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(54) Title: SARS-COV-2 ANTIGEN-BINDING PROTEINS AND USES THEREOF

(57) Abstract: The present invention provides severe acute respiratory syndrome coronaviruses (SARS-CoV), e.g., severe acute respiratory syndrome coronaviruses 2 (SARS-CoV-2) antigen-binding proteins and methods of use thereof to passively immunize and treat subjects having or at risk of having a SARS-CoV, e.g., SARS-CoV-2, infection.



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SARS-COV-2 ANTIGEN-BINDING PROTEINS AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATION**

This application claims priority to U.S. Provisional Application Serial No. 63/159,835 filed on March 11, 2021; U.S. Provisional Application Serial No. 63/111,158 filed on November 9, 2020; and U.S. Provisional Application Serial No. 63/051,219 filed on July 13, 2020. The entire contents of each of these applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The spillover of emerging viruses from their animal reservoirs into human populations continuously threatens public health. Multiple coronaviruses have emerged to cause lethal viral pneumonias in humans including, for example, MERS-CoV, SARS-CoV, and SARS-CoV-2. SARS-CoV-2, which causes COVID-19, emerged in late 2019 and has since caused a pandemic of unprecedented scale in recent history (Abraham, J. *Nat Rev Immunol.* 20:401-403, 2020). COVID-19 rapidly advanced from an epidemic to a pandemic, and as of July 8, 2021 the World Health Organization (WHO) has reported 184,820,132 confirmed cases and 4,002,209 deaths (https://covid19.who.int/?gclid=EAIaIQobChMI5amijb_y6QIViovICh2yagafEAAYASABEgIv8PD_BwE).

There is currently no vaccine to prevent infections by SARS-CoV-2, and there are no specific antiviral treatments available or proven to be effective to treat or prevent SARS-CoV-2 infection in subjects. Accordingly, there exists an immediate need for therapies to treat and prevent SARS-CoV-2 infections.

SUMMARY OF THE INVENTION

The present disclosure provides coronavirus neutralizing antigen-binding proteins and methods of use thereof to passively immunize and treat subjects having, or at risk of having, a coronavirus infection. In particular, the present disclosure provides antigen-binding proteins that specifically bind to a coronavirus spike (S) protein.

In particular, the present disclosure provides severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), neutralizing antigen-binding proteins and methods of use thereof to passively

immunize and treat subjects having, or at risk of having, a SARS-CoV, *e.g.*, SARS-CoV-2, infection. In particular, the present disclosure provides antigen-binding proteins that specifically bind to a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S; SEQ ID NO: 100).

Exemplary antigen-binding proteins of the present disclosure are listed in Table 1 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary antigen-binding proteins.

Exemplary antigen-binding proteins of the present disclosure are also shown in Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B, and 8A-8B. Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B, and/or 8A-8B sets forth the amino acid sequence of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of the exemplary antigen-binding proteins.

Accordingly in one aspect, the present disclosure provides an isolated antigen-binding protein that binds specifically to a coronavirus, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein that binds specifically to a coronavirus spike (S) protein, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein that binds specifically to a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S; SEQ ID NO: 100), comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain

CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein capable of neutralizing a coronavirus, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein capable of neutralizing a SARS-CoV-2 virus, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein that binds specifically to a coronavirus, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein that binds specifically to a coronavirus spike (S) protein, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.

In another aspect, the present disclosure provides an isolated antigen-binding protein capable of neutralizing a coronavirus, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta

coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a SARS-CoV-2 comprising a sequence and/or a mutation as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) protein.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) protein.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to coronavirus or a coronavirus spike (S) protein comprising an amino acid sequence consisting of SEQ ID NO: 100, or an amino acid sequence comprising at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a coronavirus or coronavirus (S) protein comprising at least one amino acid modification as compared to the SARS-CoV-2 (S) protein sequence of SEQ ID NO: 100.

In some embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a coronavirus or a coronavirus spike (S) protein that comprising a neutralizing antibody escape mutation. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification a position, 114, 144, 242, 243, 244, 417, 440, 453, 478, 484, 486, 489, 493, 494, 501, and/or, 614. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification as set forth in **Figure 12**. For example, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising a Y114del mutation, a L242del mutation, a A243del mutation, a L244del mutation, a D614G mutation, a K417N mutation, a N440D mutation, a Y453F mutation, a T478K mutation, a E484K mutation, a E484A mutation, a F486I mutation, a

F486L mutation, a Y489H mutation, a Q493K mutation, a Q493R mutation, a S494P mutation, and/or a N501Y mutation.

The present disclosure provides antigen-binding proteins comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% sequence identity thereto. In some embodiments, an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an HCVR of Table 1. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the HCVR of Table 1.

The present disclosure also provides antigen-binding proteins comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% sequence identity thereto. In some embodiments, an antigen-binding protein with sequence identity less than 100% comprises CDR sequences from an LCVR of Table 1. For example, such an antigen-binding protein can comprise those CDR sequences but have differences in a framework region as compared to the LCVR of Table 1.

In certain embodiments, the present disclosure provides antigen-binding proteins comprising: (i) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314, 315, and 316, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or (ii) a LCVR sequence having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

The present disclosure also provides antigen-binding proteins comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present disclosure provides antigen-binding

proteins comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary antigen-binding proteins listed in Table 1.

In certain embodiments, the present disclosure provides antigen-binding proteins comprising: (i) a HCVR having an amino acid sequence of SEQ ID NO: 1 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 8; (ii) a HCVR having an amino acid sequence of SEQ ID NO: 2 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 9; (iii) a HCVR having an amino acid sequence of SEQ ID NO: 3 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 10; (iv) a HCVR having an amino acid sequence of SEQ ID NO: 4 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 11; (v) a HCVR having an amino acid sequence of SEQ ID NO: 5 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 12; (vi) a HCVR having an amino acid sequence of SEQ ID NO: 6 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 13; (vii) a HCVR having an amino acid sequence of SEQ ID NO: 7 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 14; (viii) a HCVR having an amino acid sequence of SEQ ID NO: 112 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 128; (ix) a HCVR having an amino acid sequence of SEQ ID NO: 113 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 129; (x) a HCVR having an amino acid sequence of SEQ ID NO: 314 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 330; (xi) a HCVR having an amino acid sequence of SEQ ID NO: 315 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 331; or (xii) a HCVR having an amino acid sequence of SEQ ID NO: 316 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 332.

The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

The present disclosure also provides antigen-binding proteins comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary antigen-binding proteins listed in Table 1. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In

general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.,* Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antigen-binding protein. For example, the present disclosure includes antigen-binding proteins comprising:

(A)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:15;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:16;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:17;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:18;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:19; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:20;

(B)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:21;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:22;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:23;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:24;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:25; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:26;

(C)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:27;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:28;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:29;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:30;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:31; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:32;

(D)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:33;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:34;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:35;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:36;

- v. a LCDR2 having the sequence set forth in SEQ ID NO:37; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:38;

(E)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:39;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:40;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:41;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:42;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:43; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:44;

(F)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:45;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:46;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:47;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:48;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:49; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:50;

(G)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:51;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:52;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:53;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:54;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:55; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:56;

(H)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:199;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:200;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:201;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:202;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:203; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:204; or

(I)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:205;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:206;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:207;

- iv. a LCDR1 having the sequence set forth in SEQ ID NO:208;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:209; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:210;

(J)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:211;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:212;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:213;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:214;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:215; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:216;

(K)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:217;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:218;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:219;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:220;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:221; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:222; or

(L)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:223;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:224;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:225;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:226;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:227; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:228.

The present disclosure also provides antigen-binding proteins comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314, 315, and 316 wherein the at least one amino acid modification alters the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein and/or wherein the at least one amino acid modification alters the neutralization potency of the isolated antigen-binding protein. In certain embodiments, the at least one amino acid modification may occur at residue 23, 24, 25, 26, 27, 28, 31, 56, 58, 74, 77, 78, 79, 100 and/or 100a of the HCVR sequence. In certain embodiments, the at least one amino acid modification may

comprise A23V, A24V, S25A, G26E, F27V, F27L, F27I, T28I, S31N, S31R, S56T, S56A, A56T, Y58F, S74P, T77M, T77I, L78V, Y79F, S100aR, and/or S100aK. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) increases the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) increases the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein by about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or more. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) increases the neutralization potency of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) increases the neutralization potency of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein by about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or more. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) occurs at the binding interface between the isolated antigen-binding protein and the coronavirus or the coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the HCVR) does not occur at the binding interface between the isolated antigen-binding protein and the coronavirus or the coronavirus spike (S) protein.

In some embodiments, the present disclosure provides antigen-binding proteins comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence of SEQ ID NO: 5. The at least one amino acid modification may comprise a substitution, a deletion, an insertion and/or other modification, including a conservative amino acid substitution. In some embodiments, the at least one amino acid modification is at a position of somatic hypermutation. In some embodiments, the at least one amino acid modification is at A24, F27, T28, S31, and/or A56. In some embodiments, the at least one amino acid modification is selected from the group consisting of A24V, F27I, F27V, T28I, S31R, S31N, and A56T. In some embodiments, the present disclosure provides antigen-binding proteins comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence of SEQ ID NO: 5 at A24, F27, T28, S31, and/or A56. In some embodiments, the present disclosure provides antigen-binding proteins comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence of SEQ ID NO: 5 selected from the group consisting of A24V, F27I, F27V, T28I, S31R, S31N, and A56T. In some embodiments, the present disclosure provides antigen-binding proteins comprising a HCVR sequence having at least one amino acid modification

as compared to a HCVR sequence of SEQ ID NO: 5 selected from the group consisting of (i) A24V, T28I, S31N, and A56T; (ii) A24V, F27V, T28I, S31N, and A56T; or (iii) A24V, S31N, and A56T.

The present disclosure also provides antigen-binding proteins comprising a LCVR sequence having at least one amino acid modification as compared to a LCVR sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332, wherein the at least one amino acid modification alters the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein and/or wherein the at least one amino acid modification alters the neutralization potency of the isolated antigen-binding protein. In certain embodiments, the at least one amino acid modification may occur at residue 10, 14, 27, 42, 50, 52, 55, 56, 70, 85, 91, 92, and/or 93 of the LCVR sequence. In certain embodiments, the at least one amino acid modification may comprises T10S, S14F, Q27E, K42N, A50G, S52T, Q55E, S56N, E70D, T85S, L91V, N92I, and/or S93D. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) increases the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) increases the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein by about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or more. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) increases the neutralization potency of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) increases the neutralization potency of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein by about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or more. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) occurs at the binding interface between the isolated antigen-binding protein and the coronavirus or the coronavirus spike (S) protein. In some embodiments, the at least one amino acid modification (*e.g.*, in the LCVR) does not occur at the binding interface between the isolated antigen-binding protein and the coronavirus or the coronavirus spike (S) protein.

In some embodiments, the present disclosure provides antigen-binding proteins comprising a LCVR sequence having at least one amino acid modification as compared to a LCVR sequence of SEQ ID NO: 12. The at least one amino acid modification may comprise a substitution, a deletion, an insertion and/or other modification, including a conservative

amino acid substitution. In some embodiments, the at least one amino acid modification is at a position of somatic hypermutation. In some embodiments, the at least one amino acid modification is at N92. In some embodiments, the at least one amino acid modification comprises N92I.

In some embodiments, the present disclosure provides antigen-binding proteins which binds to an epitope on the SARS-CoV-2 receptor binding domain (RBD) comprising any one of residues 319-541. In some embodiments, the present disclosure provides antigen-binding proteins which binds to an epitope on the SARS-CoV-2 receptor binding domain (RBD) comprising K458, Y473, and/or Q474.

The present disclosure also provides antigen-binding proteins that specifically binds to a coronavirus or coronavirus spike (S) protein with an affinity of about 0.1 nM to about 100 nM (*e.g.*, about 0.1 nM, about 0.2 nM, about 0.3 nM, about 0.4 nM, about 0.5 nM, about 0.6 nM, about 0.7 nM, about 0.8 nM, about 0.9 nM, about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM, about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, or about 100 nM). In certain embodiments, the antigen-binding protein specifically binds to a coronavirus or coronavirus spike (S) protein, *e.g.*, SARS-CoV-2, with an affinity of about 9 nM to about 76 nM.

The present disclosure also provides antigen-binding proteins that specifically binds to a coronavirus or coronavirus receptor binding domain (RBD) with an affinity of about 0.1 nM to about 1 nM (*e.g.*, about 0.1 nM, about 0.2 nM, about 0.3 nM, about 0.4 nM, about 0.5 nM, about 0.6 nM, about 0.7 nM, about 0.8 nM, about 0.9 nM, about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM, about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, or about 100 nM). In certain embodiments, the antigen-binding protein specifically binds to a coronavirus spike (S) protein receptor binding domain (RBD), *e.g.*, a SARS-CoV-2 receptor binding domain (RBD), with an affinity of about 9 nM to about 76 nM.

The present disclosure also provides antigen-binding proteins that specifically binds to a SARS-CoV-2 receptor binding domain (RBD) with an affinity of about 0.1 nM to about 1 nM (*e.g.*, about 0.1 nM, about 0.2 nM, about 0.3 nM, about 0.4 nM, about 0.5 nM, about 0.6 nM, about 0.7 nM, about 0.8 nM, about 0.9 nM, about 1 nM, about 2 nM, about 3 nM, about 4 nM, about 5 nM, about 6 nM, about 7 nM, about 8 nM, about 9 nM, about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80

nM, about 90 nM, or about 100 nM). In certain embodiments, the antigen-binding protein specifically binds to a SARS-CoV-2 receptor binding domain (RBD) with an affinity of about 9 nM to about 76 nM.

The antigen-binding proteins of the disclosure may be a IGHV3-53/IGKV1-9-derived antibody. In some embodiments, the antigen-binding proteins of the disclosure may be a germline revertant antibody. For example, the germline revertant antibody may be C1A-gl or C1A-gl* or an antibody variant thereof.

The antigen-binding proteins of the disclosure may be antibodies, such as full-length antibodies, or may comprise only an antigen-binding portion of an antibody. In certain embodiments, an antigen-binding protein of the disclosure may be a Fab, a Fab', a (Fab')₂, an Fd, an Fv, a single chain Fv (scFv), a single-domain antibody (sdAb), a diabody, a triabody, a tetrabody, a minibody, or a domain antibody.

In certain embodiments, an antigen-binding protein of the disclosure is a human monoclonal antibody or an antigen-binding fragment thereof. In certain embodiments, the antigen-binding proteins of the present disclosure are monoclonal antibodies comprising a HCVR and a LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 and/or in any one of Figures 2A-2B, 4A-4J, 7A and 8A paired with any of the LCVR amino acid sequences listed in Table 1 and/or in any one of Figures 3A-3B, 4A-4J, 7B and 8B. In certain embodiments, the monoclonal antibodies comprise a Fc domain of an isotype selected from the group consisting of IgA, IgD, IgE, IgG, IgG1, IgG2, IgG3, IgG4, IgM and a variant thereof. In certain embodiments, an antigen-binding protein of the disclosure is selected from the group consisting of human monoclonal antibody C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6. In other embodiments, the antigen-binding protein is a human monoclonal C2.0 antibody or an antigen-binding fragment thereof. In particular embodiments, the antigen-binding protein is a human monoclonal C2.1 antibody or an antigen-binding fragment thereof. In other embodiments, the antigen-binding protein is a human monoclonal C2.2 antibody or an antigen-binding fragment thereof. In other embodiments, the antigen-binding protein is a human monoclonal C2.3 antibody or an antigen-binding fragment thereof. In other embodiments, the antigen-binding protein is a human monoclonal C2.4 antibody or an antigen-binding fragment thereof.

In certain embodiments, an antigen-binding protein of the disclosure is a multi-specific antibody. In certain embodiments, an antigen-binding protein of the disclosure is a bi-specific antibody. In certain embodiments, an antigen-binding protein of the disclosure is a tri-specific antibody. In certain embodiments, the antigen-binding proteins of the present

disclosure are multispecific antibodies (*e.g.*, bi-specific antibodies or tri-specific antibodies) comprising any combination of HCVR and/or LCVR amino acid sequence listed in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B. In certain embodiments, the multispecific antibodies (*e.g.*, bi-specific antibodies or tri-specific antibodies) comprise a Fc domain of an isotype selected from the group consisting of IgA, IgD, IgE, IgG, IgG1, IgG2, IgG3, IgG4, IgM and a variant thereof. In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the spike protein subunit 1 (S1) of the coronavirus spike (S) protein.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the receptor binding domain (RBD), N-terminal domain (NTD), and/or C-terminal domain (CTD) of the spike protein subunit 1 (S1).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to an epitope within a highly conserved region of the coronavirus or coronavirus (S) protein, *e.g.*, that is not protected by glycosylation and/or conformational masking.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the N-terminal domain (NTD) of the spike protein subunit 1 (S1) and/or to the spike protein subunit 2 (S2) of the coronavirus spike (S) protein.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the receptor binding domain (RBD) of the spike protein subunit 1 (S1).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the SARS-CoV-2 spike protein (S) comprising SEQ ID NO: 100.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to a coronavirus spike (S) protein subunit 1 (S1) in the “pre-fusion” conformation (“S2P”).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to a coronavirus spike (S) protein subunit 1 (S1) in the “down” and/or “up” configuration.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to a coronavirus spike (S) protein subunit 1 (S1) in the “up” configuration.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to a coronavirus spike (S) protein subunit 1 (S1) in the “down” configuration.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that are active against circulating SARS-CoV-2 variants and/or against high-risk bat coronaviruses.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to a coronavirus or coronavirus spike (S) protein at a physiological pH of about 7.0 and/or at an acidic/endosomal pH of about 6.5 to about 4.5.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that binds specifically to the SARS-CoV-2 spike protein (S; SEQ ID NO: 100).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that can bind specifically to the SARS-CoV-2 spike (S) protein. For example, in some embodiments, the antigen-binding proteins or antigen-binding fragments thereof can bind to the signal peptide (amino acids 1–13) located at the N-terminus, to the S1 subunit (14–685 residues), and/or to the S2 subunit (686–1273 residues). In some embodiments, the antigen-binding proteins or antigen-binding fragments thereof can bind to the S1 subunit, for example, to the N-terminal domain (NTD) (14–305 residues) and/or to the receptor-binding domain (RBD) (319–541 residues). In some embodiments, the antigen-binding proteins or antigen-binding fragments thereof can bind to the fusion peptide (FP) (788–806 residues), heptapeptide repeat sequence 1 (HR1) (912–984 residues), HR2 (1163–1213 residues), TM domain (1213–1237 residues), and/or cytoplasm domain (1237–1273 residues) of the S2 subunit.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that inhibit coronavirus spike (S) protein binding to angiotensin converting enzyme 2 (ACE2).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that inhibit the binding of coronavirus spike protein subunit 1 (S1) to ACE2.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that competitively inhibit SARS-CoV-2 binding to ACE2.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that are capable of inhibiting viral fusion with and/or viral entry into a cell, *e.g.*, an ACE2-expressing cell.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralizes a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2, with an IC_{50} of about 50 ng/ml to 500 ng/ml, for example, as measured by a plaque reduction neutralization test (PRNT). In certain embodiments, the present disclosure provides
5 antigen-binding proteins or antigen-binding fragments thereof neutralizes a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2. In certain embodiments, the antigen-binding proteins or antigen-binding fragments thereof neutralizes a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2 with an IC_{50} of about 62 ng/ml to 440 ng/ml, for example, as measured by a plaque reduction neutralization test (PRNT). In certain embodiments, the present
10 disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralizes SARS-CoV-2 pseudotype. In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof neutralizes SARS-CoV-2 pseudotype with greater than about 90% reduction in entry at a concentration of 100 $\mu\text{g ml}^{-1}$. In certain embodiments, the present disclosure provides antigen-binding proteins or
15 antigen-binding fragments thereof that neutralizes SARS-CoV-2 pseudotype with IC_{50} values ranging from about 0.008 to 0.671 $\mu\text{g ml}^{-1}$, for example, as measured in a dose response pseudotype neutralization assay.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralizes infectious coronavirus.

20 In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralize infectious SARS-CoV.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralize infectious SARS-CoV-2. In certain
25 embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralize infectious SARS-CoV-2 with an IC_{50} value of less than 1 $\mu\text{g ml}^{-1}$, including, for example, infectious SARS-CoV-2 strain USA/WA1/2020. In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof neutralize infectious SARS-CoV-2 strain USA/WA1/2020 and/or variants thereof.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that neutralize SARS-CoV-2 with an IC_{50} of about 62 ng/ml to 440 ng/ml, for example, as measured by a plaque reduction neutralization test (PRNT).

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that are cross-reactive and/or cross-neutralizing to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that are capable of inhibiting viral replication. In certain embodiments, the present disclosure provides antigen-binding proteins or antigen-binding fragments thereof that are capable of inhibiting transmission of a coronavirus.

In another aspect, the present disclosure provides a pharmaceutical composition comprising an isolated antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present disclosure provides pharmaceutical compositions comprising at least two isolated antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present disclosure provides pharmaceutical compositions comprising at least three isolated antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present disclosure provides pharmaceutical compositions comprising at least four isolated antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present disclosure provides pharmaceutical compositions comprising at least five or more isolated antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the present disclosure provides pharmaceutical compositions comprising an antigen-binding proteins as described herein and an additional therapeutic agent, such as a small molecule or another antibody. In some embodiments, the additional therapeutic agent may comprise a small molecule drug targeting a viral enzyme, such as a viral RNA-dependent RNA polymerase and/or a viral protease.

In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) specifically bind to non-competing epitopes on the same or different coronaviruses or coronavirus spike (S) proteins.

In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) independently bind to a neutralizing epitope and/or a non-neutralizing epitope on the same or different coronaviruses or coronavirus S proteins.

In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) are independently selected from the groups consisting of an isolated antigen-binding protein that (i) is cross-reactive to more than one coronaviruses or variant thereof, (ii) cross-neutralizes more than one strain of a coronavirus, (iii) specifically binds to a coronavirus spike (S) protein, (iv) specifically binds to a receptor binding domain (RBD) of the spike protein subunit 1 (S1), (v) specifically binds to a N-terminal domain (NTD) of the spike protein subunit 1 (S1), (vi) specifically binds to a C-terminal domain (CTD) of the spike protein subunit 1 (S1), (vii) specifically binds to a spike protein subunit 2 (S2), (viii) destabilizes the prefusion conformation of a coronavirus spike (S) protein, (ix) specifically binds a non-receptor binding domain (RBD) neutralizing epitope, (x) specifically binds an receptor binding domain (RBD) neutralizing epitope, (xi) competes with binding to ACE2, and (xii) does not compete with binding to ACE2.

In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) independently bind to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof.

In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) independently bind to a SARS-CoV-2, as described herein. In certain embodiments, the present disclosure provides pharmaceutical compositions wherein the antigen-binding proteins (*e.g.*, at least two or more antigen-binding proteins) independently bind to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of FIGS. 13-23.

In another aspect, the present disclosure provides an isolated polynucleotide molecule comprising an amino acid sequence that encodes an antigen-binding protein as described herein, *e.g.*, in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B. For example, the present disclosure provides nucleic acid molecules encoding any of the HCVR, LCVR, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 amino acid sequences listed in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B.

The present disclosure also provides nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary antigen-binding proteins listed in Table 1 and/or in any one of Figures 2A-2B, 4A-4J, 7A and 8A.

The present disclosure also provides nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary antigen-binding proteins listed in Table 1 and/or in any one of Figures 3A-3B, 4A-4J, 7B and 8B.

The present disclosure also provides nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR amino acid sequences listed in Table 1 and/or in any one of Figures 2A-2B, 4A-4J, 7A and

8A, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1 and/or in any one of Figures 3A-3B, 4A-4J, 7B and 8B. In certain embodiments according to this aspect of the disclosure, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same antigen-binding protein listed in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B.

In a related aspect, the present disclosure provides vectors, *e.g.*, recombinant expression vectors, capable of expressing a polypeptide comprising a heavy and/or or light chain variable region of an antigen-binding protein described herein. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B. Also included within the scope of the present disclosure are host cells into which such vectors have been introduced, as well as methods of producing the antigen-binding proteins by culturing the host cells under conditions permitting production of the antigen-binding proteins, and recovering the antigen-binding proteins so produced.

In another aspect, the disclosure provides methods of treating or preventing a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2, infection in a subject. The methods include administering a therapeutically effective amount of an antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof.

In another aspect, the disclosure provides methods of preventing transmission of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2. The methods include administering a therapeutically effective amount of an antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof.

In another aspect, the disclosure provides methods of providing broad spectrum immunity against circulating SARS-CoV-2 variants and high-risk bat coronaviruses coronavirus. The methods include administering a therapeutically effective amount of an antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof.

In certain embodiments, the coronavirus infection is an infection by a SARS-CoV-2 virus.

In certain embodiments, the subject has, or is at risk of having, COVID-19.

In certain embodiments, the antigen-binding protein (or pharmaceutical composition) of the disclosure is administered to the subject prior to onset of one or more manifestations of COVID-19. For example, the antigen-binding protein can be administered to the subject after the subject exhibits one or more manifestations of COVID-19.

In certain embodiments, the method disclosed herein results in the amelioration of one or more manifestations of COVID-19. Exemplary manifestations of COVID-19 include, but are not limited to, fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea.

In certain embodiments, the method disclosed herein results in passive immunity to a SARS-CoV-2 infection. The passive immunity may last for at least about 1 week to about 2 weeks, at least about 1 month to about 3 months, at least about 3 months to about 6 months, or at least about 6 months to about 12 months.

