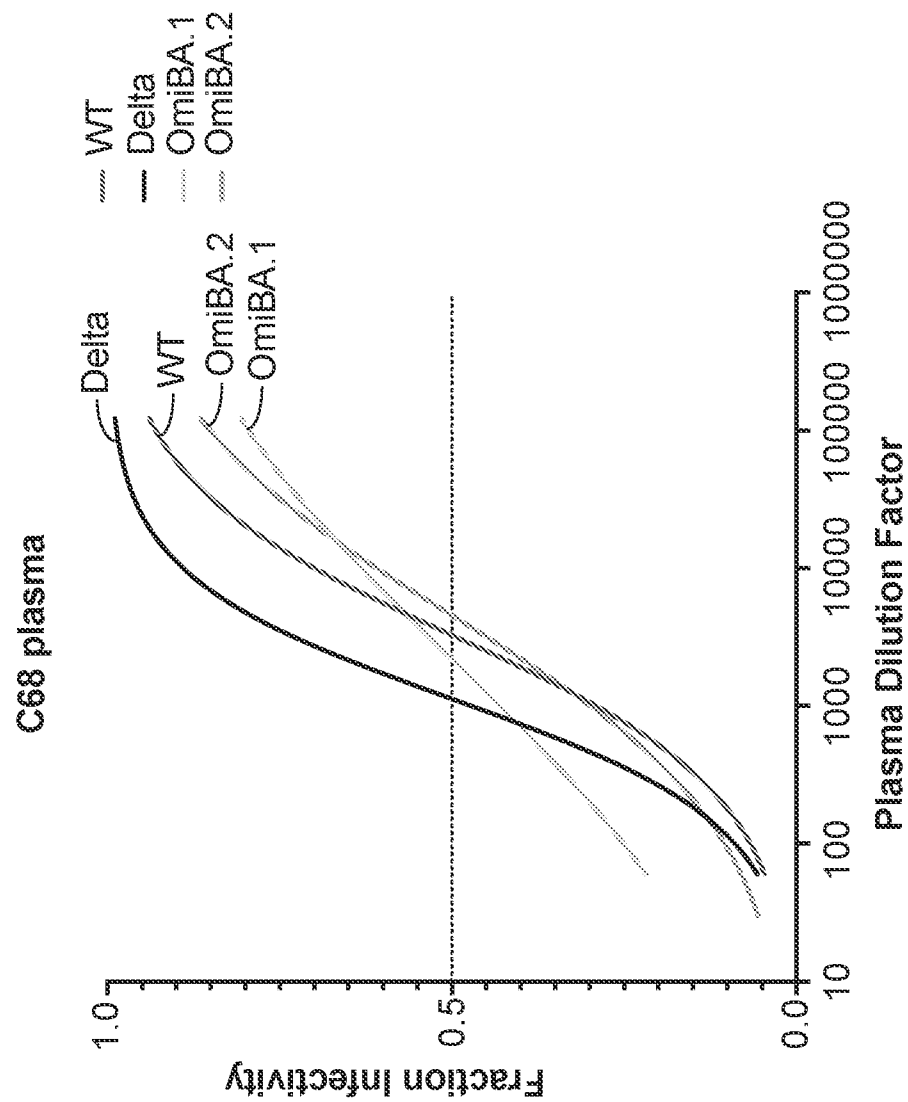


Fig. 1A

	NC50	FC
WT	2963	ref
Delta	2669	1.1