In certain embodiments, the method disclosed herein results in a reduction in the level of viral entry. For example, a reduction in the level of viral entry of at least about 80%, 85%, 90%, 95%, 99%, or 100% as compared to a control level.

In certain embodiments, the method disclosed herein results in a reduction in the level of viral titer in the subject. For example, a reduction in the level of viral titer of at least about 80%, 85%, 90%, 95%, 99%, or 100% as compared to a control level.

In certain embodiments, the method disclosed herein results in a reduction in the level of SARS-CoV-2 viral RNA in the subject. For example, a reduction in the level of SARS-CoV-2 viral RNA of at least about 80%, 85%, 90%, 95%, 99%, or 100% as compared to a control level.

The antigen-binding protein, *e.g.*, antibody, or antigen-binding fragment thereof, may be administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly, or intracranially.

In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered as a transfusion of a convalescent blood product (CBP). convalescent plasma, *e.g.*, (i) convalescent whole blood (CWB), convalescent plasma (CP) or convalescent serum (CS); (ii) pooled human immunoglobulin (Ig) for intravenous or intramuscular administration; (iii) high-titre human Ig; and (iv) polyclonal or monoclonal antibodies.

The antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered at a dose of about 0.1 mg/kg of body weight to about 300 mg/kg of body

weight of the subject. In certain embodiments, the antigen-binding protein is administered at a dosage of about 10 mg/kg to 150 mg/kg of recipient body weight. In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days after viral shedding is first detected in a sample from the subject.

In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered after prophylactic and/or therapeutic antibody administration.

In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered in combination with an additional therapeutic agent.

In certain embodiments, the subject is at higher risk for severe COVID-19. For example, the subject may be (i) 65 years or older; (ii) living in a nursing home or a long-term care facility; (iii) a first-responder; (iv) suffering from an underlying disease or condition selected from the group consisting of chronic lung disease, moderate to severe asthma, serious heart condition, cancer, poorly controlled HIV or AIDS, severe obesity (body mass index [BMI] of 40 or higher), diabetes, chronic kidney disease undergoing dialysis, and liver disease; (v) receiving, has recently received, or is about to receive a cancer treatment, a bone marrow or organ transplantation, a corticosteroid, or other immune weakening treatment; (v) a smoker; and/or (iv) immunocompromised. In certain embodiments, the methods described herein extend the subject's life span by at least about 30, 60, 90, 120, 180 or 360 days or more.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C are graphs showing that monoclonal antibodies isolated from COVID-19 convalescent peripheral blood mononuclear cells (PBMCs) potently neutralize SARS-CoV-2 and compete with ACE2 binding.

5 **Figure 1A** is a graph depicting the results of an experiment in which human lung epithelial (Calu-3) cells were infected with GFP-expressing SARS-CoV-2 or vesicular stomatitis (VSV-G) lentivirus pseudotypes in the presence of the indicated monoclonal antibodies at 100 µg/ml. Entry levels were measured by flow cytometry 48-hours post-infection. Entry percentage was normalized to a no antibody control. Error bars show
10 standard deviation. The experiment was performed twice in triplicate (n=6). This data

demonstrates that monoclonal antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6 potentially block SARS-CoV-2 entry into cells.

Figure 1B is a graph depicting the results of an experiment in which streptavidin plates were coated with biotinylated SARS-CoV-2 S2P and competitors (ACE2-IgG or an NRP2-IgG fusion control protein) were added at increasing concentrations. C2.2 IgG was then added at a fixed concentration, and this was followed by several washing steps. An anti-Fab horse radish peroxidase-coupled antibody was used to detect bound C2.2. The experiment was run once in duplicate (n=2). This data demonstrates that monoclonal antibody C2.2 competes for ACE2 binding.

Figure 1C is a graph depicting the results of an experiment in which VeroE6 cells were infected with SARS-CoV-2 USA-WA1/2020 in the presence of C2.1 at the indicated concentrations. Neutralization of plaque formation was measured 48-hours post-infection. The assay was run as three antibody dilution series performed in parallel and in duplicate. Error bars show standard deviation. This data demonstrates that monoclonal antibody C2.1 neutralizes SARS-CoV-2 *in vitro*.

Figure 2A depicts the variable heavy chain amino acid sequences for monoclonal antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6. Additionally, the variable heavy chain amino acid sequences for the B38, CC12.1, and CC12.3 antibodies are shown.

Figure 2B depicts a sequence alignment of the variable heavy chain amino acid sequences for antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, C2.6, B38, CC12.1, and CC12.3.

Figure 2C depicts a percent identity matrix for the variable heavy chain amino acid sequences for antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, C2.6, B38, CC12.1, and CC12.3.

Figure 3A depicts the variable light chain amino acid sequences for monoclonal antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6. Additionally, the variable light chain amino acid sequences for the B38, CC12.1, and CC12.3 antibodies are also shown.

Figure 3B depicts a sequence alignment of the variable light chain amino acid sequences for antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, C2.6, B38, CC12.1, and CC12.3.

Figure 3C depicts a percent identity matrix for the variable light chain amino acid sequences for antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, C2.6, B38, CC12.1, and CC12.3.

Figures 4A-4J depict the variable heavy chain and the variable light chain amino acid sequences for monoclonal antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, C2.6, C2.4opt1, C2.4opt2, C2.4opt3, C1A-gl, and C1A-gl*. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 sequences are indicated.

Figures 5A-5E depict a series of graphs showing the characteristics of SARS-CoV-2 S-reactive monoclonal antibodies from a COVID-19 convalescent individual. **Figure 5A** depicts entry levels of SARS-CoV-2 or vesicular stomatitis virus (VSV) lentivirus pseudotypes after pre-incubation with polyclonal immunoglobulins (IgG) purified from the plasma of a COVID-19 convalescent individual ("C1"), a non-immune control donor ("control" or "ctrl"), or with an ACE2-Fc fusion protein all at a concentration of 316 $\mu\text{g ml}^{-1}$. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6) are shown. One-way ANOVA with Tukey's multiple comparisons test. **** $P < 0.0001$. **Figures 5B-5C** depict violin plots showing CDR3 loop lengths and somatic hypermutation frequencies (S.H.M.) for S-reactive monoclonal antibodies. The median and quartiles are shown as dashed and dotted lines, respectively. For CDR3 loop lengths, the median and first quartile marker overlap. **Figure 5D** depict antibody heavy and light chain gene usage for SARS-CoV-2 S-reactive monoclonal antibodies. Asterisks indicate clonally related V_H3-53/V_K1-9 antibodies (referred to as "C1A-V_H3-53 antibodies"). **Figure 5E** depicts properties of the seven IGHV3-53-derived potent SARS-CoV-2 neutralizing antibodies. a.a.: amino acids. WA1/2020: SARS-CoV-2 strain USA/WA1/2020.

Figures 6A-6K depict antibody somatic mutations at the SARS-CoV-2 RBD interface. Interactions of CDR H1 residue 31 with the RBD for (**Figure 6A**) C1A-B3, (**Figure 6B**) C1A-C2, or (**Figure 6C**) BD-629 (PDB: 7CH5). Interactions of CDR H2 residue 56 with the RBD for (**Figure 6D**) B38 (PDB: 7BZ5), (**Figure 6E**) C1A-B3, or (**Figure 6F**) C1A-B12. Interactions of CDR L3 residue 92 with the RBD for (**Figure 6G**) C1A-B3, or (**Figure 6H**) C1A-B12. Interactions occurring at the base of CDR H1 near the framework regions for (**Figure 6I**) C1A-B3, (**Figure 6J**) C1A-C2, or (**Figure 6K**) BD-236 (PDB: 7CHB). The color scheme for the RBD and antibody Fab is the same as the one showed in Fig. 13b. "Germline" indicates baseline interactions occurring when a given residue is not somatically mutated; mutations are otherwise listed on top of the panel.

Figures 7A-7G depict affinity maturation and positions of somatic changes on C1A-IGHV3-53-derived (C1A-V_H3-53) antibodies. **Figure 7A** depicts an alignment of antibody variable heavy chain gene sequences. **Figure 7B** depicts an alignment of antibody variable

light chain gene sequences. In **Figure 7A** and **Figure 7B**, C1A-gl sequences shown are germline revertant sequences designed using IMGT/V-QUEST (Brochet et al., 2008). In **Figure 7A**, the CDR H3 germline sequences were challenging to predict but a possible substitution was identified (see **Figure 15A**). Panels were generated using ESPrit327 (Robert and Gouet, 2014) and modified. The Kabat numbering scheme is used. RBD contacting residues are indicated with a filled black circle. **Figure 7C** depicts a ribbon diagram of crystal structure of the C1A-B3 Fab/RBD complex showing the location of somatic mutations. See also **Figure 13**. **Figure 7D** depicts interactions for CDR H1 residue 31 with the RBD are shown for C1A-B3 (left panel) or C1A-C2 (right panel), showing the effects of the S31N_{VH} substitution. **Figure 7E** depicts interactions occurring at the base of CDR H1 near the framework regions are shown for C1A-B3 (left panel) or C1A-C2 (right panel), showing the effects of the A24V_{VH} mutation. **Figure 7F** depicts interactions of CDR H2 residue 56 with the RBD are shown for C1A-B3 (left panel), or C1A-B12 (right panel), showing the effects of the S56T/A_{VH} mutations. **Figure 7G** depicts interactions of CDR L3 residue 92 with the RBD are shown for C1A-B3 (left panel) or C1A-B12 (right panel), showing the effects of the N92I_{VL} substitution. Both sets of interactions shown occur after somatic mutations; the germline interactions at this position were not visualizes. For **Figures 7D, 7F, and 7G**, “germline” indicates baseline interactions occurring when a given residue is not somatically mutated.

Figures 8A-8G depict sequence alignments with other reported IGHV3-53/3-66-derived antibodies. **Figure 8A** depicts an alignment of variable heavy chain sequences of IGHV3-53/3-66 antibody genes reported here and elsewhere. **Figure 8B** depicts an alignment of variable light chain sequences for antibodies containing IGLVK1-9-derived light chains. Antibody sequences were obtained from the RCSB record and protein data bank (PDB) IDs listed in **Figure 14A**. Panels were generated using ESPrit3 (Robert and Gouet, 2014) and modified. The Kabat numbering scheme is used. **Figure 8C** depicts CV30-Fab/RBD complex (PDB: 6XE1) showing interactions occurring with CDR H1 mutations F27V and T28I. **Figure 8D** depicts B38 Fab/RBD complex (PDB: 7BZ5) showing interactions occurring with the CDR H1 T28I mutation. **Figure 8E** depicts BD-629 Fab/RBD complex (PDB: 7CH5)⁶ showing interactions occurring with the CDR H1 G26E and T28I mutations. **Figure 8F** depicts C1A-B3 Fab/RBD complex showing interactions occurring with the germline CDR H2 residue Y52. **Figure 8G** depicts CC12.1 Fab/RBD complex (PDB: 6XC2)⁷ showing interactions with the Y58F mutation. For panels *a* and *b*, antibody sequences were obtained from the RCSB record and protein data bank (PDB) IDs listed in **Figure 14A**.

Figures 9A-9C depict the results of monoclonal antibody isolation from a COVID-19 convalescent individual. **Figure 9A** depicts density plot from a FACS experiment to isolate memory B cells that bind phycoerythrin (PE)-labelled streptavidin tetramers coupled to a prefusion-stabilized SARS-CoV 2 S construct (S2P-PE). The approximate location of the sorting gate is shown as a box, and the percentage of cells that fall within the gate is indicated. The left panel is for a control donor and the right panel is for a COVID-19 convalescent donor. CD19 is a B-cell marker. **Figure 9B** depicts whisker plot showing ELISA values for IgG binding to S2P, the SARS-CoV-2 RBD, or the control protein Lujo virus (LUJV) GP1. Antibodies were added at a single concentration of $100 \mu\text{g ml}^{-1}$. Dashed line represents the cut off for the definition of antibodies that bind the respective protein. **Figure 9C** depicts SARS CoV 2 or VSV lentivirus pseudotypes that were pre-incubated with $100 \mu\text{g ml}^{-1}$ of the indicated IgG or ACE2-Fc fusion protein (ACE2-Fc) and the mixture was used to infect HEK293T-hACE2 cells. Entry levels were quantified 48 h later using FACS. VSV lentivirus pseudotype is included as a control. Data are normalized to a no antibody control. Dashed line indicates 10% relative entry. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6).

Figure 10 depicts a table of the properties of monoclonal antibodies isolated from a COVID-19 convalescent individual. Antibodies highlighted in gray are somatic variants of the same antibody. CDR loop lengths are shown as numbers of amino acids (a.a.). ELISA values are colored in shades according to their magnitude; darker shades are reflective of a stronger signal. S2P: prefusion stabilized version of the SARS-CoV-2 S ectodomain; RBD: receptor-binding domain; Ctrl: negative control protein Lujo virus GP1.

Figures 11A-11B depict SARS-CoV-2 pseudotype and infectious virus neutralization assays. **Figure 11A** depicts SARS-CoV-2 lentivirus pseudotypes were pre-incubated with monoclonal antibodies at the indicated concentrations and the mixture was used to infect HEK293T-hACE2 cells. Entry levels were quantified 48 h later using FACS. VSV pseudotypes are included as a negative control. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6). For some data points, error bars are smaller than symbols. IC_{50} values are shown in parentheses. **Figure 11B** depicts infectious SARS-CoV-2 (strain USA/WA1/2020) was incubated with monoclonal antibodies at the indicated concentration with infection of Vero E6 cells subsequently measured in PRNT assay (Zhang et al., 2020). Monoclonal antibody mAb114 is included as a control. Each monoclonal antibody was serially diluted in Dulbecco's Phosphate Buffered Saline (DPBS) using half-log dilutions starting at a concentration of 50

µg ml⁻¹. Means ± standard deviation from three experiments performed in triplicate (n=9) are shown. Data are normalized to a no antibody control. For some data points, error bars are smaller than symbols. IC₅₀ values are shown in parentheses.

Figure 12 depicts Fab binding kinetics to the SARS-CoV-2 receptor-binding domain.

Fab affinities for the SARS-CoV-2 RBD were measured using biolayer interferometry (BLI). Red lines represent the fit for a 1:1 binding model, and alternate colors represent response curves measured at varying concentrations. Binding kinetics were measured for six concentrations of Fab at twofold dilution ranging from 500 to 15.6 nM (for Fab C1A-B3, C1A-F10, C1A-gl, C1A-gl*), 250 to 7.8 nM (C1A-C2, C1A-H5, C1A-C4), and from 15.6 to 0.49 nM (Fab C1A-B12 and C1A-H6), ensuring that each dilution series had concentrations both above and below the dissociation constant (K_D). For affinity enhanced antibodies, binding kinetics were measured at seven concentrations of Fab at twofold dilution ranging from 100 to 1.56 nM (C1A-B12.1) or from 10 to 0.16 nM (C1A-B12.2 and C1A-B12.3). Each experiment was performed at least twice, and representative data are shown.

Figures 13A-13G depict SARS-CoV-2 receptor-binding domain recognition by C1A-IGHV3-53 antibodies. **Figure 13A** depicts BLI-based competition assay for C1A-B12 Fab, CR3022 Fab, and human ACE2-ectodomain Fc fusion protein (ACE2-Fc) binding to the SARS-CoV-2 RBD. Arrows show the time point at which the indicated protein was added. Representative results of two replicates for each experiment are shown. **Figure 13B** depicts an overlay of ribbon diagrams for X-ray crystal structures of Fab/SARS-CoV-2 RBD complexes. CDR loops contacting the RBD are indicated. **Figure 13C** depicts a ribbon diagram of the X-ray crystal structure of the SARS-CoV-2 RBD bound to the ACE2 ectodomain (PDB ID: 6M0J) (Lan et al., 2020) with the SARS-CoV-2 RBD in the same orientation as shown in **Figure 13B** for comparison. **Figures 13D-13G** depicts details of the interface between the SARS-CoV-2 RBD and the C1A-B3 antibody. The panels show significant contacts made by antibody CDR loops.

Figures 14A-14B depict a structural comparison of IGVH3-53/3-66-derived antibodies. **Figure 14A** depicts examples of gene usage and CDR H3 lengths for other IGVH3-53/3-66 (VH3-53/3-66) antibodies for which structures are available and which were included in our analysis. All antibodies, which were isolated from COVID-19 convalescent donors, engage the RBD with an essentially identical binding mode. CDR H3 length was determined using IMGT/V-QUEST definitions (Brochet et al., 2008). a.a.: amino acids. PDB ID: protein data bank identification code. **Figure 14B** depicts a structural alignment of variable heavy (V_H) and variable light (V_L) portion of Fabs derived from IGHV3-53/3-66

(VH3-53/3-66) antibodies bound to the SARS-CoV-2 RBD for all antibodies listed in **Figure 14A**. a.a.: amino acids. PDB ID: protein data bank identification code. **Figures 14C-14D** depict interactions occurring at the base of CDR H1 near with framework regions are shown for the B38 Fab/RBD complex (PDB: 7BZ5) (Wu et al., 2020) (**Figure 14C**) or CV30 Fab/RBD complex (PDB: 6XE1) (Hurlburt et al., 2020b) (**Figure 14D**). The T28IVH mutation adds a hydrophobic contact with G476RBD, and the F27VVH mutation probably makes CDR H1 more flexible, allowing local polar contacts to be optimized. **Figure 14E** depicts partial sequence alignment of C1A VH3-53 and affinity enhanced antibodies C1A-B12.1, C1A-B12.2, and C1A B12.3. **Figure 14F** shows infectious SARS-CoV-2 (strain USA/WA1/2020) was incubated with monoclonal antibodies at the indicated concentrations with infection of Vero E6 cells subsequently measured in a PRNT assay. Means $\pm$ standard deviation from three experiments performed in triplicate (n=9) are shown. Data are normalized to a no antibody control. For some data points, error bars are smaller than symbols. IC50 values are in parentheses.

Figures 15A-15E depict a germline revertant antibody neutralizes SARS-CoV-2. **Figure 15A** depicts nucleotide sequences of the D segment of C1A-IGHV3 (C1A-V_H3-53) antibodies. Changes that likely occurred at CDR H3 position 100a (S100aR or S100aK) during somatic hypermutation are highlighted. **Figure 15B** depicts a ribbon diagram of C1A-B12/RBD complex showing RBD interactions occurring with alternate side chain conformers of CDR H3 residue R100a (one conformer is labeled with an asterisk). **Figure 15C** depicts amino acid sequences for CDR H3 loops of germline revertant antibodies C1Agl and C1Agl*. **Figure 15D** depicts results of kinetic analysis of binding for Fabs on immobilized SARS-CoV-2 RBD as measured by BLI. **Figure 15E** depicts results of PRNT assay with infectious SARS-CoV-2 (strain USA/WA1/2020) and the indicated monoclonal antibodies. Data are normalized to a no antibody control. Means $\pm$ standard deviation from three experiments performed in triplicate (n=9) are shown. Error bars indicate standard deviation. For some data points, error bars are smaller than symbols.

Figure 16A depicts antibody neutralization of SARS-CoV2 lentivirus pseudotype containing the D614G spike protein mutation. Dose response neutralization assay results with SARS-CoV-2 lentivirus pseudotype with the D614G mutation. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6). For some data points, error bars are smaller than symbols.

Figure 16B depicts correlation analysis of Fab/RBD antibody affinity measurements for the indicated antibodies and SARS CoV-2 USA/WA1/2020 neutralization IC₅₀ values shows no correlation. *r*: Pearson correlation coefficient; n.s.: not significant.

Figure 17 depicts crystallography data collection and refinement statistics. ^a Numbers of crystals for C1A-B3, C1A-B12, C1A-C2 and C1A-F10 data were 1 each. ^b Values in parentheses are for the highest-resolution shell. ^c Values from program *aimless*.

Figures 18A-18M depict predicted antibody neutralization escape during persistent SARS-CoV-2 infection and comparison to other variants. **Figure 18A** depicts a timeline and sequencing interval during persistent SARS-CoV-2 infection of an immunocompromised individual as reported by Choi et al. (Choi et al., 2020). Prolonged hospitalizations are shown in gray. Sequencing on days 18 and 25 was obtained during shorter hospitalizations, which are not shown. **Figure 18B** depicts a table showing SARS-CoV-2 S RBD mutations occurring during persistent infection (Choi et al., 2020). Predicted effects of substitutions on binding of the C1A-V_H3-53 antibodies are shown in the legend. Mutations that are the focus of our analysis are highlighted. For pseudotyping, we generated S mutants for day 146 and 152 S sequences that retain the Y489H_{RBD} mutation that occurred on day 128 (these are labelled “day 146*” and “day 152*”). Sequences from variants that were first detected in the United Kingdom (“UK”, B.1.1.7), South Africa (“SA”, B.1.351), and Brazil (“BR”, P.1), and additional human-derived S sequences containing relevant mutations from samples collected in the United States (USA), are also included for comparison (see Figure 19 and Figures 18J-18M). **Figure 18C** depicts structure of the C1A-B12 Fab/RBD complex with mutated residues indicated in **Figure 18B** shown as spheres. Residues mutated during SARS-CoV-2 evolution in the immunocompromised individual are shown as dark spheres, and a residue mutated in the B.1.351 and P.1 variants (N417) is shown as a light sphere. **Figures 18D-18I** depict for each indicated mutation, interactions observed in the C1A-B12/RBD complex structure are shown in the left panels (labeled “*structure*”) and predicted effects of mutations based on modeling are shown in the right panels (labeled “*modeled*”). PyMol was used to model mutations and visualize steric clashes; short green lines or small green disks are present when nearby atoms are almost in contact, and large disks indicate significant van der Waals overlap. For modeling, only residues on the RBD were modified, and all RBD residue rotamers in the rotamer library were checked and the one that caused the least clashes was chosen. Alternate side chain rotamers for R100_{avH}, S30_{vL}, and for Y489_{RBD} are indicated with an asterisk. **Figures 18J-18M** depicts a table of human derived SARS-CoV-2 S sequences containing mutations of interest. Not all S mutations found in the respective

sequences are shown. RBD mutations of interest are shown in bold, and NTD deletions relevant to those shown in Figure S10 are shown in regular font. The Y453_{RBD} mutation found in hCoV-19/Denmark/DCGC-5481/2020 is shown because it is a REGN10933 resistance mutation detected *in vitro* (Baum et al., 2020) and has also been associated with mink-derived SARS-CoV-2 sequences.

Figures 19A-19F depicts an alignment of SARS-CoV-2 sequences. The following sequences were used for the alignment: Day 18: hCoV-19/USA/MA-JLL-D18/2020 (EPI_ISL_593478); Day 25: hCoV-19/USA/MA-JLL-D25/2020 (EPI_ISL_593479); Day 75: hCoV-19/USA/MA-JLL-D75/2020 (EPI_ISL_593480); Day 81: hCoV-19/USA/MA-JLL-D81/2020 (EPI_ISL_593553); Day 128: hCoV-19/USA/MA-JLL-D128/2020 (EPI_ISL_593554); Day 130: hCoV-19/USA/MA-JLL-D130/2020 (EPI_ISL_593555); Day 143: hCoV-19/USA/MA-JLL-D143/2020 (EPI_ISL_593556); Day 146: hCoV-19/USA/MA-JLL-D146/2020 (EPI_ISL_593557); Day 152: hCoV-19/USA/MA-JLL-D152/2020 (EPI_ISL_593558). Sequences from United Kingdom (“UK”) B.1.1.7 hCoV-19/England/205261299/2020 (EPI_ISL_754289), South Africa (“SA”) B.1.351 hCoV-19/South Africa/Tygerberg-461/2020 (EPI_ISL_745186), Brazil (“BR”) P.1 hCoV-19/Brazil/AM-20143138FN-R2/2020, United States (USA) B.1.1.7 Q493K hCoV-19/USA/FL-CDC-STM-0000013-F04/2021 (EPI_ISL_884605), UK B.1.1.7 Q493R hCoV-19/England/MILK-11C2FCD/2021 (EPI_ISL_1006449), and USA B1.1.7 Y489H hCoV-19/USA/CA-CDC-STM-A100413/2021 (EPI_ISL_850699) are included for comparison. The “day 146*” sequence shown is a version of the day 146 sequence that retains wildtype residues at positions 12-18, contains an NTD deletion spanning residues 142-144 (instead of 141-143), and contains the Y489_{RBD} mutation. The “day 152*” sequence shown is a version of the day 152 sequence that contains the Y489_{RBD} mutation. Both day 146* and day 152* sequences contain mutations in the C-terminal cytoplasmic tail to allow for efficient lentivirus pseudotyping. The figure was generated using ESPrit3 (Robert and Gouet, 2014).

Figures 20A-20I depict sequence variation and relationship to ACE2 interactions. **Figure 20A** depicts sequence alignment for S residues spanning the RBD in an immunocompromised individual (Choi *et al.*, *N Engl J Med.* 2020) at the indicated timepoints. RBD residues that interact with ACE2 only, C1A-V_H3-53 antibodies only, or both, are indicated. **Figure 20B** depicts a ribbon diagram of the X-ray crystal structure of a ACE2 ectodomain/RBD complex (PDB ID: 6M0J) (Lan et al., 2020). Residues that are mutated during SARS-CoV-2 persistent infection are shown as dark spheres. The K417_{RBD} residue, which is mutated in the B.1.351 and P.1 variants (see **Figure 18B**), is shown as light

spheres. **Figures 20C-20H** depicts views highlighting where select RBD antibody-escape mutations (see **Figures 18D-18I**) fall with respect to the ACE2 interface. **Figures 20I-20J** depict SARS-CoV-2 Day 146* (**Figure 20I**) or Day 152* (**Figure 20J**) S pseudotypes were pre-incubated with an ACE2-Fc fusion protein at the indicated concentrations and the mixture
 5 was used to infect HEK293T-hACE2 cells. Entry levels were quantified 48 h later using FACS. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6) are shown. IC₅₀ values are shown in parentheses.

Figures 21A-21E depicts neutralization escape of monoclonal antibodies and human polyclonal immunoglobulins. **Figure 21A** depicts a table showing IC₅₀ values for pseudotype
 10 neutralization tests with the indicated SARS-CoV-2 S pseudotypes. Monoclonal antibody names are abbreviated (*e.g.*, C1A-gl is “gl” and C1A-B3 is “B3”). Antibodies are listed, left to right, in order of increasing affinity. IC₅₀ values for an ACE2-Fc neutralization assay done as part of the same experiment are shown. See also **Figure 22A**. **Figure 21B** depicts a summary of results shown in **Figure 21A** highlighting the fraction of resistant monoclonal
 15 antibodies for each S pseudotype. **Figure 21C** depicts a ribbon diagram of the SARS-CoV-2 RBD bound to Fabs for antibodies REGN10987 and REGN10933 (PDB: 6XDG) (Hansen et al., 2020). Mutated residues are shown as in **Figure 18B**, with the exception that residue N439_{RBD} is shown as light spheres. **Figure 21D** depicts a table showing IC₅₀ values for SARS-CoV-2 S pseudotype neutralization tests with the indicated monoclonal antibodies.
 20 IC₅₀ values for an ACE2-Fc neutralization assay done as part of the same experiment are shown. See also **Figures 22B-22C**. **Figure 21E** depicts a dose response neutralization assay with the indicated SARS-CoV-2 S pseudotypes with polyclonal serum IgG of four COVID-19 convalescent donors (C1, C2, C3, and C4) or that of a control, non-immune donor (“ctrl”). **Figure 21F** depicts a table showing IC₅₀ values for pseudotype neutralization tests shown in
 25 **Figure 21E**.

Figure 22A depicts a dose response neutralization assay of C1A-V_H3-53 and affinity enhanced versions of C1A-B12 with the indicated S pseudotypes. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6) are shown. IC₅₀ values are shown in **Figures 21A-21B**.

Figure 22B depicts a dose response neutralization assay of REGN10933 and REGN10987 (Baum et al., 2020; Hansen et al., 2020) and CC12.1 (Rogers et al., 2020) with the indicated S pseudotypes. Data are normalized to a no antibody control. Means $\pm$ standard deviation from two experiments performed in triplicate (n=6) are shown. IC₅₀ values are shown in **Figure 21D**.

Figure 22C depicts dose response neutralization assays of monoclonal antibody B38 (Wu et al., 2020). Means $\pm$ standard deviation from two experiments performed in triplicate (n=6) are shown. IC₅₀ values are shown in **Figure 21D**.

Figures 23A-23B depicts S1 NTD deletions and predicted impact on antibody binding.

Figure 23A depicts a summary of SARS-CoV-2 S N-terminal domain (NTD) deletions occurring during persistent infection of an immunocompromised individual (Choi et al., *N Engl J Med.* 2020). Deletions found in United Kingdom (“UK”) B.1.1.7 (hCoV-19/England/205261299/2020, EPI_ISL_754289) and South Africa (“SA”) B.1.351 (hCoV-19/South Africa/Tygerberg-461/2020, EPI_ISL_745186) variants are also included for comparison.

Figure 23B depicts a ribbon diagram of the 4A8 Fab:NTD interface (PDB: 7C2L) (Chi et al., 2020). Residues 141-144, which contain mutations starting on day 75, are shown in dark gray, and residues 242-244, which are mutated in the “SA” B.1.351 SARS-CoV-2 variant (**Figures 19A-19F**), are shown in light gray. The 141-144 deletion would reposition a putative N-linked glycosylation site (N149) and potentially block epitope access.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides antigen-binding proteins that specifically bind to a coronavirus or coronavirus spike protein (S), antigen-binding protein compositions and methods of use thereof to passively immunize and treat subjects having or at risk of having a coronavirus infection. In particular, the present invention provides antigen-binding proteins that specifically bind to a severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein (S), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S), antigen-binding protein compositions and methods of use thereof to passively immunize and treat subjects having or at risk of having a SARS-CoV, *e.g.*, SARS-CoV-2, infection.

The following detailed description discloses how to make and use coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-1 and/or SARS-CoV-2, antigen-binding proteins as well as methods for treating or preventing a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection in subjects, *e.g.*, subjects susceptible to or diagnosed with a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2 infection.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”.

The term “or” is used herein to mean, and is used interchangeably with, the term “and/or,” unless context clearly indicates otherwise.

The term “about” is used herein to mean within the typical ranges of tolerances in the art. For example, “about” can be understood as about 2 standard deviations from the mean.

In certain embodiments, about means $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. When about is present before a series of numbers or a range, it is understood that “about” can modify each of the numbers in the series or range.

The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least”, and all subsequent numbers or integers that could logically be included, as clear from context. When at least is present before a series of numbers or a range, it is understood that “at least” can modify each of the numbers in the series or range.

As used herein, “no more than” or “less than” is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. When “no more than” is present before a series of numbers or a range, it is understood that “no more than” can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

As used herein, the term “coronavirus,” (“CoV”; subfamily *Coronavirinae*, family *Coronaviridae*, order *Nidovirales*), refers to a group of highly diverse, enveloped, positive-sense, single-stranded RNA viruses that cause respiratory, enteric, hepatic and neurological diseases of varying severity in a broad range of animal species, including humans. Coronaviruses are subdivided into four genera: *Alphacoronavirus*, *Betacoronavirus* (13CoV), *Gammacoronavirus* and *Deltacoronavirus*.

As use herein, the term “severe acute respiratory syndrome coronavirus” or “SARS-CoV”, refers to a coronavirus that was first discovered in 2003, which causes severe acute respiratory syndrome (SARS). SARS-CoV represents the prototype of a new lineage of coronaviruses capable of causing outbreaks of clinically significant and frequently fatal human disease. The complete genome of SARS-CoV has been identified, as well as common variants thereof. The genome of SARS-CoV is a 29,727-nucleotide polyadenylated RNA, has 11 open reading frames, and 41% of the residues are G or C. The genomic organization is typical of coronaviruses, with the characteristic gene order (5'-replicase (rep), spike (S), envelope (E), membrane (M), nucleocapsid (N)-3' and short untranslated regions at both termini. The SARS-CoV rep gene, which comprises about two-thirds of the genome, is predicted to encode two polyproteins that undergo co-translational proteolytic processing. There are four open reading frames (ORFs) downstream of rep that are predicted to encode the structural proteins, S, E, M and N. The hemagglutinin-esterase gene, which is present between ORF1b and S in group 2 and some group 3 coronaviruses was not found.

As use herein, the terms “severe acute respiratory syndrome coronavirus 2,” “SARS-CoV-2,” “2019-nCoV,” refer to the novel coronavirus that caused a pneumonia outbreak first reported in Wuhan, China in December 2019 (“COVID-19”). Phylogenetic analysis of the complete viral genome (29,903 nucleotides) revealed that SARS-CoV-2 was most closely related (89.1% nucleotide similarity) to SARS-CoV. SARS-CoV-2 has infected over 110 million individuals worldwide, resulting in over 2.4 million deaths to date. The SARS-CoV-2 spike protein (S) is a target for vaccine and drug design efforts (Abraham, 2020; Krammer, 2020). S is heavily glycosylated and forms trimers of heterodimers on the virion surface. Each S protomer has two functional subunits; S1, which contains a receptor-binding domain (RBD) that binds the cellular receptor, ACE2 (Hoffmann et al., 2020; Zhou et al., 2020), and S2, which mediates fusion of the viral and host cell membranes during viral entry. Epitopes for neutralizing antibodies include non-overlapping sites on the RBD and the S1 N-terminal domain (NTD) (Chi et al., 2020; Du et al., 2020; Hansen et al., 2020; Liu et al., 2020; Robbiani et al., 2020; Wu et al., 2020). As use herein, the term “spike protein” or “S protein”, refers to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike glycoprotein which mediates a cell surface receptor binding and fusion of the viral and host cell membranes. The S protein is a target for anti-viral antibodies produced during natural infection and comprises two functional subunits, S1 and S2. The S1 subunits of SARS-CoV-1 and SARS-CoV-2 contain a receptor-binding domain (RBD) that binds to angiotensin-converting enzyme 2 (ACE2) on the surface of host cells. S2 contains a transmembrane

anchor and mediates fusion of viral and host cell membranes after particles are internalized into acidified endosomes, although fusion at the cell surface can also occur in certain scenarios. In certain embodiments, the antigen-binding proteins, *e.g.*, neutralizing antibodies, of the present invention may block viral entry and/or viral infection by preventing the S protein from binding to host cell receptors (*e.g.*, ACE2). In certain embodiments, the antigen-binding proteins, *e.g.*, neutralizing antibodies, of the present invention may block viral entry and/or viral infection by preventing the conformational changes the S protein undergoes to mediate membrane fusion. In certain embodiments, the antigen-binding proteins, *e.g.*, neutralizing antibodies, of the present invention may block viral entry and/or viral infection by mimicking receptor binding and prematurely trigger fusogenic conformational changes in the S protein before it engages ACE2.

The amino acid sequence of the SARS-CoV-2 spike protein is provided in GenBank as accession number QJF75467.1 (SEQ ID NO:100). The term “spike protein” includes recombinant SARS-CoV-2 spike protein or a fragment thereof. The term also encompasses SARS-CoV-2 spike protein or a fragment thereof coupled to, for example, a mouse or human Fc, a signal peptide sequence, and/or a protein tag.

The term “antigen-binding protein,” “binding protein” or “binding molecule,” as used herein includes molecules that contain at least one antigen-binding portion that specifically binds to a molecule of interest, such as a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100).

In some embodiments, a binding protein is an antibody, such as a full-length antibody, or an antigen-binding fragment of an antibody, or any other polypeptide.

In some embodiments, a binding protein is a SARS-CoV-2 neutralizing antibody or an antigen-binding fragment thereof.

The term “antibody”, as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, a SARS-CoV-2 S protein). The term “antibody” includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, “full antibody molecules”), as well as multimers thereof (*e.g.*, IgM) or antigen-binding fragments thereof. Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The

light chain constant region comprises one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the antibody (or antigen-binding portion thereof) may be identical to the human germ line sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antigen binding proteins, such as 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. Empirical substitutions can be conservative or non-conservative substitutions.

The antigen-binding proteins, or the antigen-binding fragments thereof, may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antigen-binding proteins or antigen-binding domains were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and the antigen-binding domains thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions

are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as “germline mutations”). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments, which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies, or the antigen-binding domains thereof, of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies, or the antigen-binding fragments thereof, that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, *etc.* Antibodies, or the antigen-binding fragments thereof, obtained in this general manner are encompassed within the present invention.

The present invention also includes antibodies and antigen-binding molecules comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. Exemplary variants included within this aspect of the invention include variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes antibodies and antigen-binding molecules having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, *etc.* conservative amino

acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B herein.

In certain embodiments, the antigen-binding proteins of the invention are human antibodies. The term “human antibody”, as used herein, is intended to include antibodies having variable and constant regions derived from human germ line immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term “human antibody”, as used herein, is not intended to include antibodies in which CDR sequences derived from the germ line of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The antigen-binding proteins of the invention may, in some embodiments, be recombinant human antibodies. The term “recombinant human antibody”, as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor *et al.* (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, 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 V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germ line V_H and V_L sequences, may not naturally exist within the human antibody germ line repertoire *in vivo*. In some embodiments, the antigen-binding proteins of the invention may be derived from the VH3-53/VLK1-9 heavy and light chain antibody genes. In some embodiments, the antigen-binding proteins of the invention may be derived from the VH3-53 and VH3-66 antibody genes. The VH3-53 and VH3-66 antibody genes are identical except for a single amino acid change in an antibody framework region (FWR) (Lefranc and Lefranc, 2014), and potent neutralizing

antibodies derived from VH3-53 and VH3-66 germline genes have been isolated from multiple COVID-19 convalescent individuals (Du et al., 2020; Robbiani et al., 2020; Rogers et al., 2020; Seydoux et al., 2020; Shi et al., 2020; Wu et al., 2020; Yuan et al., 2020a). In some instances, antibodies derived from VH3-53 and VH3-66 germline genes engage the RBD and interfere with viral entry by blocking ACE2 engagement.

Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, CH2 or CH3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

The term “neutralizing antibody”, or the like, means that an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, can neutralize the ability of a pathogen to initiate and/or perpetuate an infection in a subject and/or in a target cell *in vitro* and/or *in vivo*.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen in 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof. In some embodiments, the present disclosure provides a neutralizing

antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to SARS-CoV-2 spike (S) protein (*e.g.*, SEQ ID NO: 100). In some embodiments, the total length of SARS-CoV-2 S is 1273 amino acids and consists of a signal peptide (amino acids 1–13) located at the N-terminus, the S1 subunit (14–685 residues), and the S2 subunit (686–1273 residues). Without wishing to be bound by any theory, the last two regions, the S1 subunit and the S2 subunit, are responsible for receptor binding and membrane fusion, respectively. In the S1 subunit, there is an N-terminal domain (NTD) (14–305 residues) and a receptor-binding domain (RBD) (319–541 residues). The fusion peptide (FP) (788–806 residues), heptapeptide repeat sequence 1 (HR1) (912–984 residues), HR2 (1163–1213 residues), TM domain (1213–1237 residues), and cytoplasm domain (1237–1273 residues) comprise the S2 subunit. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to the SARS-CoV-2 S1 subunit, for example, the NTD and/or the RBD. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to, the SARS-CoV-2 S2 subunit, for example, the FP, HR1, HR2, TM, and/or CT.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to circulating SARS-CoV-2 variants and/or high-risk bat coronaviruses.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that is cross-reactive with multiple coronaviruses or strains thereof. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that are cross-reactive to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that

causes coronavirus disease 2019, or COVID-19), and/or variants thereof. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that are cross-reactive to a SARS-CoV-2, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that are cross-reactive to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that is not cross-reactive with multiple coronaviruses or strains thereof.

Without wishing to be bound by any particular theory, the neutralizing antibodies described herein may block viral entry by preventing S from binding to host-cell receptors (*e.g.*, ACE2), or by preventing the conformational changes S must undergo to mediate fusion of the viral and host cell membranes. For example, epitopes for neutralizing antibodies on SARS-CoV-2 spike (S) protein can include at least two non-overlapping epitopes on the RBD (Wu, Y. *et al. Science*, 368(6496):1274-1278, 2020; Hansen, J. *et al. Science*, 369(6506):1010-1014, 2020; incorporated herein by reference) and the N-terminal domain (NTD) (Chi, X. *et al. Science* 369(6504):650-655, 2020; Liu, L. *et al. Nature*, 584:450-456, 2020; incorporated herein by reference). Antibodies can also bind a tertiary epitope on S that spans two RBDs, the engagement of which clamps down S into the closed conformation (Liu, L. *et al. Nature*, 584:450-456, 2020, incorporated herein by reference). In some embodiments, neutralizing monoclonal antibodies, when administered right before or after viral challenge, can decrease viral RNA lung burden or alleviate lung pathology animal models (Wu, Y. *et al. Science*, 368(6496):1274-1278, 2020; Cao, Y. *et al. Cell*, 182(1):73-84, 2020; incorporated herein by reference).

The term “specifically binds,” or “binds specifically to”, or the like, means that an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-8} M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, *e.g.*, BIACORE™, and the like. As described herein, antigen-binding proteins, *e.g.*, antibodies, have been identified which bind

specifically to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100).

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a coronavirus. In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen in 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to SARS-CoV-2 spike (S) protein (*e.g.*, SEQ ID NO: 100). In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to the SARS-CoV-2 S1 subunit, for example, the NTD and/or the RBD. In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to the SARS-CoV-2 S2 subunit, for example, the FP, HR1, HR2, TM, and/or CT.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to pre-fusion conformation stabilized proteins, including, but not limited to HexaPro, S-R/x2, or S2P DS constructs and soluble RBD domains.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to the same epitope, or an overlapping epitope, as B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and/or REGN10987, two antibodies that bind non-overlapping epitopes in the RBD; 4A8, an NTD binder; 2-43, which binds a quaternary epitope that spans two RBDs;

and/or CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that competes for binding to a coronavirus or coronavirus spike (S) protein with B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and/or REGN10987, two antibodies that bind non-overlapping epitopes in the RBD; 4A8, an NTD binder; 2-43, which binds a quaternary epitope that spans two RBDs; and/or CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that does not compete for binding to a coronavirus or coronavirus spike (S) protein with B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and/or REGN10987, two antibodies that bind non-overlapping epitopes in the RBD; 4A8, an NTD binder; 2-43, which binds a quaternary epitope that spans two RBDs; and/or CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports.

In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that is cross-reactive with multiple coronaviruses or strains thereof. In some embodiments, the present disclosure provides an antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that is cross-reactive to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that are cross-reactive to a SARS-CoV-2, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that are cross-reactive to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides a antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that is not cross-reactive with multiple coronaviruses or strains thereof.

The term “ K_D ”, as used herein, is intended to refer to the equilibrium dissociation constant of a particular antigen-binding protein-antigen interaction.

The term “antibody”, as used herein, also includes antigen-binding fragments of full antibody molecules. The terms “antigen-binding portion” of an antibody, “antigen-binding fragment” of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms “antigen-binding fragment” of an antibody, or “antibody fragment”, as used herein, refers to one or more fragments of an antibody that retain the ability to bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein.

The antigen-binding proteins of the invention may be isolated antibodies. An “isolated antibody,” as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an “isolated antibody” for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

In certain embodiments, the antigen-binding proteins, *e.g.*, isolated antibodies, of the invention may be included in a convalescent blood product (CBP) obtained, for example, by collecting whole blood or plasma from a subject who has survived a previous coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection and developed humoral immunity against the virus responsible for the disease in question (*e.g.*, COVID-19). In certain embodiments, the transfusion of CBP is able to neutralize the virus and eventually leads to its eradication from the blood circulation. Different CBP including an antigen-binding protein of the invention may be used to achieve passive immunity in a subject, including, *e.g.*, (i) convalescent whole blood (CWB), convalescent plasma (CP) or convalescent serum (CS); (ii) pooled human immunoglobulin (Ig) for intravenous or intramuscular administration; (iii) high-titre human Ig; and (iv) polyclonal or monoclonal antibodies.

The term “substantial identity” or “substantially identical,” when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is

nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

Sequence identity can be calculated using an algorithm, for example, the Needleman Wunsch algorithm (Needleman and Wunsch 1970, *J. Mol. Biol.* 48: 443-453) for global alignment, or the Smith Waterman algorithm (Smith and Waterman 1981, *J. Mol. Biol.* 147: 195-197) for local alignment. Another preferred algorithm is described by Dufresne *et al* in *Nature Biotechnology* in 2002 (vol. 20, pp. 1269-71) and is used in the software GenePAST (GQ Life Sciences, Inc. Boston, MA).

As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 95%, 96%, 97%, 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) *Methods Mol. Biol.* 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the

PAM250 log-likelihood matrix disclosed in Gonnet *et al* (1992) *Science* 256: 1443-1445. A “moderately conservative” replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410 and Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389-402.

By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

By “treatment”, “prevention” or “amelioration” of a disease or disorder, *e.g.*, a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection is meant delaying or preventing the onset of such a disease or disorder, reversing, alleviating, ameliorating, inhibiting, slowing or stopping the progression, aggravation or deterioration, the progression or severity of a condition associated with such a disease or disorder. In one embodiment, the symptoms of a disease or disorder, or pain and distress associated with an infection, are alleviated by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%. In one embodiment, the transmission of a coronavirus infection, is reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%.

As used herein, a “subject” means a human or an animal. The animal may be a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include chimpanzees, cynomolgous monkeys, spider monkeys, and macaques, *e.g.*, Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include cows, sheep, pigs, goats, birds, horses, pigs, deer, bison, buffalo, amphibians, reptiles, feline species, *e.g.*, domestic cat, canine species, *e.g.*, dog, fox, wolf, avian species, *e.g.*, chicken, emu, ostrich, and fish, *e.g.*, trout, catfish and salmon. In certain embodiments, the subject is an embryo or a fetus, where a life-long protection is elicited after vaccination with the present invention.

In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human. The terms, “patient” and “subject” are used interchangeably herein. Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, pig, sheep, goat, bird, reptile, amphibian, fish or cow. Mammals other than humans can be advantageously used as subjects that represent animal models of infectious diseases, or other related pathologies. A subject can be male or female. The subject can be an adult, an adolescent or a child. A subject can be one who has been previously diagnosed with or identified as suffering from or having a risk for developing a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection. In a preferred embodiment, the subject is a human, such as a human being treated or assessed for a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection; a human at risk for coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection; a human having a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, infection. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

The term “vaccine,” as used herein, includes any composition containing an immunogenic determinant which stimulates the immune system such that it can better respond to subsequent infections. A vaccine usually contains an immunogenic determinant, *e.g.*, an antigen, and an adjuvant, the adjuvant serving to non-specifically enhance the immune response to that immunogenic determinant. Currently produced vaccines predominantly activate the humoral immune system, *i.e.*, the antibody dependent immune response. Other vaccines focus on activating the cell-mediated immune system including cytotoxic T lymphocytes which are capable of killing targeted pathogens.

The term “adjuvant”, as used herein, refers to compounds that can be added to vaccines to stimulate immune responses against antigens. Adjuvants may enhance the

immunogenicity of highly purified or recombinant antigens. Adjuvants may reduce the amount of antigen or the number of immunizations needed to protective immunity. For example, adjuvants may activate antibody-secreting B cells to produce a higher amount of antibodies. Alternatively, adjuvants can act as a depot for an antigen, present the antigen over a longer period of time, which could help maximize the immune response and provide a longer-lasting protection. Adjuvants may also be used to enhance the efficacy of a vaccine by helping to modify the immune response to particular types of immune system cells, for example, by activating T cells instead of antibody-secreting B cells depending on the purpose of the vaccine.

II. Antigen Binding Proteins

The present disclosure provides antigen-binding proteins that include antibodies, or antigen-binding fragments thereof. Unless specifically indicated otherwise, the term “antibody,” as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (i.e., “full antibody molecules”) as well as antigen-binding fragments thereof. The terms “antigen-binding portion” of an antibody, “antigen-binding fragment” of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms “antigen-binding fragment” of an antibody, or “antibody fragment”, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100). An antigen-binding protein, such as an antibody fragment, may include a Fab fragment, a F(ab')₂ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen binding proteins, such as antigen-binding fragments of an antibody, may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, *etc.*

Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) Fab' fragments, (iii) F(ab')₂ fragments; (iv) Fd fragments; (v) Fv fragments; (vi) single-chain Fv (scFv) molecules; (vii) dAb fragments; and (viii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single-domain antibodies (sdAb), domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.*, monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

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

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

configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

As with full antibody molecules, antigen-binding proteins, *e.g.*, antigen-binding fragments of an antibody, may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present disclosure using routine techniques available in the art.

Multispecific antigen-binding proteins may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. *See, e.g.*, Tutt *et al.*, 1991, *J. Immunol.* 147:60-69; Kufer *et al.*, 2004, *Trends Biotechnol.* 22:238-244. The antigen-binding proteins, *e.g.*, antigen-binding fragments of an antibody, of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein, *e.g.*, as described herein. For example, an antibody or fragment thereof can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment, *e.g.*, as described herein, to produce a bi-specific or a multispecific antibody with a second binding specificity.

Use of the expressions “multispecific antigen-binding proteins” herein is intended to include monospecific antibodies targeting a coronavirus as well as bispecific antibodies comprising a coronavirus targeting binding arm and an arm that binds another target antigen. Thus, the present invention includes bispecific antibodies wherein one arm of an immunoglobulin binds a coronavirus, and the other arm of the immunoglobulin is specific for another target antigen. The target antigen that the other arm of the bispecific antibody binds can be any antigen expressed on or in the vicinity of a cell, tissue, organ, microorganism or virus, against which a targeted immune response is desired. The coronavirus targeting arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B herein. The other arm can comprise any of the HCVR/LCVR or CDR amino acid sequences as set forth in Table 1 herein and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B. In certain embodiments, the coronavirus arm and/or other arm binds to a coronavirus and induces neutralization and/or an immune response to the coronavirus.

As used herein, the expression “bispecific antigen-binding molecule” means a protein, polypeptide or molecular complex comprising at least a first antigen-binding domain and a second antigen-binding domain. Each antigen-binding domain within the bispecific antigen-binding molecule comprises at least one CDR that alone, or in combination with one or more additional CDRs and/or FRs, specifically binds to a particular antigen. In the context of the present invention, the first antigen-binding domain specifically binds a first antigen (*e.g.*, a coronavirus or coronavirus spike (S) protein), and the second antigen-binding domain specifically binds a second, distinct and/or noncompeting antigen (*e.g.*, on the same or a different coronavirus).