BA.1	1202	2.5
BA.2	3066	1.0

Fig. 1B

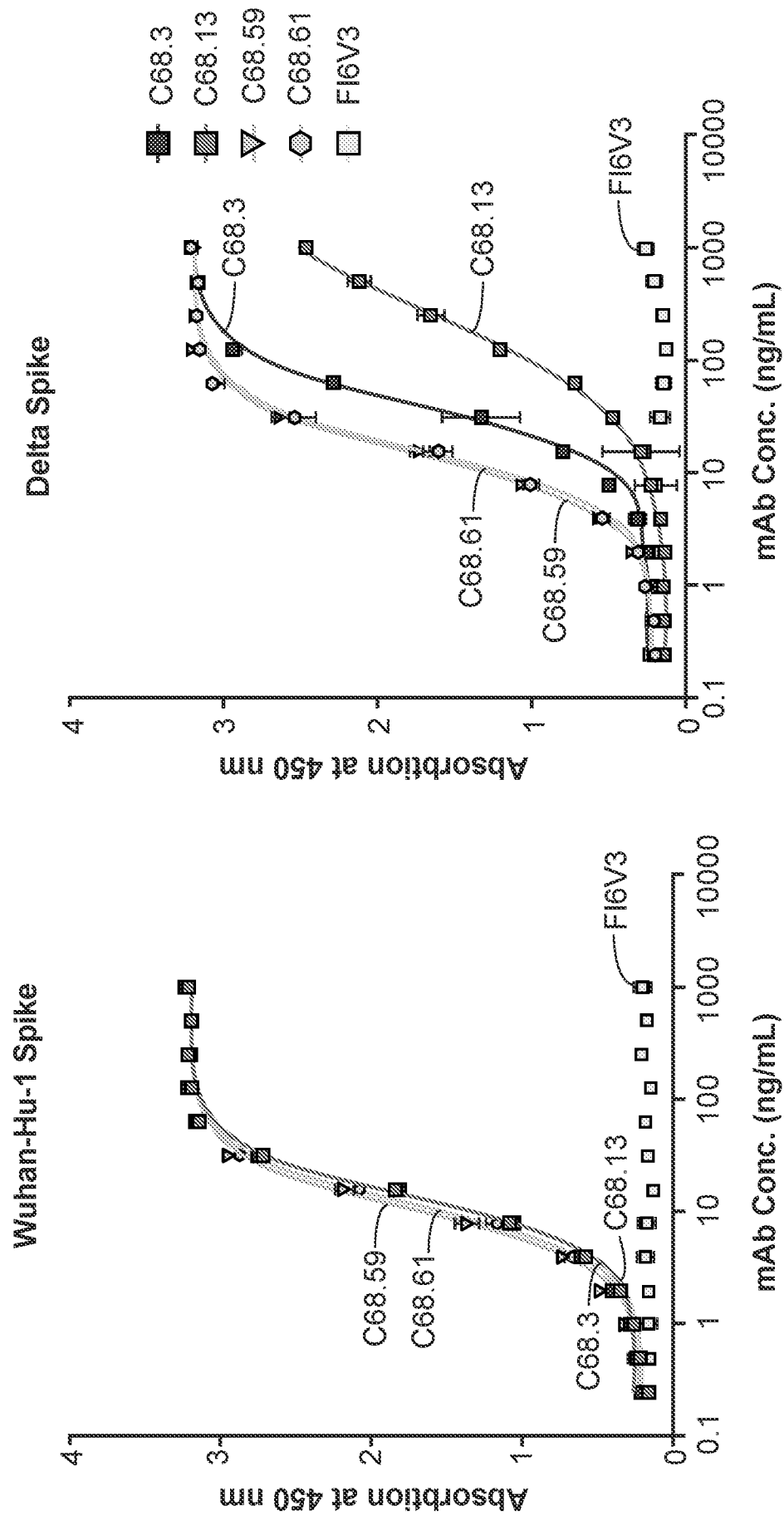


Fig. 2A

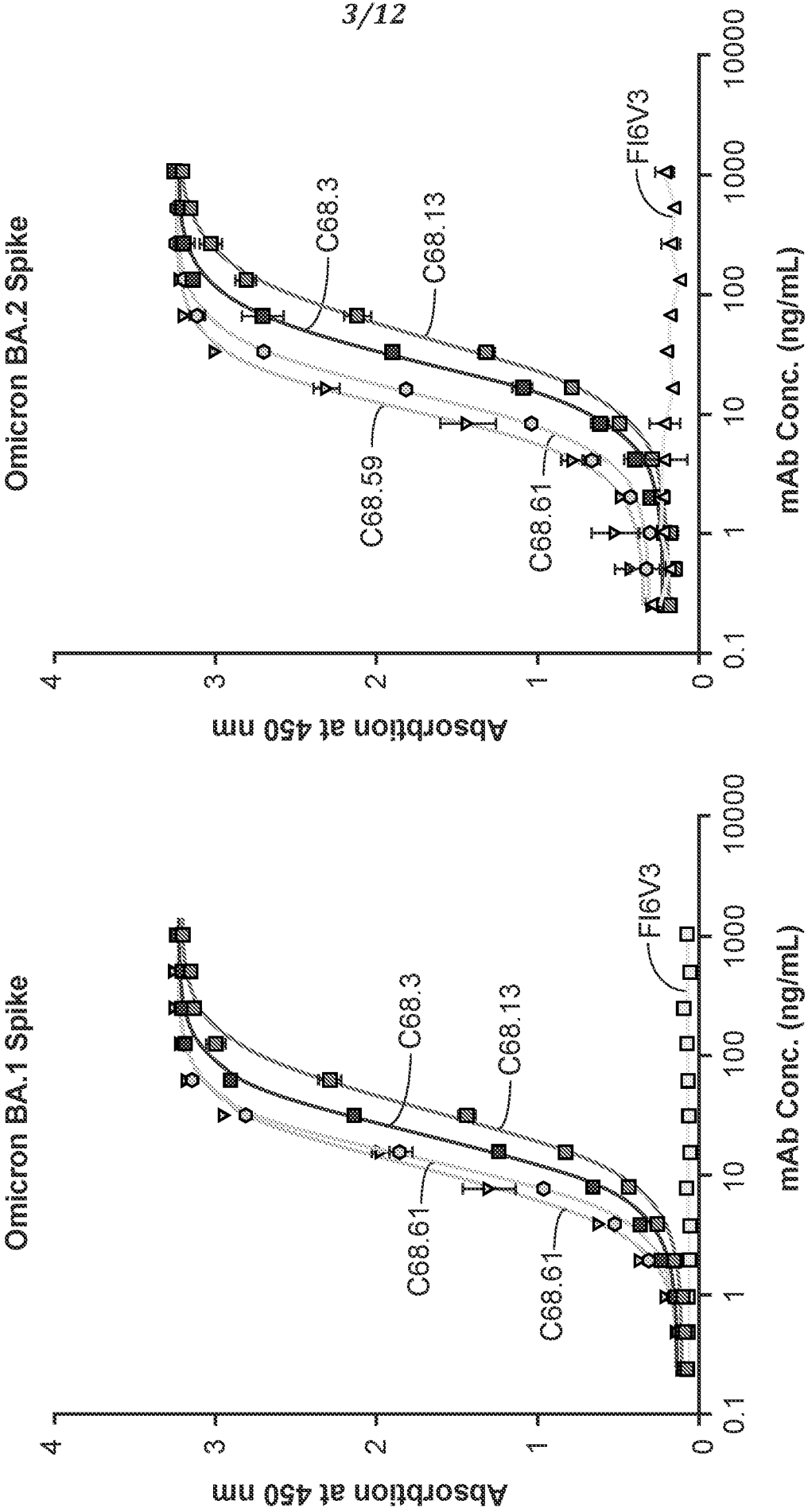


Fig. 2A (continued)