In certain exemplary embodiments of the present invention, the bispecific antigen-binding molecule is a bispecific antibody. Each antigen-binding domain of a bispecific antibody comprises a heavy chain variable domain (HCVR) and a light chain variable domain (LCVR). In the context of a bispecific antigen-binding molecule comprising a first and a second antigen binding domain (*e.g.*, a bispecific antibody), the CDRs of the first antigen-binding domain may be designated with the prefix “D1” and the CDRs of the second antigen-binding domain may be designated with the prefix “D2”. Thus, the CDRs of the first antigen-binding domain may be referred to herein as D1-HCDR1, D1-HCDR2, and D1-HCDR3; and the CDRs of the second antigen-binding domain may be referred to herein as D2-HCDR1, D2-HCDR2, and D2-HCDR3.

The first antigen-binding domain and the second antigen-binding domain may be directly or indirectly connected to one another to form a bispecific antigen-binding molecule of the present invention. Alternatively, the first antigen-binding domain and the second antigen binding domain may each be connected to a separate multimerizing domain. The association of one multimerizing domain with another multimerizing domain facilitates the association between the two antigen-binding domains, thereby forming a bispecific antigen-binding molecule. As used herein, a “multimerizing domain” is any macromolecule, protein, polypeptide, peptide, or amino acid that has the ability to associate with a second multimerizing domain of the same or similar structure or constitution. For example, a multimerizing domain may be a polypeptide comprising an immunoglobulin C_H3 domain. A non-limiting example of a multimerizing component is an Fc portion of an immunoglobulin (comprising a C_H2-C_H3 domain), *e.g.*, an Fc domain of an IgG selected from the isotypes IgG1, IgG2, IgG3, and IgG4, as well as any allotype within each isotype group.

Bispecific antigen-binding molecules of the present invention will typically comprise two multimerizing domains, *e.g.*, two Fc domains that are each individually part of a separate

antibody heavy chain. The first and second multimerizing domains may be of the same IgG isotype such as, *e.g.*, IgG1/IgG1, IgG2/IgG2, IgG4/IgG4. Alternatively, the first and second multimerizing domains may be of different IgG isotypes such as, *e.g.*, IgG1/IgG2, IgG1/IgG4, IgG2/IgG4, *etc.*

5 In certain embodiments, the multimerizing domain is an Fc fragment or an amino acid sequence of 1 to about 200 amino acids in length containing at least one cysteine residue. In other embodiments, the multimerizing domain is a cysteine residue, or a short cysteine containing peptide. Other multimerizing domains include peptides or polypeptides comprising or consisting of a leucine zipper, a helix-loop motif, or a coiled-coil motif.

10 Any multispecific, bispecific, or trispecific antibody format or technology may be used to make the bispecific antigen-binding molecules of the present invention. For example, an antibody or fragment thereof having a first antigen binding specificity can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment having a
15 second antigen-binding specificity to produce a bispecific antigen-binding molecule.

Specific exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (OVO)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, *etc.*), CrossMab, CrossFab, (SEEO)body, leucine
20 zipper, Oubody, IgG1/IgG2, dual acting Fab (OAF)-IgG, and Mab² bispecific formats (*see, e.g.*, Klein *et al.* 2012, *mAbs* 4:6, 1-11, and references cited therein, for a review of the foregoing formats).

III. Preparation of Antigen-Binding Proteins

25 Methods for generating antigen-binding proteins, such as human antibodies, in transgenic mice are known in the art. Any such known methods can be used in the context of the present disclosure to make human antibodies that specifically bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100).

Using VELOCIMMUNE® technology or any other known method for generating
30 antigen-binding proteins, *e.g.*, monoclonal antibodies, high affinity antigen-binding proteins, *e.g.*, chimeric antibodies, to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a

transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antigen-binding protein, *e.g.*, antibody, comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

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

Initially, high affinity antigen-binding proteins, *e.g.*, chimeric antibodies, are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antigen-binding proteins are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the antigen-binding proteins, *e.g.*, fully human antibodies, of the disclosure. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

IV. Bioequivalents

The antigen-binding proteins of the present disclosure encompass proteins having amino acid sequences that vary from those of the described antigen-binding proteins, *e.g.*, antibodies, but that retain the ability to bind a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100). Such variant antigen-binding proteins comprise one or more additions, deletions, or substitutions of amino acids when compared to

parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antigen-binding proteins. Likewise, the antigen-binding protein-encoding DNA sequences of the present disclosure encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antigen-binding protein that is essentially bioequivalent to an antigen-binding protein of the disclosure.

Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antigen-binding proteins or antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

In one embodiment, two antigen-binding proteins (or antibodies) are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

In one embodiment, two antigen-binding proteins (or antibodies) are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

In one embodiment, two antigen-binding proteins (or antibodies) are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antigen-binding protein or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antigen-binding protein (or its target) is measured as a function

of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antigen-binding protein.

Bioequivalent variants of the antigen-binding proteins (or antibodies) of the disclosure may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antigen-binding proteins may include antigen-binding protein variants comprising amino acid changes, which modify the glycosylation characteristics of the antigen-binding proteins, *e.g.*, mutations that eliminate or remove glycosylation.

V. Antigen Binding-Proteins Comprising Fc Variants

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

For example, the present disclosure includes antigen-binding proteins comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group

consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

VI. Biological Characteristics of the Antigen-Binding Proteins

In general, the antigen-binding proteins of the present disclosure function by binding to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100). The present disclosure includes antigen-binding proteins that bind specifically to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), or variant thereof, as described herein. In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2, wherein the SARS-CoV-2 comprises a sequence and/or a mutation as shown in any one of Figures 13-23.

The present disclosure includes antigen-binding proteins that bind specifically to a severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) protein, such as a severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) spike (S) protein and/or a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein.

The present disclosure includes antigen-binding proteins that bind specifically to a coronavirus spike (S) protein comprising an amino acid sequence consisting of SEQ ID NO: 100, or an amino acid sequence comprising at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto. In particular embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a coronavirus (S) protein comprising at least one amino acid modification as compared to the SARS-CoV-2 (S) protein sequence of SEQ ID NO: 100.

The present disclosure includes antigen-binding proteins that bind specifically to a coronavirus spike (S) protein comprising an amino acid sequence consisting of an amino acid sequence as shown in any one of Figures 13-23, or an amino acid sequence comprising at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto. In particular embodiments, the present disclosure provides antigen-binding proteins which binds specifically to a coronavirus (S) protein comprising at least one mutation as compared to an amino acid sequence as shown in any one of Figures 13-23.

In some embodiments, the present disclosure provides a neutralizing antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, that specifically binds to an antigen from a SARS-CoV-2 variant. Exemplary SARS-CoV-2 variants include, without limitation, an Alpha (lineage B.1.1.7) variant, a B.1.1.7 with E484K variant, a Delta (lineage B.1.617.2) variant, a Beta (lineage B.1.351) variant, a Gamma (lineage P.1) variant, a Eta (lineage B.1.525) variant, a Iota (lineage B.1.526) variant, a Kappa (lineage B.1.617.1) variant, a Lambda (lineage C.37) variant, a Epsilon (lineages B.1.429, B.1.427, CAL.20C) variant, a Zeta (lineage P.2) variant, a Theta (lineage P.3) variant, an R.1 variant, a Lineage B.1.1.207 variant, and/or a Lineage B.1.620 variant.

The present disclosure includes antigen-binding proteins that bind specifically to a coronavirus spike (S) protein comprising an amino acid sequence consisting of an amino acid sequence of an Alpha (lineage B.1.1.7) variant, a B.1.1.7 with E484K variant, a Delta (lineage B.1.617.2) variant, a Beta (lineage B.1.351) variant, a Gamma (lineage P.1) variant, a Eta (lineage B.1.525) variant, a Iota (lineage B.1.526) variant, a Kappa (lineage B.1.617.1) variant, a Lambda (lineage C.37) variant, a Epsilon (lineages B.1.429, B.1.427, CAL.20C) variant, a Zeta (lineage P.2) variant, a Theta (lineage P.3) variant, an R.1 variant, a Lineage B.1.1.207 variant, and/or a Lineage B.1.620 variant, or an amino acid sequence comprising at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

The present disclosure includes antigen-binding proteins that bind specifically to a coronavirus or a coronavirus spike (S) protein that comprise a neutralizing antibody escape mutation. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification at any position. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification a position 114, 144, 242, 243, 244, 417, 440, 453, 478, 484, 486, 489, 493, 494, 501, and/or, 614. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification as set forth in **Figure 12**. In some embodiments, the antigen-binding

proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising amino acid modification as set forth in any one of Figures 13-23. For examples, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising a Y114del mutation, a L242del mutation, a A243del mutation, a L244del mutation, a D614G mutation, a K417N mutation, a N440D mutation, a Y453F mutation, a T478K mutation, a E484K mutation, a E484A mutation, a F486I mutation, a F486L mutation, a Y489H mutation, a Q493K mutation, a Q493R mutation, a S494P mutation, and/or a N501Y mutation. In some embodiments, the antigen-binding proteins may bind specifically to a SARS-CoV-2 spike (S) protein comprising at least one amino acid modifications as occurring in an Alpha (lineage B.1.1.7) variant, a B.1.1.7 with E484K variant, a Delta (lineage B.1.617.2) variant, a Beta (lineage B.1.351) variant, a Gamma (lineage P.1) variant, a Eta (lineage B.1.525) variant, a Iota (lineage B.1.526) variant, a Kappa (lineage B.1.617.1) variant, a Lambda (lineage C.37) variant, a Epsilon (lineages B.1.429, B.1.427, CAL.20C) variant, a Zeta (lineage P.2) variant, a Theta (lineage P.3) variant, an R.1 variant, a Lineage B.1.1.207 variant, and/or a Lineage B.1.620 variant.

The present disclosure includes antigen-binding proteins that specifically binds to pre-fusion conformation stabilized proteins, including, but not limited to HexaPro, S-R/x2, or S2P DS constructs and soluble RBD domains.

The present disclosure includes antigen-binding proteins that bind to the same epitope, or an overlapping epitope, as B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and REGN10987, two antibodies that bind non-overlapping epitopes in the RBD; 4A8, an NTD binder; 2-43, which binds a quaternary epitope that spans two RBDs; and CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports. The present disclosure includes antigen-binding proteins that do not bind to the same epitope, or an overlapping epitope, as B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and REGN10987, two antibodies that bind non-overlapping epitopes in the RBD; 4A8, an NTD binder; 2-43, which binds a quaternary epitope that spans two RBDs; and CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports.

5 The coronavirus spike (S) protein mediates cell surface receptor binding and fusion of the viral and host cell membranes. The S protein is often a target for antiviral antibodies produced during natural infection and comprises two functional subunits, S1 and S2. The S1 subunits of SARS- CoV and SARS- CoV-2 contain a receptor-binding domain that binds to angiotensin-converting enzyme 2 (ACE2) on the surface of host cells. S2 contains a

transmembrane anchor and mediates fusion of viral and host cell membranes after particles are internalized into acidified endosomes, although fusion at the cell surface can also occur in certain scenarios. In certain embodiments, the antigen-binding proteins of the present disclosure function by blocking viral entry by preventing the S protein from binding to host cell receptors (for example, ACE2; SEQ ID NO: 100) and/or by preventing the conformational changes the S protein undergoes to mediate membrane fusion. In other embodiments, the antigen-binding proteins of the present disclosure function by mimicking receptor binding and prematurely triggering fusogenic conformational changes in the S protein before it engages ACE2.

In certain embodiments, antigen-binding proteins of the present disclosure function by binding to an epitope within the S1 subunit of SARS-CoV-2 S protein, *e.g.*, within or overlapping with the receptor-binding domain (RBD) (*e.g.*, ACE2 RBD). In certain other embodiments, antigen-binding proteins of the present disclosure function by binding to an epitope within the S2 subunit of SARS-CoV-2 S protein.

The present disclosure includes antigen-binding proteins that compete with SARS-CoV-2 S for binding to ACE2, *e.g.*, using the assay format described in Example 1 herein.

The present disclosure further includes antigen-binding proteins that neutralize and/or block SARS-CoV-2 entry into cells, *e.g.*, using the assay format described in Example 1 herein.

In certain embodiments, the antigen-binding proteins of the present disclosure are useful in preventing a coronavirus, *e.g.*, SARS-CoV-2, infection in a subject when administered prophylactically to a subject in need thereof and may increase survival of the subject. For example, the administration of an antigen-binding protein of the present disclosure may result in passive immunity to SARS-CoV-2, and/or may lead to prevention and/or amelioration of one or more manifestations of COVID-19 (*e.g.*, fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea). In certain embodiments, the antigen-binding proteins of the present disclosure are useful in treating a coronavirus, *e.g.*, SARS-CoV-2, infection in a subject when administered therapeutically to a subject in need thereof and may increase survival of the subject. For example, the administration of a therapeutically effective amount of an antigen-binding protein of the disclosure to a subject may result in passive immunity to SARS-CoV-2, and/or may lead to prevention and/or amelioration of one or more manifestations of COVID-19 (*e.g.*, fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body

ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea).

In one embodiment, the disclosure provides an isolated antigen-binding protein thereof that binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (5 *e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314, 315, and 316 or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 10 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 21, 27, 33, 39, 45, 51, and 199, and 205, 211, 217, and 223, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 22, 28, 34, 40, 46, 52, 200, and 206, 212, 218, and 224, or a substantially similar sequence thereof 20 having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (v) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 17, 23, 29, 35, 41, 47, 53, 201, and 207, 213, 219, and 225, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (vi) comprises a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO:

18, 24, 30, 36, 42, 48, 54, 202, and 208, 214, 220, and 226, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; (vii) comprises a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 19, 25, 31, 37, 43, 49, 55, 203, and 209, 215, 221, and 227, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity; and/or (viii) comprises a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 26, 32, 38, 44, 50, 56, 204, and 210, 216, 222, and 228, or a substantially similar sequence thereof having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity.

In one embodiment, the disclosure provides an isolated antigen-binding protein that specifically binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein neutralizes a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2, with an IC₅₀ of about 50 ng/ml to 500 ng/ml, for example, as measured by a plaque reduction neutralization test (PRNT).

In one embodiment, the disclosure provides an isolated antigen-binding protein that binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein neutralizes a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2, with an IC₅₀ of about 62 ng/ml to 440 ng/ml, for example, as measured by a plaque reduction neutralization test (PRNT).

In one embodiment, the disclosure provides an isolated antigen-binding protein that binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein neutralizes SARS-CoV-2 pseudotype with greater than about 90% reduction in entry at a concentration of 100 µg ml⁻¹.

In one embodiment, the disclosure provides an isolated antigen-binding protein that binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein neutralizes SARS-CoV-2 pseudotype with

IC₅₀ values ranging from about 0.008 to 0.671 µg ml⁻¹, for example, in a dose response pseudotype neutralization assay.

In one embodiment, the disclosure provides an isolated antigen-binding protein that binds to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, SEQ ID NO: 100), wherein the antigen-binding protein neutralizes infectious SARS-CoV-2, *e.g.*, strain USA/WA1/2020, with an IC₅₀ value of less than 1 µg ml⁻¹.

The antigen-binding proteins of the present disclosure may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Other biological characteristics of the antigen-binding proteins of the present disclosure will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

VII. Epitope Mapping and Related Technologies

The present disclosure includes antigen-binding proteins which interact with one or more amino acids found within one or more subunits of the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein, such as the S1 subunit and/or the S2 subunit. The epitope on the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein to which the antigen-binding proteins of the present invention bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids of a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein. Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the S1 subunit and/or S2 subunit of the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein (*e.g.*, a conformational epitope). The term “epitope”, as used herein, refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen. In particular embodiments, the epitope may located within the S1

subunit, for example, within the receptor binding domain (RBD), *e.g.*, ACE2 RBD, of the S1 subunit.

In certain embodiments, the antigen-binding proteins described herein may bind to an epitope on the SARS-CoV-2 receptor binding domain (RBD) comprising any one of residues 319-541.

Various techniques known to persons of ordinary skill in the art can be used to determine whether an antigen-binding protein “interacts with one or more amino acids” within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) *Methods Mol. Biol.* 248: 443-63), peptide cleavage analysis, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) *Prot. Sci.* 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antigen-binding protein interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antigen-binding protein to the deuterium-labeled protein. Next, the protein/antigen-binding protein complex is transferred to water and exchangeable protons within amino acids that are protected by the antigen-binding protein complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antigen-binding protein interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antigen-binding protein, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antigen-binding protein interacts. See, *e.g.*, Ehring (1999) *Analytical Biochemistry* 267: 252-259; Engen and Smith (2001) *Anal. Chem.* 73: 256A-265A.

The present disclosure includes antigen-binding proteins that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antigen-binding proteins described herein in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, or an antigen-binding protein having the CDR sequences of any of the exemplary antigen-binding proteins described in Table 1 and/or in any one of Figures 2A-2B, 3A-3B,

4A-4J, 7A-7B and 8A-8B. Likewise, the present disclosure also includes antigen-binding proteins that compete for binding to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein or a fragment thereof with any of the specific exemplary antigen-binding proteins described herein in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B, or an antigen-binding protein having the CDR sequences of any of the exemplary antigen-binding proteins described in Table 1 and/or in any one of Figures 2A-2B, 3A-3B, 4A-4J, 7A-7B and 8A-8B.

One can easily determine whether an antigen-binding protein binds to the same epitope as, or competes for binding with, a reference antigen-binding protein by using routine methods known in the art. For example, to determine if a test antigen-binding protein binds to the same epitope as a reference antigen-binding protein of the disclosure, the reference antigen-binding protein is allowed to bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein or fragment thereof under saturating conditions. Next, the ability of a test antigen-binding protein to bind to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein is assessed. If the test antigen-binding protein is able to bind to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein following saturation binding with the reference antigen-binding protein, it can be concluded that the test antigen-binding protein binds to a different epitope than the reference antigen-binding protein. On the other hand, if the test antigen-binding protein is not able to bind to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein following saturation binding with the reference antigen-binding protein, then the test antigen-binding protein may bind to the same epitope as the epitope bound by the reference antigen-binding protein of the disclosure.

To determine if an antigen-binding protein competes for binding with a reference antigen-binding protein, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antigen-binding protein is allowed to bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein under saturating conditions followed by assessment of binding of the test antigen-binding protein to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein. In a second orientation, the test antigen-binding protein is allowed to bind to a coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein under saturating conditions followed by assessment of binding of the reference antigen-binding protein to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein. If, in both orientations, only the first (saturating) antigen-binding protein is capable of binding to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-

2, spike (S) protein, then it is concluded that the test antigen-binding protein and the reference antigen-binding protein compete for binding to the coronavirus, *e.g.*, SARS-CoV, *e.g.*, SARS-CoV-2, spike (S) protein. As will be appreciated by a person of ordinary skill in the art, an antigen-binding protein that competes for binding with a reference antigen-binding protein may not necessarily bind to the identical epitope as the reference antigen-binding protein, but may sterically block binding of the reference antigen-binding protein by binding an overlapping or adjacent epitope.

Exemplary reference antibodies that may be used according to the methods described herein include, but are not limited to, B38, a VH3-53-derived RBD-ACE2 competitor; REGN10933 and REGN10987, two antibodies that bind non-overlapping epitopes in the receptor binding domain (RBD); 4A8, an N-terminal domain (NTD) binder; 2-43, which binds a quaternary epitope that spans two receptor binding domains (RBDs), and CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports.

Two antigen-binding proteins bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antigen-binding protein inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans et al., Cancer Res. 1990 50:1495-1502). Alternatively, two antigen-binding proteins have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other. Two antigen-binding proteins have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antigen-binding protein reduce or eliminate binding of the other.

Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antigen-binding protein is in fact due to binding to the same epitope as the reference antigen-binding protein or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antigen-binding protein-binding assay available in the art.

VIII. Therapeutic Administration and Formulations

The disclosure provides therapeutic compositions comprising the antigen-binding proteins, *e.g.*, antibodies, or antigen-binding fragments thereof, of the present disclosure.

Therapeutic compositions in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists:

Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

The dose of the antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When an antigen-binding protein of the present disclosure is used for treating a disease or disorder in an adult patient, or for preventing such a disease, it is advantageous to administer the antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, of the present disclosure normally at a dosage, *e.g.*, single dose, of about 0.1 to about 300 mg/kg body weight, more preferably about 10 mg/kg to 150 mg/kg body weight. In certain embodiments, the antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, of the present disclosure are administered at a dosage of about 5 to about 60, about 20 to about 50, about 10 to about 50, about 1 to about 10, or about 0.8 to about 11, about 25 to about 75, about 50 to about 100, about 75 to about 125, about 100 to about 200, about 150 to about 250, about 200 to about 300 mg/kg body weight. Depending on the severity of the infection, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments thereof, of the disclosure can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antigen-binding protein, *e.g.*, antibody, or antigen-binding fragments

thereof, in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks. In certain embodiments, the antigen-binding protein is administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days after viral shedding is first detected in a sample from the subject.

In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered as a transfusion of a convalescent blood product (CBP). convalescent plasma, *e.g.*, (i) convalescent whole blood (CWB), convalescent plasma (CP) or convalescent serum (CS); (ii) pooled human immunoglobulin (Ig) for intravenous or intramuscular administration; (iii) high-titre human Ig; and (iv) polyclonal or monoclonal antibodies.

Various delivery systems are known and can be used to administer the pharmaceutical composition of the disclosure, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

The use of nanoparticles to deliver the antigen-binding proteins, *e.g.*, antibody, or antigen-binding fragments thereof, of the present disclosure is also contemplated herein. Antigen binding protein-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antigen binding protein-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 (“Antibody-conjugated nanoparticles for biomedical applications” in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles may be developed and conjugated to antigen-binding proteins contained in pharmaceutical compositions to target tumor cells or autoimmune tissue cells or virally infected cells.

Nanoparticles for drug delivery have also been described in, for example, U.S. Patent No. 8,257,740, or U.S. Patent No. 8,246,995, each incorporated herein in its entirety.

In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antigen-binding protein or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)), etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

A pharmaceutical composition of the present disclosure can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present disclosure. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland),

5 HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of

10 disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present disclosure include, but certainly are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the

15 HUMIRA™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the antigen-binding protein

20 contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the antigen-binding protein is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

In certain embodiments, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered in combination with an additional therapeutic agent.

25 For example, the antigen-binding protein, *e.g.*, antibody or antigen-binding fragment thereof, may be administered in combination with a small molecule or another antibody. In some embodiments, the additional therapeutic agent may comprise a small molecule drug targeting a viral enzyme, such as a viral RNA-dependent RNA polymerase and/or a viral protease.

30 IX. Therapeutic Uses of the Antigen-Binding Proteins

The antibodies of the disclosure are useful, *inter alia*, for the treatment, prevention and/or amelioration of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2, infection. For example, the present disclosure provides methods for treating a coronavirus, *e.g.*, a SARS-

CoV, *e.g.*, SARS-CoV-2, infection by administering an antigen-binding protein (or pharmaceutical composition comprising an antigen-binding protein) as described herein to a patient in need of such treatment, and antigen-binding proteins (or pharmaceutical composition comprising antigen-binding protein) for use in the treatment of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2, infection. The antigen-binding proteins of the present disclosure are useful for the treatment, prevention, and/or amelioration of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2, infection and/or for ameliorating at least one symptom associated with such infection (*e.g.*, at least one symptom associated with COVID-19). In the context of the methods of treatment described herein, the antigen-binding protein may be administered as a monotherapy (*i.e.*, as the only therapeutic agent) or in combination with one or more additional therapeutic agents (*e.g.*, an antiviral agent, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, a steroid, and a combination of any of the foregoing).

In some embodiments, the antibodies described herein are useful for treating subjects having, or at risk of having, COVID-19. In certain embodiments, the subject may be suffering from one or more manifestations of COVID-19, including, for example, fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea. In some embodiments, the antibodies described herein are useful for treating subjects at higher risk for severe COVID-19. For example, a subject that 65 years or older; a subject living in a nursing home or a long-term care facility; a subject who is a first-responder; a subject is suffering from an underlying disease or condition selected from the group consisting of chronic lung disease, moderate to severe asthma, serious heart condition, cancer, poorly controlled HIV or AIDS, severe obesity (body mass index [BMI] of 40 or higher), diabetes, chronic kidney disease undergoing dialysis, and liver disease; a subject who is receiving, has recently received, or is about to receive a cancer treatment, a bone marrow or organ transplantation, a corticosteroid, or other immune weakening treatment; a subject who is a smoker; and/or a subject who is immunocompromised.

One or more antibodies of the present disclosure may be administered to relieve or prevent or decrease the severity of one or more of the symptoms or conditions of the disease or disorder, *e.g.*, a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2, infection (COVID-19).

It is also contemplated herein to use one or more antibodies of the present disclosure prophylactically to patients at risk for developing a disease or disorder a SARS-CoV, *e.g.*, SARS-CoV-2, infection (COVID-19).

5 In a further embodiment of the disclosure, the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from a SARS-CoV, *e.g.*, SARS-CoV-2, infection (COVID-19).

X. Methods Of the Invention

10 The antigen-binding proteins of the present invention are useful for the prophylaxis and treatment of SARS-CoV, *e.g.*, SARS-CoV-2, infection (COVID-19).

Accordingly, the present invention, in one aspect, provides a method of passively immunizing a subject against severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), infection. The method includes administering the antigen-binding protein of the invention to the subject, thereby
15 passively immunizing the subject against a SARS-CoV, *e.g.*, SARS-CoV-2, infection.