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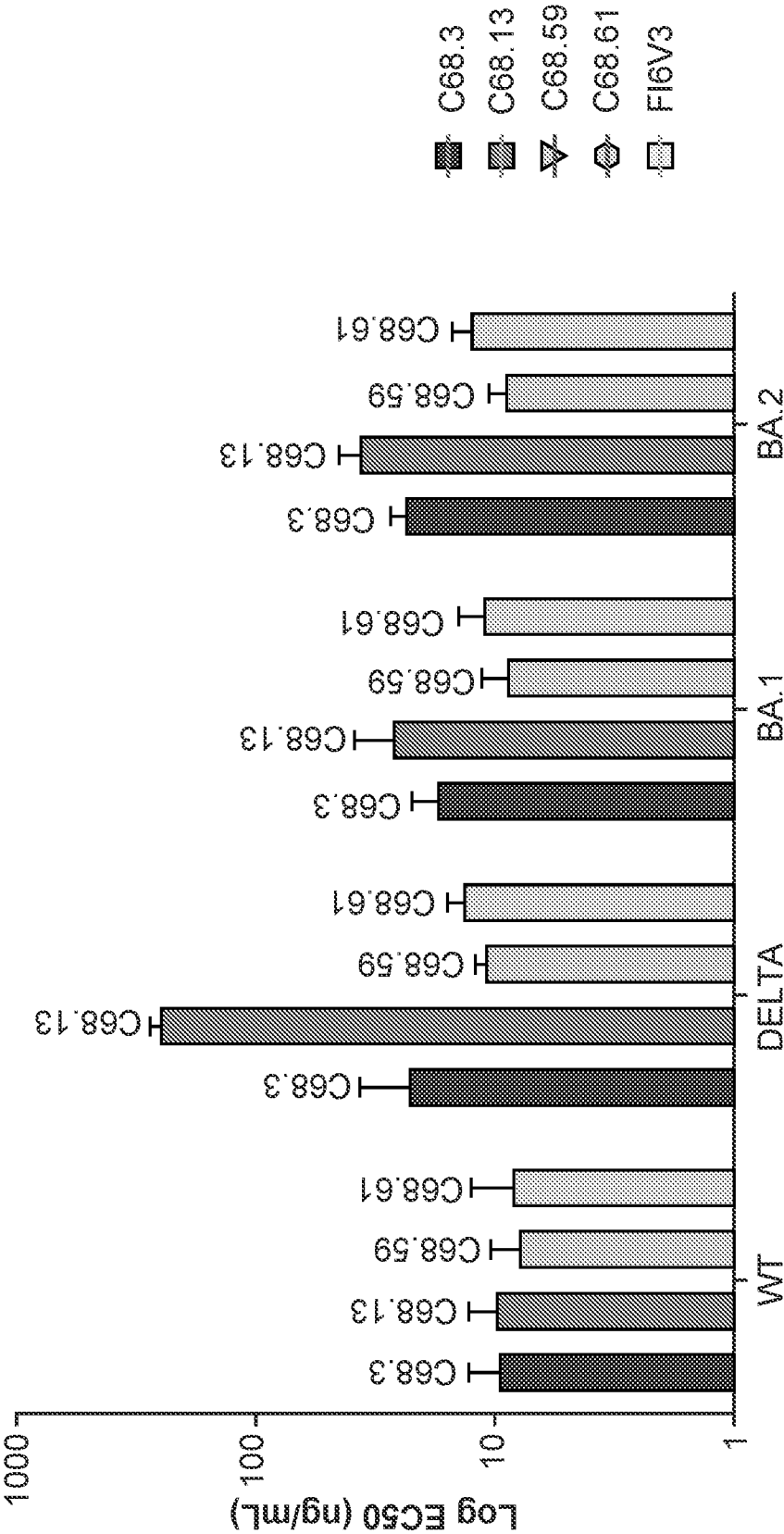


Fig. 2B

	WT		Delta		Omicron BA.1		Omicron BA.2	
	EC50	FC	EC50	FC	EC50	FC	EC50	FC
mabs								
C68.3	10	ref	23	2.4	17	1.8	24	2.5
C68.13	10	ref	251	26	27	2.7	37	3.8
C68.59	8	ref	11	1.4	8	1.0	9	1.1
C68.61	8	ref	14	1.6	11	1.3	13	1.6