In another aspect, the present invention provides a method of treating or preventing a coronavirus, *e.g.*, a severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), infection in a subject. The method includes administering the antigen-binding protein of the invention to the subject,
20 thereby treating or preventing the SARS-CoV, *e.g.*, SARS-CoV-2, infection.

In another aspect, the present invention provides a method of treating a subject having a severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), infection. The method includes administering the antigen-binding protein of the invention to the subject, thereby treating the subject having the
25 SARS-CoV, *e.g.*, SARS-CoV-2, infection.

In yet another aspect, the present invention provides a method of protecting a subject against a severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), infection. The method includes administering the antigen-binding protein of the invention to the subject, thereby protecting
30 the subject against a SARS-CoV, *e.g.*, SARS-CoV-2, infection.

In one aspect, the present invention provides a method of decreasing the level of severe acute respiratory syndrome coronavirus (SARS-CoV), *e.g.*, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in a subject having a SARS-CoV, *e.g.*, SARS-CoV-

2 infection. The method includes administering the antigen-binding protein of the invention to the subject, thereby decreasing the level of SARS-CoV, *e.g.*, SARS-CoV-2, in the subject.

In another aspect, the disclosure provides methods of preventing transmission of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, SARS-CoV-2. The methods include administering a therapeutically effective amount of an antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof.

In another aspect, the disclosure provides methods of providing broad spectrum immunity against circulating SARS-CoV-2 variants and high-risk bat coronaviruses
5 coronavirus. In some embodiments, the disclosure provides methods of providing broad spectrum immunity against an Alpha (lineage B.1.1.7) variant, a B.1.1.7 with E484K variant, a Delta (lineage B.1.617.2) variant, a Beta (lineage B.1.351) variant, a Gamma (lineage P.1) variant, a Eta (lineage B.1.525) variant, a Iota (lineage B.1.526) variant, a Kappa (lineage B.1.617.1) variant, a Lambda (lineage C.37) variant, a Epsilon (lineages B.1.429, B.1.427,
10 CAL.20C) variant, a Zeta (lineage P.2) variant, a Theta (lineage P.3) variant, an R.1 variant, a Lineage B.1.1.207 variant, and/or a Lineage B.1.620 variant. The methods include administering a therapeutically effective amount of an antigen-binding protein of the disclosure or a pharmaceutical composition of the disclosure to the subject in need thereof.

In certain embodiments, the method disclosed herein results in the amelioration of one or more manifestations of COVID-19. Exemplary manifestations of COVID-19 include, but are not limited to, fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea.

In certain embodiments, the method disclosed herein results in passive immunity to a SARS-CoV-2 infection. The passive immunity may last for at least about 1 week to about 2 weeks, at least about 1 month to about 3 months, at least about 3 months to about 6 months, or at least about 6 months to about 12 months.

In certain embodiments, the method disclosed herein results in a reduction in the level of viral entry. For example, a reduction in the level of viral entry of at least about 80%, 85%, 90%, 95%, or 99% as compared to a control level.

In certain embodiments, the method disclosed herein results in a reduction in the level of viral titer in the subject. For example, a reduction in the level of viral titer of at least about 80%, 85%, 90%, 95%, or 99% as compared to a control level.

In certain embodiments, the method disclosed herein results in a reduction in the level of SARS-CoV-2 viral RNA in the subject. For example, a reduction in the level of SARS-

CoV-2 viral RNA of at least about 80%, 85%, 90%, 95%, or 99% as compared to a control level.

In one embodiment, the method of the invention further comprise administering to the subject an additional agent or a therapy suitable for treatment or prevention of a SARS-CoV, *e.g.*, SARS-CoV-2, infection, *e.g.*, an antiviral agent, an immune stimulator, a therapeutic vaccine, a viral entry inhibitor, a steroid, and a combination of any of the foregoing.

5 The methods of the present invention may include administering the antigen-binding proteins separately or as part of a therapeutic regimen or combination therapy. The terms "administer," "administering," or "administration," as used herein refer to transfusing, implanting, absorbing, ingesting, injecting, or inhaling, the antigen-binding proteins of the present invention, regardless of form. In some embodiments, a single administration of the
10 antigen-binding proteins of the invention is sufficient for methods as described herein. A single dose of the antigen-binding proteins of the invention can result in a passive immunity to a SARS-CoV, *e.g.*, SARS-CoV-2, infection. In other embodiments, the antigen-binding proteins may be administered in multiple administrations. In certain embodiments, the antigen-binding protein is administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
15 16, 17, 18, 19, or 20 days after viral shedding is first detected in a sample from the subject.

Any suitable route of administration is encompassed by the methods of the invention, *e.g.* transfusion, intradermal, subcutaneous, intravenous, intramuscular, or mucosal. Mucosal routes of administration include, but are not limited to, oral, rectal, vaginal, and nasal administration. In some embodiments, the antigen-binding protein is administered
20 transdermally, intradermally, subcutaneously, orally, rectally, vaginally or by inhalation. In other embodiments, the antigen-binding protein is administered as a convalescent blood product (CBP), *e.g.*, (i) convalescent whole blood (CWB), convalescent plasma (CP) or convalescent serum (CS); (ii) pooled human immunoglobulin (Ig) for intravenous or intramuscular administration; (iii) high-titre human Ig; and (iv) polyclonal or monoclonal
25 antibodies.

The methods disclosed herein can be applied to a wide range of subjects. In some embodiments, the subject is a mammal, *e.g.*, a human, an embryo, a horse, a dog, a cat, a cow, a sheep, a pig, a fish, an amphibian, a reptile, a goat, a bird, a monkey, a mouse, a rabbit, and a rat. In other embodiments, the subject is a human. In certain embodiments, the
30 subject is an embryo.

The terms "treat" or "treating," as used herein, refer to partially or completely alleviating, inhibiting, ameliorating, and/or relieving the SARS-CoV, *e.g.*, SARS-CoV-2,

infection. In some instances, treatment can result in the continued absence of the SARS-CoV, *e.g.*, SARS-CoV-2, infection.

In some instances, treatments methods can include a single administration, multiple administrations, and repeating administration as required for the prophylaxis or treatment of a coronavirus, *e.g.*, a SARS-CoV, *e.g.*, a SARS-CoV-2, infection. In some instances treatment methods can include assessing a level of infection in the subject prior to treatment, during treatment, and/or after treatment. In some instances, treatment can continue until a decrease in the level of disease in the subject is detected.

The methods herein include administration of an effective amount of an antigen-binding protein of the disclosure to achieve the desired or stated effect, *e.g.*, ameliorating and/or eliminating SARS-CoV, *e.g.*, SARS-CoV-2, infection subject, thereby treating the subject. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, condition or symptoms, the patient's disposition to the infection, condition or symptoms, and the judgment of the treating physician.

Following administration, the subject can be evaluated to detect, assess, or determine the level of SARS-CoV, *e.g.*, SARS-CoV-2, infection. In some instances, treatment can continue until a change, *e.g.*, reduction, in the level of infectious disease in the subject is detected.

Upon improvement of a patient's condition, *e.g.*, a change, *e.g.*, decrease, in the level of disease in the subject, a maintenance dose of a an antigen-binding protein composition of the present invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In some embodiments, an effective amount of an antigen-binding protein composition of the invention is the amount sufficient to reduce the severity of SARS-CoV, *e.g.*, SARS-CoV-2, infection in a subject having a SARS-CoV, *e.g.*, SARS-CoV-2, infection, or the amount sufficient to reduce or ameliorate the severity of one or more symptoms thereof, or the amount sufficient to prevent the progression of the SARS-CoV, *e.g.*, SARS-CoV-2, infection, or the amount sufficient to enhance or improve the therapeutic effect(s) of another

therapy or therapeutic agent administered concurrently with, before, or after an antigen-binding protein composition of the invention.

In some embodiments, the effective amount of the antigen-binding protein composition administered to the subject, at a dosage of about 0.1 to about 300 mg/kg body weight, more preferably about 10 mg/kg to 150 mg/kg body weight. In certain embodiments, antigen-binding protein composition administered to the subject can be administered to a subject at low doses (<10 mg/kg) through an advantageous route (*e.g.*, subcutaneously).

V. Kits

The invention also provides a kit for passively immunizing a subject against SARS-CoV, *e.g.*, SARS-CoV-2, infection. Such kits can include a composition described herein. Such kits can also facilitate performance of the methods described herein.

In one aspect, the kit comprises the antigen-binding proteins of the invention and instructions for administering the antigen-binding proteins to a subject. In some embodiments, the antigen-binding proteins is prepackaged in a sterile container.

The composition in each container may be in the form of a pharmaceutically acceptable solution, *e.g.*, in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the composition may be lyophilized or desiccated. The kit optionally further comprises in a separate container a pharmaceutically acceptable solution (*e.g.*, saline, dextrose solution, *etc.*), preferably sterile, to reconstitute the composition to form a solution for injection purposes.

The kits of the invention may optionally comprise additional components useful for performing the methods of the invention. The kit may comprise one or more reusable or disposable device(s) for administration (*e.g.*, syringes, needles, dispensing pens), preferably packaged in sterile form, and/or a packaged alcohol pad.

In certain embodiments, kits can be supplied with instructional materials which describe performance of the methods of the invention. Kits may include instructions for administration or delivery of an antigen-binding protein by a clinician or by the patient. In another embodiment, the kits may include instructions for proper storage and handling of the antigen-binding protein compositions.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention be-longs. Although methods and materials similar or equivalent to those described herein can

be used, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The present invention is further illustrated by the following examples, which are not intended to be limiting in any way. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Figures, are hereby incorporated herein by reference.

EXAMPLES

Example 1. Characterization of antibodies that neutralize SARS-CoV-2 virus

Peripheral blood mononuclear cells (PBMCs) were obtained from an individual that had recovered from COVID-19 more than 4 weeks prior to sampling. This individual had mild disease and did not require hospitalization. From their PBMCs, 7 monoclonal antibodies were isolated, referred to herein as C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6, that are clonally related and use the VH3-53/VLK1-9 heavy and light chain antibody genes. Thus far, seven out of the seven C2 antibodies tested neutralize SARS-CoV-2 pseudotypes (**Figures 1A-1C**). Competition studies with an Fc-fusion protein comprising part of the soluble ectodomain of the SARS-CoV-2 cellular receptor (ACE2-IgG) show that C2.2 blocks ACE2 receptor binding, suggesting that the mechanism of viral entry inhibition is competitive inhibition of receptor binding (**Figure 1B**). A confirmatory test performed with C2.1 and infectious SARS-CoV-2 showed that the antibody is active against authentic SARS-CoV-2 (**Figure 1C**). The amino acid sequences of the variable heavy chains (HCVR), variable light chains (LCVR), and CDRs of monoclonal antibodies C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6 are listed in **Table 1** (*e.g.*, underlined in the HCVR and LCVR sequences) and shown in **Figures 2A-2B**, **Figures 3A-3B**, and **Figures 4A-4G**, respectively. B38, an antibody isolated from a COVID-19 convalescent individual in China, is also derived from VH3-53/VLK1-9 germline genes¹. CC12.1 and CC12.3 are also neutralizing antibodies isolated from COVID-19 convalescent donors². B38 and CC12.1 bind to the SARS-CoV-2 ACE2-receptor binding, potently neutralizes the virus, and are also protective in animal models including transgenic mice expressing ACE2 and Syrian hamsters¹. The sequence of the C2 panel of VH3-53/VLK1-9 antibodies significantly differs in sequence – greater than 7% sequence

divergence in variable heavy chain and greater than 4.5% sequence divergence in the light chain – from B38 and CC12.1 and CC12.3 (**Figures 2C and 3C**). The C2 antibodies are also significantly different from B38 and CC12.1 and CC12.3 in VH CDRH3; differences include a five amino acid insert that makes the C2 antibody CDRH3s longer (“DVSGY”).

5

Table 1. Exemplary Antigen-Binding Protein and Target Sequences

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.0 (HCVR) Also referred to herein as C2.0_VH and C1A-H6	EVQLVESGGGLIQPGGSLRLSCVAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSV <u>IYSGGT</u> TYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYC <u>ARGDVSGYKYGLDY</u> WGQGTLVTVS	1
C2.1 (HCVR) Also referred to herein as C2.1_VH and C1A-C2	EVQLVESGGGLIQPGGSLRLSCAVS <u>GFTVSNNY</u> MSWVRQAP GKGLEWVSV <u>IYSGGT</u> TYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	2
C2.2 (HCVR) Also referred to herein as C2.2_VH and C1A-B3	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSV <u>IYSGGT</u> TYADSVKGRFTISRDN SKNTVFLQMN SLRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	3
C2.3 (HCVR) Also referred to herein as C2.3_VH and C1A-C4	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSV <u>IYSGGT</u> TYADSVKGRFTISRDN SKNLYLQMN LRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	4
C2.4 (HCVR) Also referred to herein as C2.4_VH and C1A-B12	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSV <u>IYSGGAT</u> TYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	5

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.5 (HCVR) Also referred to herein as C2.5_VH and C1A-F10	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSVI <u>IYSGGTT</u> YYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	6
C2.6 (HCVR) Also referred to herein as C2.6_VH and C1A-H5	EVQLVESGGGLIQPGGSLRLSCAAAG <u>ITVSSNY</u> MSWVRQAPG KGLEWVSVI <u>IYSGGTT</u> YYADSVKGRFTISRDNPKNMLYLQMN SLRAEDTAVYYC <u>ARGDVSGYRYGLDY</u> WGQGTLVTVS	7
C2.0 (LCVR) Also referred to herein as C2.0_VL and C1A-H6	DIQLTQSPSSLSASVGDRVITITCRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIYA <u>AAS</u> TLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC <u>QQLISYPGT</u> FGQGTKLEIK	8
C2.1 (LCVR) Also referred to herein as C2.1_VL and C1A-C2	DIQLTQSPSSLSAFVGDRVITITCRASE <u>EGISSY</u> LAWYQQKPGNA PKLLIYA <u>AAS</u> TLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC <u>QQLNSYPGT</u> FGQGTKLEIK	9
C2.2 (LCVR) Also referred to herein as C2.2_VL and C1A-B3	DIQLTQSPSSLSASVGDRVITITCRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIYA <u>AAS</u> TLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY <u>CQQLNSYPGT</u> FGQGTKLEIK	10
C2.3 (LCVR) Also referred to herein as C2.3_VL and C1A-C4	DIQLTQSPSSLSASVGDRVITITCRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIY <u>GAS</u> TLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYY <u>CQQVNDYPGT</u> FGQGTKLEIK	11

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.4 (LCVR) Also referred to herein as C2.4_VL and C1A-B12	DIQLTQSPSSLSASVGDRVITICRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIY <u>AAS</u> TLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY <u>CQQLISYPGT</u> FGQGTKLEIK	12
C2.5 (LCVR) Also referred to herein as C2.5_VL and C1A-F10	DIQLTQSPSSLSASVGDRVITICRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIY <u>AAS</u> TLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY <u>CQQLNSYPGT</u> FGQGTKLEIK	13
C2.6 (LCVR) Also referred to herein as C2.6_VL and C1A-H5	DIQLTQSPSSLSASVGDRVITICRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIY <u>GAT</u> TLQSGVPSRFSGSGSGTDFTLTISLQPEDFASY <u>CQQLNSYPGT</u> FGQGTKLEIK	14
C2.0_VH: (CDRH1)	GFTVSSNY	15
C2.0_VH: (CDRH2)	IYSGGTT	16
C2.0_VH: (CDRH3)	ARGDVSGYKYGLDY	17
C2.0_VL: (CDRL1)	QGISSY	18
C2.0_VL: (CDRL2)	AAS	19
C2.0_VL: (CDRL3)	QQLISYPGT	20
C2.1_VH: (CDRH1)	GFTVSNNY	21
C2.1_VH: (CDRH2)	IYSGGTT	22

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.1_VH: (CDRH3)	ARGDVSGYRYGLDY	23
C2.1_VL: (CDRL1)	EGISSY	24
C2.1_VL: (CDRL2)	AAS	25
C2.1_VL: (CDRL3)	QQLNSYPGT	26
C2.2_VH: (CDRH1)	GFTVSSNY	27
C2.2_VH: (CDRH2)	IYSGGTT	28
C2.2_VH: (CDRH3)	ARGDVSGYRYGLDY	29
C2.2_VL: (CDRL1)	QGISSY	30
C2.2_VL: (CDRL2)	AAS	31
C2.2_VL: (CDRL3)	QQLNSYPGT	32
C2.3_VH: (CDRH1)	GFTVSSNY	33
C2.3_VH: (CDRH2)	IYSGGTT	34
C2.3_VH: (CDRH3)	ARGDVSGYRYGLDY	35
C2.3_VL: (CDRL1)	QGISSY	36
C2.3_VL: (CDRL2)	GAS	37

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.3_VL: (CDRL3)	QQVNDYPGT	38
C2.4_VH: (CDRH1)	GFTVSSNY	39
C2.4_VH: (CDRH2)	IYSGGAT	40
C2.4_VH: (CDRH3)	ARGDVSGYRYGLDY	41
C2.4_VL: (CDRL1)	QGISSY	42
C2.4_VL: (CDRL2)	AAS	43
C2.4_VL: (CDRL3)	QQLISYPGT	44
C2.5_VH: (CDRH1)	GFTVSSNY	45
C2.5_VH: (CDRH2)	IYSGGTT	46
C2.5_VH: (CDRH3)	ARGDVSGYRYGLDY	47
C2.5_VL: (CDRL1)	QGISSY	48
C2.5_VL: (CDRL2)	AAS	49
C2.5_VL: (CDRL3)	QQLNSYPGT	50
C2.6_VH: (CDRH1)	GITVSSNY	51
C2.6_VH: (CDRH2)	IYSGGTT	52

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.6_VH: (CDRH3)	ARGDVSGYRYGLDY	53
C2.6_VL: (CDRL1)	QGISSY	54
C2.6_VL: (CDRL2)	GAT	55
C2.6_VL: (CDRL3)	QQLNSYPGT	56
SARS-CoV-2 spike protein (GenBank accession number QJF75467.1)	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKV FRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFD NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSL LIVNNAT NVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANN CTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHT PINLVRDLPQGFSALEPLVDLPIGINITRFQT LLALHRSYLT PGDSSSGW TAGAAAYYVGYLQPRTFLLKYNE NGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTE SIVRFPNITNLC PFGEVFNATRFASVYAWBRKRISNCVADYSV LYNSASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQI APGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY LYRLFRKSNLKP FERDISTEIIYQAGSTPCNGVEGFNCYFPLQSY GFQPTNGVGYQP YRVVVL SFELLHAPATVCGPKKSTNLVKN KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRD PQTL EILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPV AIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNN SYECDI PIGAGICASYQTQ TNSPRRARSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSM TKTSVDCTMYICGDSTECSNLL LQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKD FGGFNFSQILPDPSKPSKRSFIEDLLFNKVT LADAGFIKQYGDC LGDIAARDLICAQKFENGLTVLPPLL TDEMIAQYTSALLAGTITS GWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIAN QFNSAIGKIQDSL SSTA SALGKLQDVVNQNAQALNTLVKQLS	100

Identifier	Amino Acid Sequence	SEQ ID NO.
	SNFGAISSVLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQ LIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFP QSAPHGVVFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVF VSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVY DPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQK EIDRLNEVAKNLNESLIDLQELGKYEYIKWPWYIWLGFIAGL IAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKG VKLHYT	
C1A-gl (HCVR) Also referred to herein as C1A-gl VH	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSVIYSGGSTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYC <u>CARGDVSGYSYGLDY</u> WGQGTLLTVS	112
C1A-gl* (HCVR) Also referred to herein as C1A-gl* VH	EVQLVESGGGLIQPGGSLRLSCAAS <u>GFTVSSNY</u> MSWVRQAPG KGLEWVSVIYSGGSTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYC <u>CARGDVSGYRYGLDY</u> WGQGTLLTVS	113
C1A-gl (LCVR) Also referred to herein as C1A-gl VL	DIQLTQSPSFLSASVGDRVTITCRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIYA <u>AAS</u> TLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYC <u>QQLNSYPGT</u> FGQGTKLEIK	128
C1A-gl* (LCVR) Also referred to herein as C1A-gl* VL	DIQLTQSPSFLSASVGDRVTITCRAS <u>QGISSY</u> LAWYQQKPGKA PKLLIYA <u>AAS</u> TLQSGVPSRFSGSGSGTEFTLTISLQPEDFATYYC <u>QQLNSYPGT</u> FGQGTKLEIK	129
C1A-gl VH: (CDRH1)	GFTVSSNY	199
C1A-gl VH:	IYSGGST	200

Identifier	Amino Acid Sequence	SEQ ID NO.
(CDRH2)		
C1A-gl VH: (CDRH3)	ARGDVSGYSYGLDY	201
C1A-gl VL: (CDRL1)	QGISSY	202
C1A-gl VL: (CDRL2)	AAS	203
C1A-gl VL: (CDRL3)	QQLNSYPGT	204
C1A-gl* VH: (CDRH1)	GFTVSSNY	205
C1A-gl* VH: (CDRH2)	IYSGGST	206
C1A-gl* VH: (CDRH3)	ARGDVSGYRYGLDY	207
C1A-gl* VL: (CDRL1)	QGISSY	208
C1A-gl* VL: (CDRL2)	AAS	209
C1A-gl* VL: (CDRL3)	QQLNSYPGT	210
C2.4opt1 VH: (CDRH1)	GFTVSNNY	211
C2.4opt1 VH: (CDRH2)	IYSGGTT	212
C2.4opt1 VH: (CDRH3)	ARGDVSGYRYGLDY	213
C2.4opt1 VL:	QGISSY	214

Identifier	Amino Acid Sequence	SEQ ID NO.
(CDRL1)		
C2.4opt1 VL: (CDRL2)	AAS	215
C2.4opt1 VL: (CDRL3)	QQLISYPGT	216
C2.4opt2 VH: (CDRH1)	GFIVSNNY	217
C2.4opt2 VH: (CDRH2)	IYSGGTT	218
C2.4opt2 VH: (CDRH3)	ARGDVSGYRYGLDY	219
C2.4opt2 VL: (CDRL1)	QGISSY	220
C2.4opt2 VL: (CDRL2)	AAS	221
C2.4opt2 VL: (CDRL3)	QQLISYPGT	222
C2.4opt3 VH: (CDRH1)	GVIVSNNY	223
C2.4opt3 VH: (CDRH2)	IYSGGTT	224
C2.4opt3 VH: (CDRH3)	ARGDVSGYRYGLDY	225
C2.4opt3 VL: (CDRL1)	QGISSY	226

Identifier	Amino Acid Sequence	SEQ ID NO.
C2.4opt3 VL: (CDRL2)	AAS	227
C2.4opt3 VL: (CDRL3)	QQLISYPGT	228
C2.4opt1 (HCVR) Also referred to herein as C2.4opt1 VH	EVQLVESGGGLIQPGGSLRLSCAVSGFTVSNNYMSWVRQAPG KGLEWVSVIYSGGTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARGDVSGYRYGLDYWGQGTLVTVS	314
C2.4opt2 (HCVR) Also referred to herein as C2.4opt2 VH	EVQLVESGGGLIQPGGSLRLSCAVSGFIVSNNYMSWVRQAPG KGLEWVSVIYSGGTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARGDVSGYRYGLDYWGQGTLVTVS	315
C2.4opt3 (HCVR) Also referred to herein as C2.4opt3 VH	EVQLVESGGGLIQPGGSLRLSCAVSGVIVSNNYMSWVRQAPG KGLEWVSVIYSGGTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARGDVSGYRYGLDYWGQGTLVTVS	316
C2.4opt1 (LCVR) Also referred to herein as C2.4opt1 VL	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKA PKLLIYAAS TLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYY CQQLISYPGTFGQGTKLEIK	330
C2.4opt2 (LCVR) Also referred to herein as C2.4opt2 VL	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKA PKLLIYAAS TLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYY CQQLISYPGTFGQGTKLEIK	331
C2.4opt3 (LCVR)	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKA PKLLIYAAS TLQSGVPSRFSGSGSGTDFTLTIS SLQPEDFATYY CQQLISYPGTFGQGTKLEIK	332

Identifier	Amino Acid Sequence	SEQ ID NO.
Also referred to herein as C2.4opt3 VL		

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Example 2. Development of therapeutic antibodies for the treatment and/or prevention of coronavirus infections

This experiment aims to rapidly develop therapeutic antibodies or biologics, or technologies that accelerate the advancement of biologics against emerging pathogens including, but not limited to, SARS-CoV-2. Emphasis will be placed on conserved epitopes that are difficult to target and on interventions with a potentially broader spectrum of activity that may protect a subject from future coronavirus outbreaks and against additional groups of emerging viruses.

Multiple methods for the identification of highly effective therapeutic antibodies will be used, including, for example, instrument assisted in-depth B cell repertoire analysis of human samples, microbial display, and antigen-specific single B cell sorting. To define modes of action (MOAs) and optimize leads, various structural, biophysical, and computational methods, including hydrogen-deuterium exchange (HDX), X-ray crystallography, cryo-EM, and molecular dynamics simulation will be used. Numerous testing modalities (*e.g.*, *in vitro* testing with virus pseudotypes and assays with infectious virus under containment, etc.) and efficacy testing in animals are envisioned. *In vivo* studies will be used to designed best in class therapeutics and characterize their MOAs. The study will include a patient-derived monoclonal antibody (mAb) “deep dive”, secondary optimization (*e.g.*, FcR and FcRn, and glyco-engineering, bi- and tri-specific antibodies), scale up, and characterization of key reagents (*e.g.*, antigens, small scale production of large

numbers of mAbs) and generation of material suitable for pre-clinical *in vivo* characterization and for early clinical studies in humans.