EC50 = ng/mL

Fig. 2C

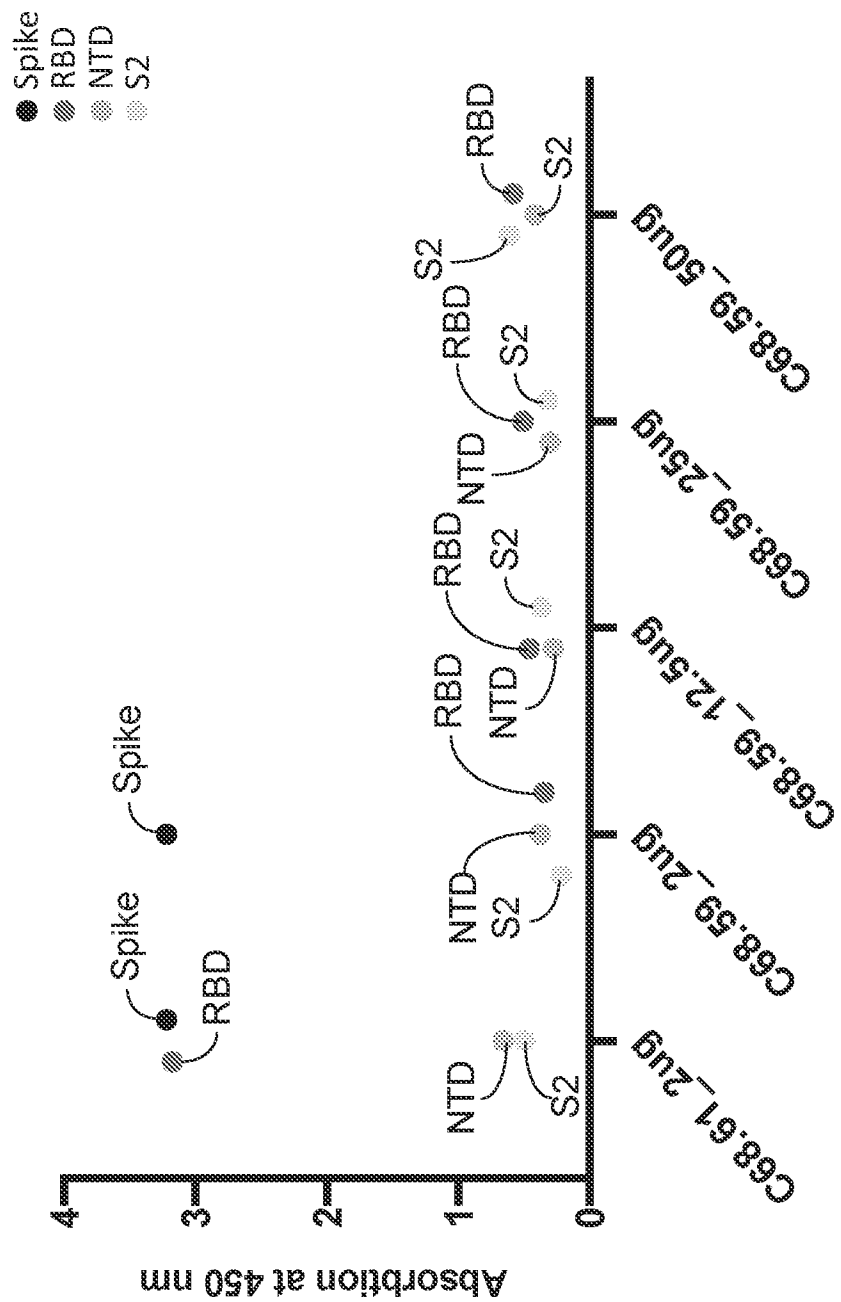


Fig. 3

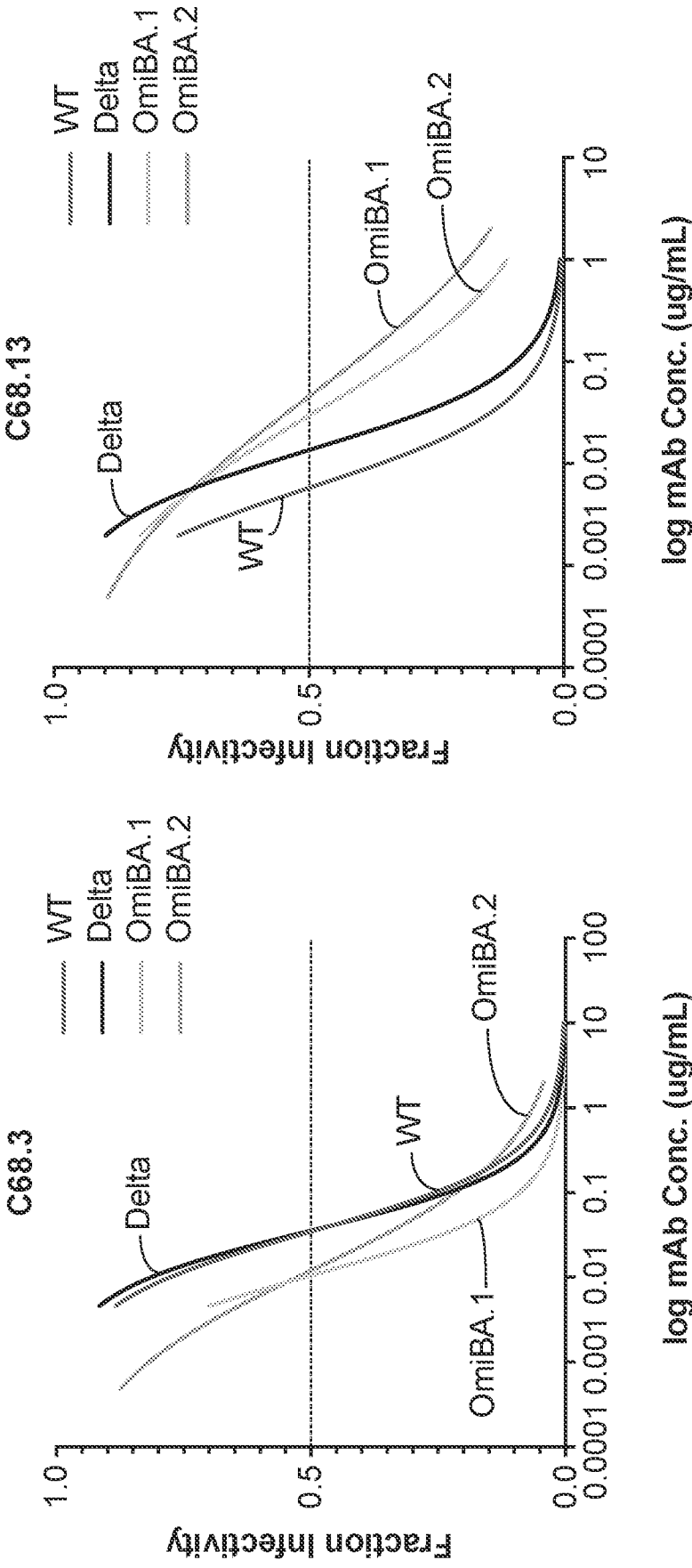


Fig. 4B

Fig. 4A

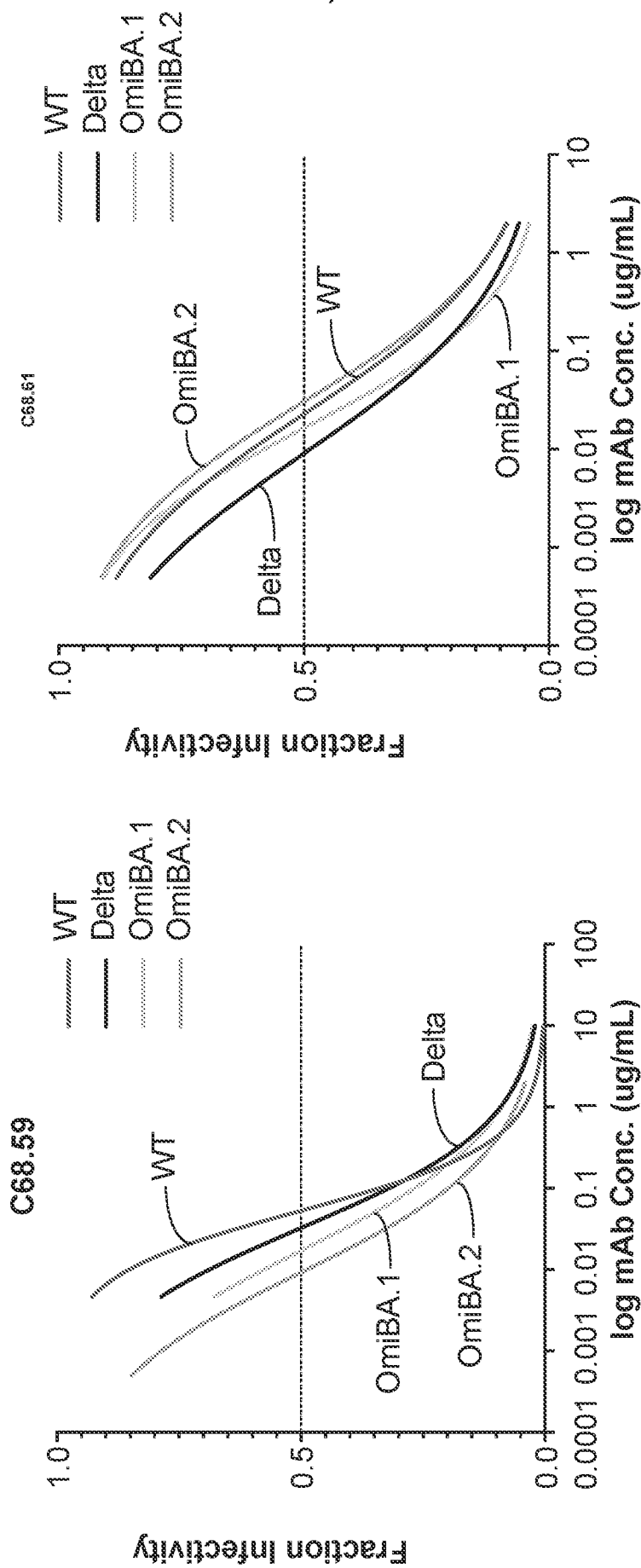


Fig. 4D

Fig. 4C

	WT		Delta		Omicron BA.1		Omicron BA.2	
	NC50	FC	NC50	FC	NC50	FC	NC50	FC
C68 plasma	2963	ref	2669	1.1	1202	2.5	3066	1.0
mabs	IC50	FC	IC50	FC	IC50	FC	IC50	FC
C68.3	50	ref	41	1.2	12	4.3	9	5.6
C68.13	8	ref	14	1.8	28	3.4	45	5.5
C68.59	31	ref	25	1.2	16	1.9	9	3.4
C68.61	178	ref	156	1.1	125	1.4	84	2.1
LY-CoV016	9	ref	8	1.1	>2000		>2000	
LY-CoV555	4	ref	>2000		>2000		>2000	
LY-CoV01404	3	ref	4	1.3	2	1.5	2	1.5
S309	1518	ref	457	3.3	129	12	1613	1.1
REGN10987	6	ref	39	6.5	>2000		265	44

Fig. 4E

	WT + D614G		Alpha		Delta		Omicron BA.1	
	IC50	FC	IC50	FC	IC50	FC	IC50	FC
mabs								
C68.3	246	ref	61	4.0	17	15	11	23
C68.13	55	ref	11	5.1	8	6.7	7	7.7
C68.59	901	ref	217	4.2	199	4.5	146	6.2
C68.61	1725	ref	634	2.7	482	3.6	843	2.0
REGN10933	5	ref	4	1.2	9	1.8	>10000	
REGN10987	13	ref	2	5.4	5	2.7	>10000	

Fig. 5

		C68 RBD-specific mabs			Key
	RBD Epitope/ Class	3	13	61	
Commercial mab	LY-CoV016	+	+	---	< 25%
	LY-CoV555	+++	---	---	
	LY-CoV1404	+	+++	---	25-50%
	REGN10987	---	++	---	51-75%
	S309	---	+++	---	> 76%
	CR3022	---	---	---	