Introduction

5 Coronaviruses are positive strand RNA viruses with large genomes responsible for multiple outbreaks of lethal lower respiratory tract infection in humans. SARS-CoV-2, which causes COVID-19, emerged late in 2019 and has since caused a pandemic of unprecedented scale in recent history. Therapeutic and prophylactic interventions against SARS-CoV-2 are urgently needed. The coronavirus spike (S) protein mediates cell surface receptor binding and fusion of the viral and host cell membranes and is a target for antiviral antibodies elicited during natural infection. S is large and requires proteolytic processing at two sites: the S1/S2 junction and at an S2 site (S2') that is upstream of its fusion peptide¹. S forms club-shaped trimers of S1/S2 heterodimers on the virion surface. S1 mediates binding to cell surface receptors, and S2 contains the fusion peptide and a transmembrane segment and mediates fusion of viral and host cell membranes¹. Fusion at the cell surface can also occur in certain instances when S is exposed to extracellular proteases¹. The S1 subunit of SARS-CoV-2 contains a receptor-binding domain (RBD) that binds angiotensin converting enzyme 2 (ACE2)^{2,3}. S1 contains an additional subdomain called the N-terminal domain (NTD). S2 contains a fusion peptide in its N-terminal region and a transmembrane segment that anchors S in the viral membrane.

Neutralizing antibodies could block viral entry by preventing S from binding to host-cell receptors (e.g., ACE2), or by preventing the conformational changes S must undergo to mediate fusion of the viral and host cell membranes. Epitopes for neutralizing antibodies on SARS-CoV-2 S include at least two non-overlapping epitopes on the RBD^{4,5} and the N-terminal domain (NTD)^{6,7}. Antibodies can also bind a tertiary epitope on S that spans two RBDs, the engagement of which clamps down S into the closed conformation⁷. Neutralizing monoclonal antibodies, when administered right before or after viral challenge, can decrease viral RNA lung burden or alleviate lung pathology animal models^{4,8}.

Multiple techniques now allow the rapid recovery of antiviral monoclonal antibodies or antibody-derivatives. These include in vitro selection approaches with yeast or phage display, animal immunization with subsequent antibody humanization, antigen-specific single B-cell sorting, or Epstein-Barr virus B-cell immortalization. The latter two approaches rely on access to peripheral blood mononuclear cells (PBMCs) obtained from recovered individuals. Monoclonal antibodies can also be rapidly scaled up for testing during outbreaks.

Notable examples include mAb114, which comprises a single antibody developed using EBV B-cell immortalization⁹, and REGN-EB3, a three-antibody cocktail derived from immunizing mice engineered to express human Ig heavy and light chain variable regions¹⁰. mAb114 and REGN-EB3 were shown to be effective against Ebola virus disease in a randomized clinical trial¹¹.

Several antiviral monoclonal neutralizing antibodies are now entering human clinical trials for both the prevention and treatment of COVID-19. The outlined project is an effort to develop the next generation of safe, effective, dose-sparing, and broadly active antibody-based therapeutics against COVID-19. The long-term vision is to adapt these approaches to also counter threats posed by other emerging pathogens with pandemic potential.

To develop monoclonal antibodies for the treatment or prevention of COVID-19

From the starting point of peripheral blood mononuclear cells (PBMCs) obtained from COVID-19 convalescent individuals, antibody isolation technology will be used to isolate large panels of antibodies that target multiple sites on the coronavirus spike (S) protein to potentially neutralize infection. Detailed biophysical and structural analysis of antibody-glycoprotein interactions along with molecular dynamics simulations to optimize antibody potency will be performed. Antibody generation is expected to encompass a first-generation process that will drive biologics discovery with criteria for potential transition to development based on current best science, and a second-generation process that incorporates insights from structure/function analyses of correlates with highest possible potency, analyses of Fc-avoidance of antibody dependent enhancement (ADE), and analyses of optimal dosing on an ongoing basis. Antibody neutralization escape as mode of drug resistance is a significant concern, particularly as multiple therapeutics programs are targeting the receptor binding domain (RBD), a site on SARS-CoV-2 spike (S) protein that is diverse in sequence and contains known resistance mutations to antiviral antibodies. *In vitro* and *in vivo* studies will be used to identify, predict, and preemptively counter resistance mutations through carefully designed, highly potent antibody-cocktails.

Through the proposed project, multiple technologies will be used to create SARS-CoV-2 therapeutic antibody candidates, including cloning of human antibodies from patient-derived B-cells, transgenic mice, microbial display, and others. The initial focus will be SARS-CoV-2 and include all globally emerging spike protein mutations (*e.g.*, D614G and/or a Q493K spike mutant and neutralizing antibody escape mutants), but will shift to a wider breadth to include therapeutic antibody candidates that are also active against circulating

SARS-CoV-2 variants and high-risk bat coronaviruses. The SARS-CoV-2 spike protein (S) is a heavily glycosylated protein and is a target of neutralizing antibodies elicited during natural infection. Multiple reports describe the SARS-CoV-2 spike protein as a difficult target of neutralizing antibodies¹²⁻¹⁴. This may be due to host factors (*e.g.*, dysregulated B-cell responses¹⁵) and properties of the virus (*e.g.*, immune evasion by the viral spike (S) protein). For example, spike (S) protein is extensively glycosylated, conformationally heterogeneous, and can mask its receptor binding domain (RBD) in a pH-dependent manner¹⁶. The study will focus on generating antibodies not only against the receptor binding domain (RBD) but also against highly conserved regions that are not protected by glycosylation or conformational masking (*e.g.*, the N-terminal domain (NTD) or the spike protein subunit 2 (S2)). This goal requires the generation of properly folded, high quality antigens (*e.g.*, mammalian cell derived recombinant proteins, nanodisc-embedded proteins, virus like particles, etc.) and suitable screening assays. Expected analytics will include routine characterization of antigens and antibodies, glycoproteomics, subunit mass spectrometry analysis (*e.g.*, glycan profiles, released glycan profiles, and glycopeptides), and additional biophysical assessments as required (*e.g.*, multi-angle light scattering, analytical ultracentrifugation, etc.). As a central component of these efforts, spike (S) proteins will be generated that are stabilized to adopt various spike (S) protein conformations¹⁷⁻¹⁹. Additionally, virus like particles (VLPs) will be made containing the spike (S) protein and imaging these by cryo-electron microscopy (*e.g.*, cryo-EM).

To confirm the *in vivo* activity of VH3-53 antibodies

Seven clonotypes (*e.g.*, C1A-B3, C1A-F10, C1A-C2, C1A-H5, and C1A-C4) of a single antibody that each potentially neutralizes SARS-CoV-2 (IC₅₀ values of 62 ng/ml to 440 ng/ml) have been identified (**Figures 5A-5E**). To demonstrate that these antibodies are active in an *in vivo* animal model of SARS-CoV-2 infection, the DNA sequences and/or expression plasmids of these antibodies will be used to produce tens of milligrams of endotoxin-free material to be evaluated in a rat pharmacokinetics study. A Syrian hamster model²⁰ may also be used to evaluate the *in vivo* activity of these antibodies.

To increase antibody potency using molecular dynamic studies at Abbvie

VH3-53 (or closely related, VH3-66)-derived antibodies have been identified that potentially neutralize SARS-CoV-2, and molecular structures for Fabs of these antibodies bound to the SARS-CoV-2 RBD are available for some of these^{4,21}. VH3-53 antibodies

require low frequencies of somatic mutation but are nonetheless exquisitely potent. Seven related somatic hypermutation variants of a single VH3-53 neutralizing antibody have been identified. Neutralizing activity against infectious SARS-CoV-2 measured by plaque reduction neutralization tests (PRNT) have IC₅₀ values ranging from 62 ng/ml to 440 ng/ml (Figure 5E). A germline antibody, C1A-gl*, that contains a single somatic mutation in CDR H3 (Ser to Arg) also potently neutralizes SARS-CoV-2 (IC₅₀ value of 102 ng/ml, Fig. 15E insert). A high-resolution X-crystal structures (2.1 to 2.8 Å) of Fabs C1A-B3, C1A-F10, C1A-C2, and C1A-B12 bound to the SARS-CoV-2 RBD has also been obtained. Fabs derived from these antibodies bind the SARS-CoV-2 RBD with affinities that range from 66 to 1 nM (Figure 5E). The structures have helped identify key mutations that are likely to further increase the potency of C1A-B12 (e.g., VH changes A24V, F27I, F27V, T28I, S31R, S31N, A56T, and VK N92I) (Figures 6A-6K, 7A-7C, and 8A-8G); a construct including the substitutions has been generated and awaits experimental validation. To explore the impact of all mutations on affinity, the crystal structures, as refined PDB coordinates and antibody sequences, will be used to conduct molecular dynamic studies and clonal lineage analysis to understand affinity maturation pathways against the SARS-CoV-2 receptor binding domain (RBD). A series of antibodies with various combinations of the mutations to explore affinity and efficacy/potency (e.g., in pseudotype assays and for experiments with infectious virus) and possible molecular structures will be generated.

Deep dive antibody cloning of human peripheral blood mononuclear cells (PBMCs)

Direct cloning of anti-COVID-19 patient derived antibodies will be performed. Pilot experiments have been conducted and have resulted in the isolation of a panel of SARS-CoV-2 neutralizing antibodies from a COVID-19 convalescent individual. Another portion of the same patient peripheral blood mononuclear cells (PBMCs) will be used for “deep dive” screening and antibody isolation (e.g., using a Beacon® Optofluidic system, Berkeley Lights). It is anticipated that PBMCs from five convalescent donors who are more than 4 weeks out from infection initially will also be analyzed, and as well as additional samples as the project evolves. As early and late antibody repertoires may differ, samples from more recently infected individuals may be used, while adhering to the appropriate biosafety protocols/rules. Primary screening, sequencing, and molecular cloning will be performed, and antibodies will be expressed at small-medium scale for sequence and material characterization and analyses. Antibody neutralization assays using viral pseudotypes will be performed and as well as *in vitro* neutralization assays with authentic SARS-CoV-2. Initially,

SARS-CoV-2 generated from the US index case (USA-WA1/2020) will be used in neutralization assays, but this will be followed by studies using viruses representing currently circulating variants to account for virus evolution during the pandemic. The kinetic parameters of antibody Fab binding to SARS-CoV-2 S will be determined using biolayer interferometry (BLI), and will confirm antibody binding at both physiological and acidic/endosomal pH, given that low pH locking of the receptor binding domains (RBDs) in the down conformation is a recently reported mechanism of antibody-neutralization evasion¹⁶.

To identify additional epitopes for cocktail design

SARS-CoV-2 spike (S) protein mutations that lead to neutralization escape of antibodies targeting the spike (S) protein have been described, favoring use of antibody cocktails that bind non-competing epitopes on S²⁴. A significant concern is evolution of antibody-escape mutations either inside a given infected host, or as the virus circulates at large in the broader population, particularly should SARS-CoV-2 become endemic and/or seasonal. The development of prophylactic or therapeutic interventions against COVID-19 have converged on antibodies that target the ACE2-engaging epitope on the RBD. Without wishing to be bound by any particular theory, if only such agents are used clinically, the likelihood of viral neutralization escape would be further be increased, making it critical to diversify lead candidates against additional epitopes. For these reasons, a therapeutic strategy that targets multiple epitopes on spike (S) protein is likely critical for long-term therapeutic efficacy. High throughput Carterra-based epitope binning will be used to define the epitopes for all antibodies already isolated and additional antibodies isolated as described herein. As antigens for the assay, SARS-CoV-2 spike (S) protein constructs will be designed, expressed, and purified that are stabilized in relevant conformations (*e.g.*, HexaPro¹⁷, S-R/x2¹⁸ and S2P DS constructs¹⁹). These proteins will contain the relevant tags for downstream assays (*e.g.*, BirA-ligase tag for site specific biotinylation). Stable cell lines will be generated for these antigens. Reference antibodies to be included in the Carterra experiments to help define epitope bins include: B38, a VH3-53-derived RBD-ACE2 competitor⁴; REGN10933 and REGN10987, two antibodies that bind non-overlapping epitopes in the receptor binding domain (RBD)⁵; 4A8, an N-terminal domain (NTD) binder⁶; 2-43, which binds a quaternary epitope that spans two receptor binding domains (RBDs)⁷, and CR3022, an antibody that has been described as either neutralizing or non-neutralizing in various reports^{16,27}. Carterra epitope binning studies will be conducted using the antibodies describes herein (*e.g.*, Table 1)

and the listed reference antibodies. Epitope relationships of the antibodies will be determined. Antibody binding sites will be determined using hydrogen-deuterium exchange (HDX). Molecular structures of Fabs bound to spike (S) protein or the receptor binding domain (RBD) will also be determined using either X-ray crystallography or cryo-EM.

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Exemplary monoclonal antibody composition

One aim of this experiment is to produce a therapeutic with the following target product profile (TPP); a cocktail comprising two or three monoclonal antibodies binding non-overlapping epitopes on the spike (S) protein of SARS-CoV-2, indicated for the prevention or early treatment of serious lower respiratory track viral infection by this coronavirus. Safety and efficacy would be established in adults with and without comorbid conditions with administration of the agent as pre-exposure prophylaxis, post-exposure prophylaxis, or early treatment illness. Efforts will be taken to increase potency so that the drug could be administered at low doses (<10 mg/kg) through an advantageous route (e.g., subcutaneously).

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Towards this goal, the studies outlined herein will be performed. An early emphasis will be placed on lead optimization by iteratively engineering and testing antibody variants and cocktail optimizations. Research activities to be carried out early in parallel include next-generation biologic design, and at late stage antibody isolation for non-SARS-CoV-2 high risk viruses.

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Example 3. SARS-CoV-2 evolution in an immunocompromised host reveals shared neutralization escape mechanisms

Introduction

The SARS-CoV-2 viral spike (S) protein mediates attachment and entry into host cells and is a major target of vaccine and drug design. Potent SARS-CoV-2 neutralizing antibodies derived from closely related antibody heavy chain genes (IGHV3-53 or 3-66) have been isolated from multiple COVID-19 convalescent individuals¹⁻⁷. These usually contain minimal somatic mutations and bind the S receptor-binding domain (RBD) to interfere with attachment to the cellular receptor angiotensin-converting enzyme 2 (ACE2). In this experiment, antigen-specific single B cell sorting was used to isolate S-reactive monoclonal antibodies from the blood of a COVID-19 convalescent individual. Seven potent neutralizing antibodies were somatic variants of the same IGHV3-53-derived antibody and bind the RBD with varying affinity. The x-ray crystal structures of four Fab variants bound to the RBD are described herein and are used to explain the basis for changes in RBD affinity. In this experiment it is shown that a germline revertant antibody binds tightly to the SARS-CoV-2 RBD and neutralizes virus, and that gains in affinity for the RBD do not necessarily correlate with increased neutralization potency, suggesting that somatic mutation is not necessarily required to exert robust antiviral effect. This studies clarifies the molecular basis for a heavily germline-biased human antibody response to SARS-CoV-2.

Additionally, coronaviruses encode a viral exonuclease that increases replication fidelity (Denison et al., 2011), which probably makes antigenic drift in SARS-CoV-2 less significant than in other enveloped RNA viruses. Changes in SARS-CoV-2 S have nonetheless occurred over time and become fixed among circulating variants; the D614Gs

mutation is a prime example (Yurkovetskiy et al., 2020). This mutation, however, does not seem to impact the activity of RBD-targeting neutralizing antibodies (Yurkovetskiy et al., 2020). Ultimately, evolution of S antibody escape mutations could impact the long-term effectiveness of vaccines and monoclonal antibody-based therapeutics that target S.

In the present efforts to study SARS-CoV-2 antibody neutralization and to predict escape mutations, this experiment examined sequences of S variants that evolved in a persistently infected individual receiving B-cell depleting therapy (Choi *et al.*, *N Engl J Med.* 2020). This study shows that mutations acquired in S during persistent infection confer pseudotype resistance to a large panel of clonally related V_H3-53-derived neutralizing antibodies that were isolated from a healthy COVID-19 convalescent donor. Resistance also extended to B38 (Wu et al., 2020) and CC12.1 (Rogers et al., 2020), two V_H3-53-derived antibodies isolated from other COVID-19 convalescent donors, to the two components of the REGN-COV2 antibody cocktail (Baum et al., 2020; Hansen et al., 2020), and to the polyclonal immunoglobulins (IgG) of four out of four healthy convalescent donors tested. Antibody affinity enhancements, which we performed based on X-ray crystal structures we determined of V_H3-53-derived antibody Fabs bound to the RBD, can in part counter neutralization escape caused by S changes that occurred in the immunocompromised host. Notably, the S mutations we studied foreshadowed the appearance of emerging SARS-CoV-2 variants.

Results

SARS-CoV-2 S is a large and heavily glycosylated protein that forms trimers of heterodimers on the surface of virions. Each S protomer has two functional subunits; S1, which contains a receptor-binding domain (RBD) that binds to cellular receptor, ACE2^{8,9}, and S2, which mediates fusion of the viral and host cell membranes during viral entry. IGHV3-53 or IGHV3-66 antibody genes are identical except for a single amino acid mutation in an antibody framework region (FWR)¹⁰, and potent SARS-CoV-2 neutralizing antibodies derived from these two germline genes have been isolated from multiple COVID-19 convalescent individuals¹⁻⁷. The S RBDs can be in “down” or “up” conformations^{11,12}, and ACE2 and IGHV3-53/3-66 neutralizing antibodies may only bind the RBD when it is “up”^{6,13}.

Isolated VH3-53-derived neutralizing antibodies bind the RBD with varying affinity

To study neutralizing antibody responses to SARS-CoV-2, a peripheral blood sample was obtained from a healthy individual (“C1”) who had been infected by SARS-CoV-2 five weeks prior to sampling. Polyclonal immunoglobulin G (IgG) purified from the blood of this individual neutralized SARS-CoV-2 lentivirus pseudotype but not vesicular stomatitis virus (VSV) lentivirus pseudotype (**FIG. 5A**). A soluble SARS-CoV-2 S construct was generated that is stabilized through mutations and the addition of trimerization tag to adopt and remain in the S “pre-fusion” conformation (“S2P”)¹¹ and used it as an antigen to isolate 116 memory B cells (CD19⁺, IgG⁺) by FACS (**FIG. 9A**). 48 recombinant monoclonal antibodies were produced in sufficient amount for further characterization. Forty-three of these antibodies bound S2P by ELISA, and 18 also bound the RBD (**FIG. 9B** and **FIG. 10**). Most antibodies were derived from the IGHV3 (V_H3) heavy chain subgroup and had kappa light chains (**FIG. 5D**). Antibody CDR H3 and CDR L3 loops had an average length of 15 and 9 amino acids, respectively, with low frequencies of somatic hypermutation in variable heavy and light chain sequences (**FIGS. 5C-5D** and **FIG. 10**).

Of the 43 antibodies tested, only eight neutralized SARS-CoV-2 pseudotype with greater than 90% reduction in entry at a screening concentration of 100 µg ml⁻¹ (**FIG. 9C**). IC₅₀ values ranged from 0.008 to 0.671 µg ml⁻¹ in dose response pseudotype neutralization assays (**FIG. 5E** and **FIG. 11A**). These eight antibodies also neutralized infectious SARS-CoV-2, but authentic virus was more resistant to antibody neutralization than pseudotype (**FIG. 5E** and **FIG. 11B**).

The potent neutralizing antibodies having an IC₅₀ value of less than 0.5 µg ml⁻¹ against infectious SARS-CoV-2 – C1A-B3, -F10, -C2, -H5, -C4, -B12, and -H6 – were somatic variants of the same IGHV3-53/IGKV1-9-derived (V_H3-53/V_K1-9) antibody (referred to as “C1A-VH3-53 antibodies” herein) (**FIG. 5E**, **FIGS. 7A-7B**, **FIG. 10**, **FIG. 11**). Each had a low number of amino acid substitutions in the heavy and light chain variable genes (**FIG. 5E**). Monomeric Fabs derived from these antibodies bound tightly to the RBD, with affinities ranging from 76 nM to 0.9 nM (**FIG. 5E**, **FIG. 12**). C1A-B12, which was used as a representative member of the C1A-IGHV3 antibodies, prevented an ACE2-Fc fusion protein from binding to the RBD in a biolayer interferometry (BLI)-based competition assay (**FIG. 13A**). The Fab of CR3022, a human antibody that does not compete with ACE2-binding¹⁴, did not affect C1A-B12 Fab or ACE2-Fc binding to the RBD (**FIG. 13A**).

Structural basis for affinity maturation of C1A-V_H3-53 antibodies

To better understand the basis for affinity maturation and the effects of somatic mutation of C1A-V_H3-53 antibodies on RBD affinity, X-ray crystal structures were determined of the RBD bound to four Fabs: C1A-B3, -B12, -C2, and -F10 (also referred to as “C1A-V_H3-53 Fab/RBD complexes”) (**FIG. 13B**). The Fabs engage the RBD through an identical binding mode with root mean square deviations (r.m.s.d.) of 0.36-0.39 Å by structural superposition. As with other IGHV3-53/IGHV3-66-derived antibodies (also referred to as “V_H3-53/3-66-derived antibodies”) ^{1,5-7,15}, CDR loops H1, H2, H3, and L1 make the most significant contacts with the RBD on a surface that overlaps with the ACE2 binding site (**FIGS. 13B-13C**). As suggested by the results of competition assays (**FIG. 13A**), the antibodies and ACE2 bind the same site on the RBD (**FIG. 13C**). Most of the contacts are polar and involve backbone and sidechain atoms on both sides of the interface (**FIGS. 13D-13G**). Somatic mutations in the C1A-IGHV3-3 antibodies occurred in CDR loops and FWRs, and in the structure, some (*e.g.*, the F10S and S14F mutations in the light chain) are positioned far from the RBD and are unlikely to impact antigen affinity (**FIG. 7C**).

High-resolution X-ray crystal structures of multiple clonotypes allowed us to examine the effects of somatic mutations on the interaction interface (**FIGS. 7C-7G**). The analysis also included eight additional IGHV3-53/3-66-derived SARS-CoV-2 neutralizing antibodies isolated in other studies from multiple donors (B38, CC12.1, CC12.3, CV30, C105, BD-236, and BD-629) ^{1,5-7,15}. These antibodies have an essentially identical binding mode on the RBD (**FIG. 14**).

As examples of mutations at the Fab/RBD interface mutations that likely increase affinity for the RBD, the V_H S31N and the S31R mutations, which are found in C1A-C2 and BD-629, respectively, provide new contacts with RBD Q474 and K458 (**FIG. 6A-C**, **FIG. 7D**). The V_H S56T mutation, which occurs in most of the C1A-IGHV3-53 antibodies (**FIG. 7F**), provides additional hydrophobic contacts with the RBD and with neighboring tyrosines on the antibody and, for example, positions a methyl group in van der Waals contact with RBD T415 and the side chains of Y52 and Y58 on the antibody (**FIGS. 6D-6E**); and the V_H S56A mutation in C1A-B12 removes a polar contact with RBD D420 (**FIG. 7A**, **FIG. 7F**). On the light chain, the N92I substitution in the two highest affinity binding antibodies, C1A-H6 and C1A-B12, provides a new hydrophobic contact with RBD Y505 (**FIG. 7A**, **FIG. 7G**, **FIG. 6G-H**). The V_H T28I somatic mutation, which was not observed in C1A-IGHV3-53 antibodies, is probably important as it independently occurred in CV30 ¹⁵, B38 ¹, and BD-629 ⁶ (**FIG. 8A**). This change adds a hydrophobic contact with the Cα atom of RBD G476 and

probably also helps orient CDR H1 to optimize neighboring polar contacts (**FIG. 8C-E**). The residue at position 26 in the CDR H1 loop of IGHV3-53/3-66 antibodies is almost uniformly a glycine, but BD-629 contains a unique substitution (G26E) that provides a new set of polar contacts with the RBD (**FIG. 8E**).

As has been well described in antibody responses to influenza virus and HIV^{16,17}, somatic hypermutation that are not at antibody/antigen interfaces can nevertheless substantially contribute to affinity gains by influencing CDR loop configuration and flexibility. Although the V_H A24V mutation is not at the RBD/Fab interface (**FIG. 7C**), it is a pocket-filling mutation that, through hydrophobic interactions with the side chain of V_H F27, would rigidify CDR H1 or “pre-configure” it in a conformation that is compatible with RBD binding (**FIG. 6I-6J, FIG. 7E**). V_H F27 is frequently mutated to a smaller hydrophobic residues during somatic hypermutation; it is replaced by an isoleucine in C1A-H5, BD-604, and BD-236⁶, by a leucine in CC12.1⁷, and by a valine in CV30¹⁵ (**FIG. 6K and FIG. 8A and 8C**). In contrast to the V_H A24V mutation, replacing V_H F27 with smaller hydrophobic residue would likely make CDR H1 more flexible as opposed to rigidifying it, and this added flexibility could allow optimization of local polar contacts, particularly as additional mutations are introduced during affinity maturation (the T28I change in addition to the F27V mutation in CV30)¹⁵ (**FIG. 8C**).

Affinity is not the only property that may be beneficial to an effective antibody response¹⁸, and antibody combining site diversity may provide broader protection against pathogens that are antigenically variable and evolve over time¹⁹. As examples of BCR diversification that could result in a loss of RBD affinity, the of V_H S56A mutation in C1A-B12 removes a polar contact with RBD D420, and the Y58F mutation in CC12.1 removes a polar contact with the backbone carbonyl of RBD T415 (**FIG. 6F and FIG. 8F-8G**).

IGHV3-53/3-66-derived SARS-CoV-2 neutralizing antibodies usually have short CDR H3 loops to avoid clashes with the RBD surface⁷ (**FIG. 14**). Although it is challenging to predict germline CDR H3 sequences, this study identified a potential mutation located centrally in the D5-18*01 gene segment (also referred to as the “D segment”) from which the CDR H3 loop could be derived²⁶ (**FIG. 15A**). In six out of seven of the clonally related antibodies, the inferred mutation replaces a germline serine with an arginine, for which two rotamers anchor an extensive network of polar interactions with the RBD (**FIG. 15B**). This network includes RBD Q493, a residue that is relevant to antibody neutralization escape as described further herein. More specifically, six of the seven clonally related IGH/V3-53 antibodies that were isolated contain the S100aR mutation in CDR H3 with independent

substitutions at the nucleotide level (**FIG. 15A**), suggesting that this adaptation was recurrently selected for during the affinity maturation process. Two alternate conformations were observed for the R100a side chain in the C1A-B12 Fab/RBD structure; it can either contact the side chain of RBD Q493 or the backbone carbonyl of RBD S494 (**FIG. 15B**). The R100a side chain also helps position neighboring antibody residues to make additional contacts with the RBD as part of a larger network of polar interactions involving water molecules.

To better understand the selective pressure driving the expansion of the IGHV3-53 class of SARS-CoV-2 neutralizing antibodies and the role of the S100aR somatic change in affinity maturation, germline revertant antibodies that contain germline V_H and V_L sequences but vary with either having a serine or an arginine at this CDR H3 position (C1A-gl and C1A-gl*, respectively; **FIGS. 7A-7B, FIG. 15C**) were generated. More specifically, an antibody revertant was generated in which all positions are reverted to their germline counterparts (C1A-gl), and another that only retains the S100aR substitution (C1A-gl*) (**FIG. 15C, FIGS. 7A-7B, FIG. 4H, and FIG. 4I**). Monomeric C1A-gl and C1A-gl* Fabs bound the SARS-CoV-2 S RBD with affinities of 127 nM and 46 nM, respectively (**FIG. 15D and FIG. 12**). The effect of the mutation was most pronounced on antibody on-rate, suggesting that the CDR H3 S100aR substitution allows the antibody to more effectively dock onto the RBD. Despite the difference in RBD affinity, C1A-gl and C1A-gl* IgG neutralized infectious SARS-CoV-2 with comparable IC₅₀ values of 0.126 and 0.102 µg ml⁻¹, respectively (**FIG. 15E, FIG. 5E**).

Next, the question of whether C1A-IGHV3 antibodies could neutralize SARS-CoV-2 containing the D614G mutation in S, which is found in a circulating SARS-CoV-2 strain with increased infectivity²⁰⁻²² was addressed. C1A-gl, which binds the RBD with 126 nM affinity, and C1A-B12, which binds the RBD with thirty-fold higher affinity (KD of 4 nM), neutralized SARS-CoV-2 S D614G pseudotypes with comparable IC₅₀ values (74 and 34 ng ml⁻¹, respectively) (**FIG. 16A**). While RBD affinities varied over a hundredfold for the antibodies in this study (**FIG. 5E**), this study observed no statistically significant correlation between RBD binding affinity and infectious SARS-CoV-2 neutralization (**FIG. 16B**). Taken together, these results suggest that once a threshold RBD affinity is reached for IGHV3-53 antibodies, gains in affinity are not necessarily associated with more potent virus neutralization.

Our ability to detect robust binding of monomeric Fab to the SARS-CoV-2 S RBD, a format that does not take into account the avidity that would be observed with B cell

receptors engaging trimeric S, suggests strong selective pressure for evolution of human antibody responses against this epitope. These results stand in contrast to those observed with antibody CV30, an IGHV3-53/IGVK3-20 antibody for which reversion of its only two substitutions with respect to the germline antibody, V_H F27V and T28I, results in a change in affinity from 3.6 nM to 407 nM and in an almost sixty fold change in neutralization IC₅₀ value (from 0.030 µg ml⁻¹ to 16.5 µg ml⁻¹)¹⁵. As described in our analysis, the V_H F27V and T28I mutations may respectively affect loop dynamicity and help optimize the geometry of CDR H1 contacts with the RBD¹⁵. The lack of a drastic change in affinity with reversion of germline antibody sequences with C1A-IGHV3-53 antibodies suggest that these take better advantage of antigen complementarity afforded by their CDR H3 loop and light chain gene (IGVK1-9 for C1A-IGHV3-53 antibodies and IGVK3-20 for CV30) (**FIG. 14**).

Although at this stage a correlation between RBD affinity and virus neutralization was not observed in this aspect of the current experiment, it is noted that affinities measurements with full length SARS-CoV-2 S was not evaluated. IGHV3-53/3-66 neutralizing antibodies may only bind the RBD when it is “up”^{6,13}, and efficient virus neutralization by engagement of an epitope that is transiently exposed likely requires optimization of binding on- and off-rates in addition to gains in overall affinity.

Conclusions that can specifically be drawn from this work include that a germline revertant of some IGVH3-33 neutralizing antibodies can bind and neutralize virus, that a single residue substitution in the CDR H3 loops of a germline or near-germline antibody can drastically enhance its affinity to the RBD, but that gains in affinity are not necessarily associated with more potent neutralization once a certain affinity has been reached. Mutations that allow SARS-CoV-2 S to escape neutralization by antibodies that compete with ACE2 binding have been observed *in vitro*²³. Although SARS-CoV-2 encodes an exonuclease that increases the fidelity of replication of its large RNA genome, recurrence of an identical antibody response in multiple COVID-19 convalescent individuals suggests that selective pressure on this epitope is significant. To avoid evolution of neutralization escape over time as the virus circulates in humans, vaccine design efforts may need to focus on potent neutralizing antibodies binding additional sites on SARS-CoV-2 S, rather than on clonal expansion of one or a limited set of IGVH3-53/3-66-derived antibodies, as occurred during natural infection of the convalescent donor studied.

Structural predictions of neutralization antibody escape

The convergence of nearly identical responses against the RBD in multiple COVID-19 convalescent individuals (**Figures 14A-14B**) led this study to hypothesize that SARS-CoV-2 could evolve resistance to V_H3-53/3-66 antibodies as the virus continues to circulate in humans. A recent report described significant evolution of SARS-CoV-2 in an individual receiving profound immunosuppression (Choi et al., 2020). The individual had antiphospholipid syndrome complicated by diffuse alveolar hemorrhage and received glucocorticoids, cyclophosphamide, rituximab, and eculizumab as part of their immunosuppression; they ultimately experienced at least three episodes of symptomatic disease (Choi et al., 2020). COVID-19 was diagnosed on day 0 of infection by RT-PCR, and SARS-CoV-2 whole genome viral sequencing was performed from nasopharyngeal specimens at various time points and up to day 152 (**Figure 18A**) (Choi et al., 2020). There was evidence of pronounced RBD sequence evolution by the later time points, with a total of eight mutations (**Figures 18B-18C**). This study predicted that five of these eight mutations would impact C1A-V_H3-53 antibody binding (**Figure 18B**). The Q493K_{RBD} mutation would introduce a substantial clash with CDR H3 residue R100_{avH} found in most of the antibody clones (**Figures 7A and Figure 18D**). C1A-H6 is the only antibody clone that contains a lysine at position 100_{avH} (**Figure 7A**); although this study did not obtain a crystal structure of the RBD bound to the C1A-H6 Fab to visualize its contacts, K100_{avH} would probably also clash with K493_{RBD}. The N501Y_{RBD} mutation would introduce minor clashes with CDR L1 residue S30_{vH}, a V_K1-9 germline residue (**Figures 7B and Figure 18E**). This germline residue is conserved in the other V_H3-53-derived neutralizing antibodies we examined that also contain the V_K1-9 light chain and for which X-ray crystal structures are available (**Figures 14A-14B, and Figure 8B**). Alternate rotamers observed in the structures for residues R100_{avH} and S30_{vL} would partially accommodate the Q493K_{RBD} and N501Y_{RBD} mutations (**Figures 18D-18E**). The other mutations (E484K/_A_{RBD}, F486I_{RBD}, and Y489H_{RBD}) would alter polar or hydrophobic antibody contacts (**Figure 18F-8H**). In particular, the Y489H_{RBD} change detected on day 128 sequencing would alter an extensive network of polar interactions with antibody residue R94_{vH}, a germline antibody residue that is conserved in all V_H3-53/3-66-derived antibodies (**Figures 18H and Figure 8A**). Mutations at position E484_{RBD}, however, would be better tolerated, as the network of polar interactions with the antibodies, which includes water molecules, would either be lost (E484A_{RBD}) or possibly be reorganized (E484K_{RBD}) (**Figure 18F**).

Evolved spike variants escape V_H3-53 antibody neutralization

This next sought to validate the structural predictions for the effects of RBD mutations on V_H3-53 antibody neutralization using lentivirus pseudotypes bearing variant S proteins. Because variants detected at the two latest time points contained the most RBD mutations, we pseudotypes with S proteins that contain mutations observed on days 128 through 152 (**Figures 18B and Figure 19**) were generated. The original day 146 sequence contains a seven-residue deletion at the N terminus of S1 near the expected signal peptide juncture that is of unclear significance, so this segment was preserved as WT for viral pseudotyping (**Figure 19**). The Y489H_{RBD} mutation found on day 128 was also retained given its predicted impact on the antibody-RBD interface and because it has also been detected in additional human derived SARS-CoV-2 S sequences (**Figures 18B, 18H, 18J-18L**), generating S mutants denoted “day 146*” and “day 152*” (**Figure 19**). This study used D614G_S pseudotypes as the wild type (WT) control for these experiments because all sequences recovered from the immunocompromised individual included it, suggesting that the initial infecting SARS-CoV-2 virus contained this substitution (**Figure 19**). Day 146* and day 152* pseudotypes were neutralized by an ACE2-Fc fusion protein (**Figures 20I-20J**) but were resistant to neutralization by C1A-V_H3-53 antibodies (**Figures 21A-21B, and Figure 22A**). C1A-H6, whose Fab binds the tightest to the RBD (0.9 nM; **Figure 5E**), had some activity against the day 152* S pseudotype, but with a forty-fold decrease in potency (IC₅₀ value of 4.5 µg ml⁻¹) (**Figure 21A and Figure 22A**).

The Q493K_{RBD} mutation, which was observed in sequences obtained on day 128, 130, and 146 (**Figure 18B**), has previously been described through in vitro resistance mapping efforts with recombinant vesicular stomatitis virus expressing SARS-CoV-2 S (rVSV-S) (Weisblum et al., 2020). The Q493K_{RBD} change or a similar mutation at the same position (Q493R_{RBD}) have been recently described in other human-derived SARS-CoV-2 sequences (**Figure 18B and 18J-18L**). To determine the role of the Q493K/R_{RBD} mutations in resistance to C1A-V_H3-53 antibodies, this study generated pseudotypes containing either mutation in addition to the D614G_S change. This study also included an N439K_{RBD} variant, a recently described antibody neutralization escape mutant (Thomson et al., 2021). The Q493K_{RBD} mutation caused substantial resistance to the C1A-V_H3-53 antibodies that bind the most weakly to the RBD (**Figures 21A-21B**). Similar findings were observed with the Q493R_{RBD} pseudotypes, although the decrease in neutralization sensitivity was more severe. The only exception was C1A-gl, which neutralized Q493K/R_{RBD} pseudotypes better than C1A-gl*, likely because a serine instead of an arginine at CDR H3 position 100a would better

accommodate these RBD mutations (**Figure 18D**). The N439K_{RBD} mutation had no effect on pseudotype neutralization by C1A-V_{H3}-53 antibodies, which was expected because this mutation falls outside of the V_{H3}-53 antibody epitope on the RBD.

To determine if resistance extends to V_{H3}-53-derived antibodies isolated from different COVID-19 convalescent donors, this study also tested antibodies B38 (Wu et al., 2020) and CC12.1 (Rogers et al., 2020; Yuan et al., 2020a). The Q493K/R_{RBD} mutations conferred decreased sensitivity to B38 but had no effect on neutralization by CC12.1 (**Figures 21D, Figures 22B-22C**). Day 146* and day 152* S pseudotypes, however, were completely resistant to both of these monoclonal antibodies (**Figures 21D, Figures 22B-22C**).

Resistance to therapeutic antibodies in clinical use

The monoclonal antibody cocktail REGN-COV2 comprises two antibodies that bind non-overlapping sites on the RBD to suppress the emergence of antibody neutralization escape mutations (Baum et al., 2020; Hansen et al., 2020). REGN10933 binds a region of the RBD that overlaps significantly with the ACE2-binding site, while REGN10987 binds a region that has little to no overlap (**Figure 21C**). Of the S mutations that evolved during persistent SARS-CoV-2 infection in the immunocompromised individual, the Q493K_{RBD} change, found in day 146 sequencing, was previously detected in tissue cell culture passaging experiments using REGN10933 and rVSV-S (Baum et al., 2020). In these experiments, the Q493K_{RBD} mutation decreased REGN10933 pseudotype neutralization potency by fifteenfold (**Figures 21D and Figure 22B**). Day 146* and day 152* S pseudotypes, however, were completely resistant (**Figures 21D and Figure 22B**). Notably, the day 152* variant lacks the Q493K_{RBD} substitution, but its F486I_{RBD} mutation is similar to a known REGN10933 resistance mutation (F486V_{RBD}) (**Figures 18B and 18G**) (Baum et al., 2020).

The N440D_{RBD} mutation, which was only detected on day 146 sequencing (**Figure 18A**), falls on the REGN10987 RBD-binding site. It is adjacent to a N439K_{RBD} mutation that is found in circulating variants with reported REGN10987 resistance (Thomson et al., 2021) (**Figure 21C**). The day 146* variant had a fourfold decrease in REGN10987 neutralization sensitivity, while the N439K_{RBD} mutation caused fourteenfold decrease in sensitivity (**Figure 21D and Figure 22B**). REGN10987, therefore, is the only antibody this study tested that had demonstrable activity against the day 146* S pseudotypes, but a single substitution at an adjacent position (N439K_{RBD}) found in circulating variants (Thomson et al., 2021) could result in additional neutralization escape.

Evolved S variants are resistant to convalescent donor polyclonal IgG

All of the potent SARS-CoV-2 neutralizing antibodies that were isolated from the healthy COVID-19 convalescent donor (C1) were clonotypes of a single V_H3-53/V_K1-9 antibody, suggesting that this individual's memory B cell response was narrowly focused on this class of neutralizing antibodies (**Figures 5A-5D, Figures 9A-9C; Figure 10**). While purified C1 IgG could neutralize WT (D614G_S) pseudotypes, day 146* and 152* S pseudotypes were resistant to C1 serum IgG neutralization (**Figures 21E-21F**). The Q493K/R_{RBD} mutations also conferred near complete resistance to C1 IgG (**Figures 21E-21F**). The N439K_{RBD} mutation (Thomson et al., 2021), an escape mutation that falls outside of the RBD epitope for V_H3-53-derived neutralizing antibodies, had no effect on C1 polyclonal IgG neutralization (**Figures 21E-21F**). To determine whether our findings extended to other COVID-19 convalescent donors that may have less epitope biased antibody responses, this study performed similar experiments with purified IgG from three additional donors ("C2", "C3", and "C4") (**Figures 21E-21F**). The neutralizing activity of purified IgG from these donors was mostly unaffected by the single mutations (Q493K/R_{RBD} or N439K_{RBD}), but day 146* and 152* S pseudotypes were resistant to neutralization.

V_H3-53 antibody affinity maturation partially overcomes neutralization escape

Although the benefits of antibody RBD affinity are limited in SARS-CoV-2 neutralization (**Figure 16B**), affinity gains, in principle, could compensate for losses of contacts or potential clashes that are caused by escape mutations. Indeed, the highest affinity binding antibodies were seemingly the least impacted by neutralization escape mutations (**Figures 21A-21B, and Figure 22A**). We selected C1A-B12, our most potent neutralizing antibody against infectious SARS-CoV-2 (**Figure 5E**), to directly test whether additional affinity enhancing mutations could overcome neutralization escape. To generate affinity enhanced versions of C1A-B12, we introduced into its sequence somatic hypermutation changes found in other V_H3-53/3-66 antibodies, including antibodies described elsewhere (Hurlburt et al., 2020; Wu et al., 2020) (**Figure 14C-14E and Figure 8A**). Fabs for the resulting antibodies (C1A-B12.1, C1A-B12.2, and C1A-B12.3) bound to the RBD with a six- to-ten-fold increase in affinity as compared to the parent C1A-B12 Fab (**Figure 5E; Figure 12**). Affinity enhanced variants potentially neutralized D614G_S pseudotypes and infectious SARS-CoV-2 (**Figure 5E; Figures 21A-21B, Figure 14F, and Figure 22A**). Remarkably, while C1A-B12 had no activity against day 152* S pseudotype, all three affinity optimized versions were active; the antibody containing the most mutations, C1A-B12.3, was the most

potent ($IC_{50} < 0.5 \mu g ml^{-1}$) (**Figures 21A-21B, and Figure 22A**). Day 146* S pseudotype, however, was still resistant to neutralization by the affinity enhanced antibodies (**Figures 21A-21B, and Figure 22A**).

5 Discussion

The finding that the day 146* and day 152* S pseudotypes escape neutralization by all unmodified V_H3-53 antibodies that were tested and REGN10933, an antibody derived from a different germline gene (V_H3-11) (Baum et al., 2020; Hansen et al., 2020), suggest that SARS-CoV-2 can evolve solutions to bind ACE2 while escaping neutralization by a major class of human neutralizing antibodies. Perhaps the most striking finding is that the polyclonal antibody response in a convalescent individual we studied (C1) is so focused on an RBD epitope that single mutations (Q493K/R_{RBD}) can confer substantial resistance to serum IgG neutralization (**Figures 21E-21F**). The Q493K/R_{RBD} mutations, however, had less of an effect on the serum IgG of three additional COVID-19 convalescent donors (**Figures 21E-21F**), suggesting that the C1 donor may be a rare example of an overly focused antibody response.

The immunocompromised individual we studied received REGN-COV2 (REGN10933 and REGN10987) on day 145 of their illness, so the S mutations detected on days 146 and 152 could have been influenced by selective pressure from this therapeutic antibody cocktail, as described in a recent report (Starr et al., 2021). Nonetheless, several of the RBD mutations studied were detected well before day 146 (**Figure 18B**), suggesting that they could have arisen through selective pressure from the individual's weakened neutralizing antibody response (Choi et al., 2020). Furthermore, based on publicly available sequences as of February 19, 2021 in the GISAID database (Elbe and Buckland-Merrett, 2017), seven of eight RBD mutations examined in the immunocompromised individual have been detected in additional human derived SARS-CoV-2 sequences (**Figures 18B, 18J-18L**). The only unique mutation is F486I_{RBD}, although a nearly identical mutation, F486L_{RBD}, has been observed in another human derived SARS-CoV-2 S sequence (**Figures 18J-18L**). Importantly, Q493K_{RBD}/N501Y_{RBD}, Q493R_{RBD}/N501Y_{RBD}, and Y489H_{RBD}/N501Y_{RBD} S variants have recently been reported in the GISAID database, albeit with very low frequency for the time being (**Figure 18B and Figures 18J-18L**). Additional mutations that we did not study directly but that could also substantially impact neutralization by V_H3-53-derived antibodies are the K417N/T_{RBD} mutations observed in S variants initially detected in South Africa (B.1.351) and Brazil (P.1); like the Y489H_{RBD} mutation, these changes would alter an

extensive network of polar contacts with V_H3-53/V_K1-9 antibodies (**Figures 18B, 18H, and 18I**).

A detailed understanding of the human antibody response to SARS-CoV-2 and of virus-host co-evolution will be required to design countermeasures that anticipate changes in the virus as it continues to circulate in humans. The portion of the coronavirus S RBDs that interacts with ACE2, called the “receptor-binding motif,” can be thought of as a hypervariable region within an otherwise conserved domain (Li et al., 2005a). The RBD of the closely related SARS-CoV, within its receptor-binding motif, contains two “hotspots” for host co-adaptation that are centered on N479_{RBD} and T487_{RBD} (SARS-CoV numbering). Mutations at these positions regulate cross-species transmission and neutralizing antibody escape (Li et al., 2005a; Sui et al., 2008; Wu et al., 2012). Interestingly, two of the residues we pinpointed in our analysis (Q493_{RBD} and N501_{RBD}) are in the equivalent hotspot positions on the SARS-CoV-2 S RBD (Wan et al., 2020).

The N501Y_{RBD} mutation, in particular, is involved in SARS-CoV-2 adaptation to murine ACE2 binding (Gu et al., 2020) and has been observed with increasing frequency among circulating variants originally detected in the United Kingdom (B.1.1.7), South Africa (B.1.351), and Brazil (P.1) (**Figure 18B**). Examination of the structure of an RBD/ACE2 ectodomain complex (Shang et al., 2020) suggest that the N501Y_{RBD} change could introduce favorable hydrophobic contacts with Y41_{ACE2} and K353_{ACE2} (**Figures 20A and 20D**). The SARS-CoV-2 Q493K_{RBD} change is also involved in adaptation to murine ACE2 (Leist et al., 2020) and is analogous to the SARS-CoV N479K_{RBD} mutation, which allows preferential engagement of palm civet ACE2 (host reservoir) over human ACE2 (Li et al., 2005b; Wu et al., 2012). The RBD sequence changes we studied, therefore, are likely a combination of neutralizing antibody escape mutations and adaptations to human ACE2 binding.

In certain instances, antibody escape mutations could also negatively impact ACE2 binding. While D614G_S and day 146* S pseudotypes had comparable IC₅₀ values in neutralization tests with an ACE2-Fc fusion protein, day 152* S pseudotype typically had a more than tenfold increase, with some variability in the absolute value depending on the experiment (**Figures 21A, 21D, 20I, and 20J**). These observations suggest that the affinity of the day 152* S for ACE2 may have been compromised. A loss in receptor-binding affinity for the day 152* S may be explained by the F486I_{RBD} mutation it contains, which would remove prominent hydrophobic contacts with ACE2 (**Figure 20F**). While we used pseudotypes for our studies and focused on the RBD, additional studies with authentic viruses will be required to determine the consequences of the S mutations we studied on viral fitness

and potential for transmission. For example, it is unclear how a deletion detected at the S1 N terminus/signal peptide juncture on the original day 146 sequence (**Figure 19**) would impact S processing, and whether potentially decreased ACE2 binding by a day 152*-like S variant would affect viral replication and transmission.

While these were not the focus of our studies, non-RBD binding neutralizing antibodies can target the SARS-CoV-2 S1 NTD. 4A8, an antibody isolated from a COVID-19 convalescent individual, is a representative member of this class (Chi et al., 2020). Examination of evolved S sequences reveal that they contain internal deletions within the S NTD (spanning residues 141-144) that would disrupt part of 4A8's epitope (**Figures 19 and Figure 23A-23B**). The deletions would also reposition a nearby N-linked glycan and potentially block 4A8 epitope access (**Figure 23B**). A recent report described persistent SARS-CoV-2 infection in another immunocompromised individual who had acquired hypogammaglobulinemia, with detectable viral RNA more than one hundred days after infection (Avanzato et al., 2020). In this individual, S evolution also led to a deletion of a similar segment in the S1 NTD (spanning residues 139-145) (Avanzato et al., 2020). Notably, S1 NTD deletions found in the B.1.1.7 and B.1.351 S variants are in, or near, the NTD deletion found in the variants studied (**Figures 23A-23B and Figure 19**). Although NTD internal deletions could substantially impact 4A8 neutralization, we could not directly test this hypothesis because 4A8 has very weak neutralizing activity against S lentivirus pseudotypes on HEK293T cells overexpressing human ACE2 (Chi et al., 2020), which we also used in our assays.

The value of our study and additional studies examining immune responses in immunocompromised individuals (Kemp et al., 2021; Starr et al., 2021) is obtaining insight from a dynamic immune system over time as opposed to only studying viral escape mutations in vitro. In vitro studies have indeed been highly informative in predicting the range of S mutations that can be acquired for antibody neutralization escape, but studying S sequence evolution during persistent SARS-CoV-2 infection may help highlight the mutations that have the most potential to spread in emerging variants.

Lastly, while single mutations are unlikely to confer substantial resistance to polyclonal antibody responses in many individuals, multiple mutations, as are observed in the late stage evolved S variants we studied, are likely to have an impact. To fully understand the consequences of SARS-CoV-2 S genetic drift, including its potential implication to ongoing vaccination campaigns, our study underscores the importance of studying multiple mutations that can concomitantly be found in S, as opposed to single S mutations in isolation.