% inhibition (vs max binding control)	Key
< 25%	---
25-50%	+
51-75%	++
> 76%	+++

Fig. 6A

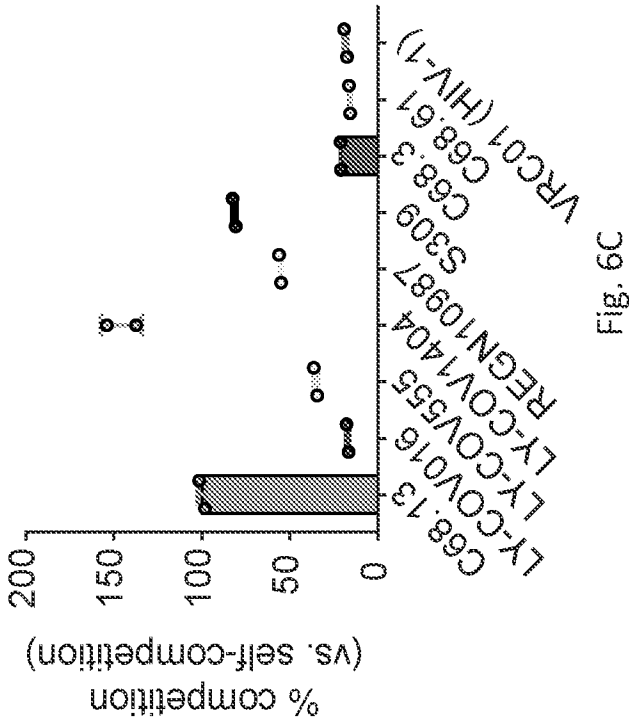


Fig. 6C

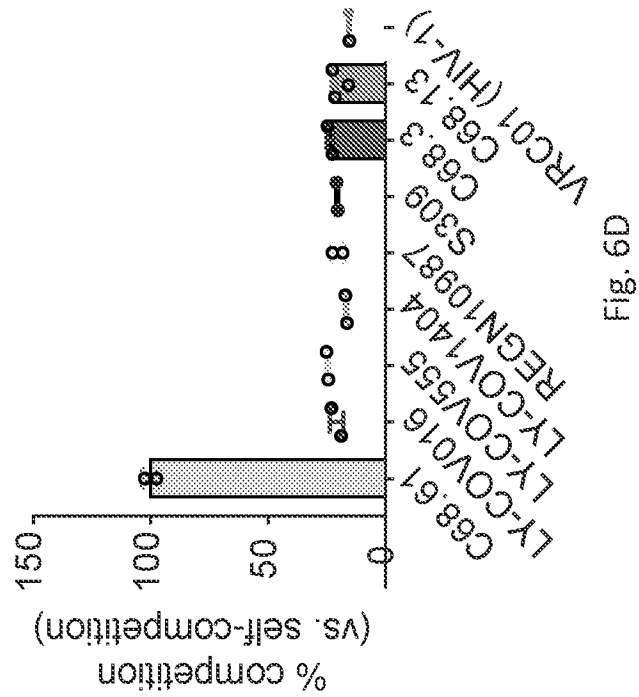


Fig. 6D

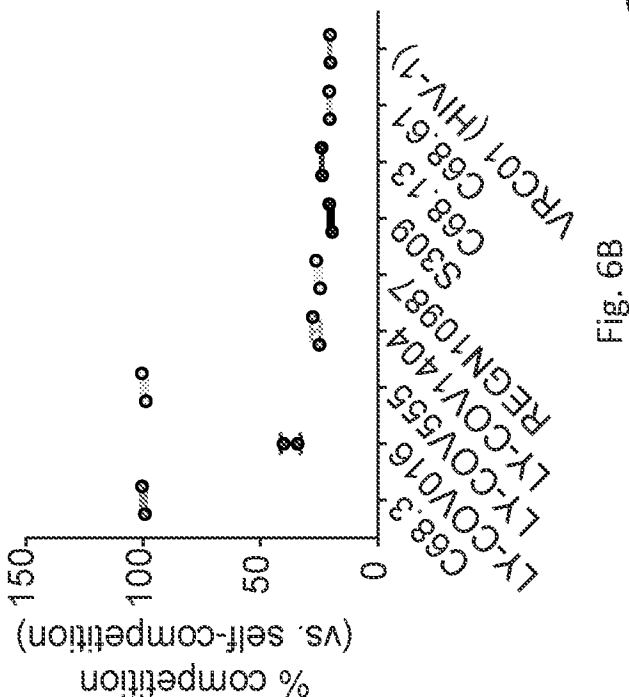


Fig. 6B