Other considerations

The S mutations studied are only from one immunocompromised individual (n = 1) (Choi et al., 2020). While some of the S mutations discussed are also now found in other human-derived SARS-CoV-2 sequences available in public data bases (e.g., B.1.1.7 variants containing the Q493K/R_{RBD} mutations; see Table S3), these variants for the time being are rare and the context in which they arose is also not defined based on public information (e.g., whether they occurred in a healthy person or an immunocompromised individual, or whether the individual received treatment with convalescent plasma or therapeutic antibodies prior to sampling, etc.). While this study used infectious SARS-CoV-2 in some assays, replication defective pseudotyped lentiviruses was used as a surrogate system for studying the effects of S mutations. The replication fitness of infectious SARS-CoV-2 carrying the S mutations that were studied remains to be determined. This study used multiple V_H3-53-derived monoclonal antibodies isolated from a healthy donor (n=1), and single antibodies from two additional donors (B38 from (Wu et al., 2020) and CC12.1 (Rogers et al., 2020)), but did not test all V_H3-53-derived monoclonal antibodies identified to date. Other V_H3-53-derived antibodies may be differently impacted by specific mutations because of differences in their light chain genes and CDR H3 loops. Lastly, while we predicted that residue R100_{avH} was a serine in the germline C1A-V_H3-53 antibody based on our analysis using the IMGT/V-QUEST database (Brochet et al., 2008), this database is likely missing alleles. To prove that the described D gene assignment was accurate, this would have had to sequence D gene segments in the PBMC donor C1, and this did not perform this analysis. There is, therefore, the possibility that an arginine or lysine would be found at position 100_{avH} in a germline C1A-V_H3-53 antibody.

Materials and Methods**Protein Data Bank (PDB) identification numbers**

Protein Data Bank (PDB) identification numbers for the C1A-B3/RBD, C1A-F10/RBD, C1A-C2/RBD, and C1A-B12 RBD complexes are 7KFW, 7KFY, 7KFX, and 7KFV, respectively.

Cells and viruses

HEK293T cells (ATCC CRL-11268) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin. HEK293T-hACE2 stable cells were maintained in DMEM

supplemented with 10% (v/v) fetal bovine serum (FBS), 25 mM HEPES, and 1% (v/v) penicillin-streptomycin with the addition of 1 $\mu\text{g ml}^{-1}$ puromycin. HEK293T cells were maintained grown in suspension in FreeStyle 293 Expression Medium (Gibco) and HEK293S GnTI^{-/-} cells (ATCC CRL-3022) in Freestyle 293 Expression Medium supplemented with 2% ultra-low IgG FBS (Gibco). Expi293FTM (Thermo Fisher Scientific) cells were maintained in Expi293TM expression medium (Gibco) supplemented with 1% (v/v) penicillin-streptomycin. An Expi293F stable cell line that expresses His₆-tagged SARS-CoV-2 S2P was maintained in adherent culture with DMEM supplemented with 1% (v/v) GlutaMax (Gibco), 1% (v/v) penicillin-streptomycin, 10% (v/v) FBS and 1 $\mu\text{g ml}^{-1}$ puromycin. The cell line was then adapted to suspension culture and maintained in Expi293TM expression medium supplemented with 1% (v/v) penicillin-streptomycin and 1 $\mu\text{g ml}^{-1}$ puromycin (Gibco).

Passage 4 SARS-CoV-2 USA/WA1/2020²⁸ was received from the University of Texas Medical Branch. A T225 flask of VeroE6 cells was inoculated with 90 μl starting material in 15 ml DMEM containing 2% (v/v) of heat inactivated FBS (HI-FBS) and incubated in a humidified incubator at 37 °C with periodic rocking for 1 h. After 1 h, 60 ml of DMEM / 2% (v/v) HI-FBS was added without removing the inoculum and incubated again at 37 °C. The flask was observed daily for progression of cytopathic effect and stock was harvested at 66 h post-inoculation. Stock supernatant was harvested and clarified by centrifugation at 5,250 relative centrifugal field (RCF) at 4°C for 10 min and the HI-FBS concentration was increased to 10% (v/v) final concentration.

Single B cell sorting and antibody cloning

This study was approved by the Harvard Medical School Office of Human Research Administration Institutional Review Board (IRB20-0365) as was the use of healthy donor control blood (IRB19-0786). Informed, written consent was received from a healthy adult male participant (C1) who recovered from confirmed SARS2-CoV-2 infection, with mild illness not requiring hospitalization, five weeks before blood donation. C1 and control donor PBMCs were isolated by Ficoll-Plaque (GE Healthcare) density centrifugation. Single memory B cells were stained and sorted as previously described²⁹ using a MoFlo Astrios EQ Cell Sorter (Beckman Coulter). Briefly, B cells were enriched by incubating PBMCs with anti-CD20 MicroBeads (Miltenyi Biotec) followed by magnetic separation on a MACS LS column (Miltenyi Biotec) according to the manufacturer's instructions. The B cells were washed, counted, and resuspended in phosphate buffered saline (PBS) containing 2% (v/v) FBS. The B cells were adjusted to a density of 1×10^7 cells and incubated cells with

biotinylated SARS CoV-2 spike (S2P) at a concentration of 5 $\mu\text{g ml}^{-1}$ on ice for 30 min. After washing three times and resuspending the cells, anti-IgG-APC antibody (BD Biosciences catalog number 550931), anti-CD19-FITC antibody (BD Biosciences catalog number 340864), and streptavidin-PE (Invitrogen) was added. After incubating the cells on ice for 30 min, the cells were washed three times in PBS containing 2% (v/v) FBS and passed the suspension through a cell strainer before sorting.

Single cell cDNA synthesis was performed using SuperScriptTM III reverse transcriptase (Invitrogen) followed by nested PCR amplification to obtain the IgH, Ig λ , and IgK variable segments from memory B cells as previously described³⁰. IMGT/V-QUEST²⁶

(<http://www.imgt.org>) was used to analyze IgG gene usage and the extent of variable segment somatic hypermutation. The variable segments were cloned into the pVRC8400 vector for expression of the IgG and Fab constructs as previously described³¹.

Protein production

For single B cell sorting a construct comprising human codon optimized was cloned for SARS-CoV-2 S (GenBank ID: QHD43416.1 residues 16-1208) with a “GSAS” substitution at the furin cleavage site (residues 682-685), stabilized in the prefusion conformation through proline substitutions at residues 986 and 987¹¹, and a C-terminal foldon trimerization motif followed by a BirA ligase site, a Tobacco Etch Virus (TEV) protease site, a FLAG tag, and a His₆-tag into a pHLsec vector³², which contains its own secretion signal sequence. It is noted that two N-terminal S residues (residues 14 and 15) downstream of the native S signal peptide were inadvertently omitted from the S2P construct during subcloning. Expi293FTM cells were transfected using an ExpiFectamineTM (Thermo Fisher Scientific) transfection kit according to the manufacturer’s protocol. The protein was purified using anti-FLAG M2 Affinity Gel (Sigma) according to manufacturer’s protocol and the FLAG tag and His₆-tag were removed with TEV digestion followed by reverse nickel affinity purification and size-exclusion chromatography on a Superose 6 Increase column (GE Healthcare Life Sciences). The protein was biotinylated with BirA ligase as previously described³¹.

To obtain recombinant S2P for ELISAs, Ni Sepharose[®] Excel (GE Healthcare Life Sciences) was used to purify His₆-tagged SARS-CoV-2 S2P from the supernatant of Expi293F cells stably expressing this protein. The protein was further purified using size exclusion chromatography on a Superpose 6 Increase column.

Human codon optimized cDNA was synthesized for antibodies based on publicly available sequences; 4A8 (Chi et al., 2020) (PDB: 72CL), B38 (PDB:7BZ5) (Wu et al., 2020)

, CC12.1 (Yuan et al., 2020a) (PDB: 6XC2), and REGN10933 and REGN10987 (Hansen et al., 2020) (PDB 6XDG). Recombinant monoclonal antibodies and Fab fragments were expressed and purified using the pVRC8400 vector as previously described³¹. To generate the CR3022 control Fab, its variable heavy chain and light chain gene regions (GenBank IDs: DQ168569.1 and DQ168570.1) were amplified from cDNA and subcloned into the pVRC8400 vector. Expi293FTM cells were transfected using an ExpiFectamine™ transfection kit according to the manufacturer's protocol. The IgG and Fabs were affinity purified using MabSelect SuRE Resin (GE Healthcare) using the manufacturer's protocol. All Fabs were further purified by size exclusion chromatography on a Superdex 200 Increase column (S200, GE Healthcare Life Sciences)s, which eluted as single peaks at the expected retention volume.

Constructs for the SARS-CoV-2 S RBD (GenBank ID: QHD43416.1 residues 319-541) were subcloned into the pHLsec³² vector for use in ELISAs, BLI binding studies, and X-ray crystallography. For ELISAs and crystallography the construct includes an N-terminal His₆-tag, a TEV protease site and a short linker (amino acids SGSG). For BLI-binding assays, the construct includes an N-terminal His₆-tag, followed by a TEV protease site, a BirA ligase site, and a 7-residue linker (amino acids GTGSGTG). Protein was produced for ELISA and BLI-binding assays by using linear polyethylenimine (PEI) to transfect HEK293T cells grown in suspension and purified by nickel affinity purification. For BLI-binding assays the protein was digested with TEV protease to remove the His₆-tag followed by reverse nickel affinity purification. Protein was biotinylated with BirA ligase as previously described³³, followed by a reverse nickel affinity purification step to remove BirA ligase, which contains a His₆-tag and cannot be separated by size exclusion chromatography from the SARS-CoV-2 RBD due to its similar size. For crystallography, the RBD protein was produced by PEI transfection of GnTI^{-/-} HEK293S cells grown in suspension or HEK293T cells grown in suspension and also in presence of kifunensine (5 μM), purified by nickel affinity purification, and removed the His₆-tag by TEV digestion followed by reverse nickel affinity purification. As a final step, size exclusion was used on a Superdex 200 Increase column, in which each recombinant RBD protein ran as a single peak at the expected retention volume.

The ectodomain of human ACE2 (GenBank ID: BAB40370.1) residues 18-740, with cDNA, with a C-terminal Fc tag was subcloned into a pVRC8400 vector containing human IgG1 Fc. The protein was expressed in Expi293F™ cells using an ExpiFectamine™ transfection kit according to the manufacturer's protocol, and purified the protein using MabSelect SuRE Resin using the manufacturer's protocol, followed by size exclusion

chromatography on a Superose 6 Increase column, with the protein eluting at the expected retention volume.

Lentivirus pseudotype production

5 The human codon optimized SARS-CoV-2 S protein (Genbank ID: QJR84873.1 residues 1-1246) with a modified cytoplasmic sequence that includes HIV gp41 residues (NRVRQGYS) replacing C-terminal residues 1247-1273 of the S protein was subcloned into the pCAGGS expression vector. With this human codon optimized modified S construct as a starting point, a Gibson assembly was used to introduce to generate the D614Gs,
 10 D614Gs/N439K_{RBD}, D614Gs/Q493K_{RBD}, D614Gs/Q493R_{RBD}, Day 146*, and Day 152 S variants. Day 146 S is derived from hCoV-19/USA/MA-JLL-D146/2020 (EPI_ISL_593557) but contains WT sequences at positions 11-18 and at residue 144 (Figure S6). Day 152 S is derived from hCoV-19/USA/MA-JLL-D152/2020 (EPI_ISL_593558). A pCAGGS expressor plasmid for VSV G was previously described³⁴. To package lentivirus, HEK293T cells were
 15 co-transfected using lipofectamineTM 3000 (Thermo Fisher Scientific) with an envelope gene-encoding pCAGGS vector, a packaging vector containing HIV Gag, Pol, Rev, and Tat (psPAX2, Addgene #12260), and a transfer vector containing GFP (lentiCas9-EGFP, Addgene #63592³⁵) in which Cas9 was deleted. After 18 h, the supernatant was changed to DMEM containing 2 % FBS (v/v). At 48 and 72 h supernatants were harvested, samples were
 20 centrifuged at 3000 x g for 5 min, and filtered through a 0.45 µm filter. All pseudotypes except for SARS-CoV-2 Day 152 were concentrated. To concentrate lentivirus pseudotypes, the supernatant was layered on top of a 10% (v/v) sucrose cushion in 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 0.5 mM EDTA and spun samples at 10,000 x g for 4 h at 4 °C. Supernatants were removed and virus pellets were resuspended in Opti-MEM containing 25
 25 mM HEPES and 5% (v/v) FBS and stored these at -80 °C.

Pseudotype neutralization experiments

Polyclonal IgG was purified from human plasma samples using PierceTM Protein G Ultra LinkTM Resin (Thermo Fisher Scientific) and by following the manufacturer's protocol.
 30 Polyclonal serum IgG, monoclonal antibodies or an ACE2-Fc fusion protein were pre-incubated with SARS-CoV-2 S, SARS-CoV-2 S variants, or VSV G lentivirus pseudotypes in the presence of 0.5 µg ml⁻¹ of polybrene for 1 h at 37 °C. Virus antibody mixtures were added to HEK293T-hACE2 with incubation on cells at 37 °C for 24 h, and the media replaced with DMEM containing 10% (v/v) FBS, 1% (v/v) penicillin-streptomycin (v/v), and 1 µg ml⁻¹

puromycin. The percent of GFP positive cells was determined by FACS with an iQue Screener PLUS (Intellicyt) 48 h after initial infection. The percent relative entry was calculated by using the following equation: Relative Entry (%) = (% GFP positive cells in antibody well/%GFP positive cells in no antibody control) x 100. Percent antibody

neutralization was calculated using the following equation: Neutralization (%) = [1 - (% GFP positive cells in nanobody well/% GFP positive cells in PBS alone well)] x 100.

Live virus PRNT experiments

Monoclonal antibody samples were serially diluted in Dulbecco's Phosphate-

Buffered Saline (DPBS, Gibco) using half-log dilutions starting at a concentration of 50 µg ml⁻¹. Dilutions were prepared in triplicate for each sample and plated in triplicate. Each dilution was incubated at 37 °C for 1 h with 1,000 plaque-forming units ml⁻¹ (PFU ml⁻¹) of SARS-CoV-2 (isolate USA-WA1/2020). 200 µl of each dilution was added to the confluent monolayers of NR-596 Vero E6 cells (ATCC) in triplicate and incubated in a 5% CO₂

incubator at 37 °C for 1 hour. The cells were rocked gently every 15 min to prevent monolayer drying. Cells were then overlaid with a 1:1 solution of 2.5% (v/v) Avicel® RC-591 microcrystalline cellulose and carboxymethylcellulose sodium (DuPont Nutrition & Biosciences) and 2x Modified Eagle Medium (MEM - Temin's modification, Gibco) supplemented with 100 X antibiotic-antimycotic (Gibco) and 100X GlutaMAX (Gibco) both to a final concentration of 2X, and 10% (v/v) FBS (Gibco). The plates were then incubated at 37 °C for two days. After two days, the monolayers were fixed with 10% (v/v) neutral buffered formalin for at least 6 h (NBF, Sigma-Aldrich) and stained with 0.2% (v/v) aqueous Gentian Violet (RICCA Chemicals) in 10% (v/v) neutral buffered formalin for 30 min, followed by rinsing and plaque counting.

ELISA experiments

NUNC Maxisorp plates (Thermo Fisher Scientific) were coated with His₆-tagged SARS-CoV-2 S2P, SARS-CoV-2 RBD, or LUJV GP1 in PBS overnight at 4 °C, followed by a blocking step with PBS containing 3% (v/v) BSA 0.02% (v/v) Tween. Monoclonal antibodies were incubated at a concentration of 100 µg ml⁻¹ for one hour. Samples were washed three times with PBS containing 0.02 % (v/v) Tween. Bound antibody was detected with horseradish peroxidase (HRP)-coupled anti-human (Fc) antibody (Sigma Aldrich catalog number A0170).

Biolayer interferometry assays

BLI experiments were performed with an Octet RED96e (Sartorius). For affinity measurements, biotinylated SARS-CoV-2 RBD was loaded onto a streptavidin (SA) sensor (ForteBio) at $1.5 \mu\text{g ml}^{-1}$ in kinetic buffer (PBS containing 0.02% Tween and 0.1% BSA) for 100 s. After a baseline measurement for 60 s in kinetic buffer, antibody Fabs were associated for 300 s followed by a 300 s dissociation step.

For ACE2-Fc competition experiments, biotinylated SARS-CoV-2 RBD was loaded onto SA sensors (ForteBio) at $1.5 \mu\text{g ml}^{-1}$ for 80 s. C1A-B12 Fab or CR3022-Fab was associated at 250 nM or buffer for 180 s followed by an association with ACE2-Fc or CR3022 Fab at a concentration of 250 nM for 180 s. Complexes were allowed to dissociate for 180 s.

Crystallization and Structure Determination

Each Fab:SARS-CoV-2 RBD complex was prepared by mixing RBD with 1.5 molar excess of Fab. The mixtures were incubated at 4°C for 1 h prior to purification on a Superdex 200 Increase column (GE Healthcare Life Sciences) in buffer containing 150 mM NaCl, 25 mM Tris-HCl, pH 7.5. Each complex co-eluted as a single peak at expected retention volume. The concentration of each complex was adjusted to 13 mg ml^{-1} and screened for crystallization conditions in hanging drops containing $0.1 \mu\text{l}$ of protein and $0.1 \mu\text{l}$ of mother liquor using a Mosquito protein crystallization robot (SPT Labtech) with commercially available screens (Hampton Research). Crystals grew within 24 h for the C1A-B12 Fab:RBD complex in 0.1 M Bicine pH 8.5, 20% (w/v) polyethylene glycol 10,000, for the C1A-B3 Fab:RBD complex in 0.2 M Ammonium phosphate dibasic, 20% (w/v) polyethylene glycol 3,350; for the C1A-C2 Fab:RBD complex in 0.03 M citric acid, 0.07M BIS-TRIS propane pH 7.6, 20% (w/v) polyethylene glycol 3,350, and for C1A-F10 Fab:RBD complex in 0.10% (w/v) n-Octyl-B-glucoside, 0.1 M Sodium citrate tribasic dihydrate pH 4.5, and 22% (w/v) polyethylene glycol 3,350.

All crystals were flash frozen in mother liquor supplemented with 15% (v/v) glycerol as cryoprotectant. Single crystal X-ray diffraction data was collected on Eiger X 16M pixel detectors (Dectris) at a wavelength of $0.979180 \text{ \AA}$ at the Advanced Photon Source (APS, Argonne, IL) NE-CAT beamline 24-ID-E for the C1A-B12 Fab:RBD and C1A-B3 Fab:RBD complexes and NE-CAT beamline 24-ID-C for the C1A-C2 Fab:RBD and C1A-F10 Fab:RBD complexes. Diffraction data were indexed and integrated using XDS (build 202 00131)³⁶ and merged using AIMLESS (v0.5.32)³⁷. The structure of C1A-B12 Fab:RBD

(space group $P2_12_12_1$) was determined by molecular replacement using Phaser (v2.8.3)³⁸, with coordinates for the B38 Fab variable domain, constant domain and RBD (PDB ID: 7BZ5¹) used as search models. Three copies were found in the asymmetric unit (ASU). Iterative modeling was performed using O³⁹ and refinement in Phenix (v1.18.2-3874)⁴⁰ and
 5 Buster (v2.10.3)⁴¹, during which alternative conformations were also built where density was apparent. During refinements, TLS groups calculated using PHENIX⁴⁰ and a python script were updated, as well as occupancy restraints calculated in Buster. During model building, geometry restraints were also customized to prevent large displacement of unambiguous
 10 molecules were automatically picked and updated in Buster, followed by manual examination and adjustment till late stage refinement. The structures of C1A-B3:RBD (space group $P2_12_12_1$, 3 copies per ASU), C1A-C2:RBD (space group $C222_1$, 1 copy per ASU) and C1A-F10:RBD (space group $C222_1$, 1 copy per ASU) were determined using RBD and the C1A-B12 Fab variable and constant domains as search ensembles with CDR and flexible loops
 15 truncated, with iterative model building and refinement as described above. Data collection, processing and refinement statistics are summarized in **FIG. 17**. Structures and figures were analyzed and generated using PyMOL (Schrödinger).

Protein Data Bank (PDB) identification numbers

20 Protein Data Bank (PDB) identification numbers for the C1A-B3/RBD, C1A-F10/RBD, C1A-C2/RBD, and C1A-B12 RBD complexes are 7KFW, 7KFY, 7KFX, and 7KFV, respectively.

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We claim:

1. An isolated antigen-binding protein that binds specifically to a coronavirus, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.
2. An isolated antigen-binding protein that binds specifically to a coronavirus spike (S) protein, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.
3. An isolated antigen-binding protein capable of neutralizing a coronavirus, comprising three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2, and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences listed in Table 1, and three light chain CDRs (LCDR1, LCDR2, and LCDR3) contained within any one of the light chain variable region (LCVR) sequences listed in Table 1.
4. An isolated antigen-binding protein that binds specifically to a coronavirus, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.
5. An isolated antigen-binding protein that binds specifically to a coronavirus spike (S) protein, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.

6. An isolated antigen-binding protein capable of neutralizing a coronavirus, comprising a heavy chain variable region (HCVR) sequence having at least one amino acid modification as compared to any one of the HCVR sequences listed in Table 1, and/or a light chain variable region (LCVR) sequence having at least one amino acid modification as compared to any one of the light chain variable region (LCVR) sequences listed in Table 1.

7. The isolated antigen-binding protein of any one of claims 1-6, which binds specifically to a severe acute respiratory syndrome coronavirus (SARS-CoV) spike (S) protein.

8. The isolated antigen-binding protein of any one of claims 1-6, which binds specifically to a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein.

9. The isolated antigen-binding protein of any one of claims 1-8, wherein the coronavirus or coronavirus spike (S) protein comprises an amino acid sequence consisting of SEQ ID NO: 100, or an amino acid sequence comprising at least about 75%, 80%, 85%, 90%, 95%, or 99% sequence identity thereto.

10. The isolated antigen-binding protein of any one of claims 1-9, wherein the coronavirus or the coronavirus spike (S) protein comprises a neutralizing antibody escape mutation.

11. The isolated antigen-binding protein of any one of claims 1-10, wherein the coronavirus or coronavirus (S) protein comprises at least one amino acid modification as compared to the SARS-CoV-2 spike (S) protein sequence of SEQ ID NO: 100.

12. The isolated antigen-binding protein of claim 11, wherein the coronavirus spike (S) protein comprises a Y114del mutation, a L242del mutation, a A243del mutation, a L244del mutation, a D614G mutation, a K417N mutation, a N440D mutation, a Y453F mutation, a T478K mutation, a E484K mutation, a E484A mutation, a F486I mutation, a F486L mutation, a Y489H mutation, a Q493K mutation, a Q493R mutation, a S494P mutation, and/or a N501Y mutation.

13. The isolated antigen-binding protein of any one of claims 1-12, wherein said HCVR sequence comprises an amino acid sequence selected from the group consisting of

SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314, 315, and 316; and/or wherein said LCVR sequence comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332.

14. The isolated antigen-binding protein of any one of claims 1-13, comprising:

(A)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:15;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:16;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:17;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:18;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:19; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:20;

(B)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:21;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:22;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:23;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:24;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:25; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:26;

(C)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:27;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:28;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:29;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:30;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:31; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:32;

(D)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:33;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:34;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:35;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:36;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:37; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:38;

(E)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:39;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:40;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:41;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:42;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:43; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:44;

(F)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:45;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:46;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:47;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:48;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:49; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:50;

(G)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:51;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:52;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:53;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:54;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:55; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:56;

(H)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:199;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:200;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:201;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:202;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:203; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:204; or

(I)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:205;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:206;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:207;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:208;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:209; and

- vi. a LCDR3 having the sequence set forth in SEQ ID NO:210;

(J)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:211;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:212;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:213;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:214;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:215; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:216;

(K)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:217;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:218;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:219;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:220;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:221; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:222; or

(L)

- i. a HCDR1 having the sequence set forth in SEQ ID NO:223;
- ii. a HCDR2 having the sequence set forth in SEQ ID NO:224;
- iii. a HCDR3 having the sequence set forth in SEQ ID NO:225;
- iv. a LCDR1 having the sequence set forth in SEQ ID NO:226;
- v. a LCDR2 having the sequence set forth in SEQ ID NO:227; and
- vi. a LCDR3 having the sequence set forth in SEQ ID NO:228.

15. The isolated antigen-binding protein of any one of claims 1-14, comprising:

(i) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314, 315, and 316, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto; and/or

(ii) a LCVR sequence having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332, or an amino acid sequence having at least about 85%, 90%, 95%, or 99% sequence identity thereto.

16. The isolated antigen-binding protein of any one of claims 1-14, comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 112, 113, 314,

315, and 316, wherein the at least one amino acid modification alters the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein and/or wherein the at least one amino acid modification alters the neutralization potency of the isolated antigen-binding protein.

17. The isolated antigen-binding protein of claim 16, wherein the at least one amino acid modification occurs at residue 23, 24, 25, 26, 27, 28, 31, 56, 58, 74, 77, 78, 79, 100 and/or 100a of the HCVR sequence.

18. The isolated antigen-binding protein of claim 17, wherein the at least one amino acid modification comprises A23V, A24V, S25A, G26E, F27V, F27L, F27I, T28I, S31N, S31R, S56T, S56A, A56T, Y58F, S74P, T77M, T77I, L78V, Y79F, S100aR, and/or S100aK.

19. The isolated antigen-binding protein of claim 16, comprising a HCVR sequence having at least one amino acid modification as compared to a HCVR sequence of SEQ ID NO: 5.

20. The isolated antigen-binding protein of claim 19, wherein the at least one amino acid modification is selected from the group consisting of A24V, F27I, F27V, T28I, S31R, S31N, and A56T.

21. The isolated antigen-binding protein of any one of claims 1-19, comprising a LCVR sequence having at least one amino acid modification as compared to a LCVR sequence selected from the group consisting of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 128, 129, 330, 331, and 332, wherein the at least one amino acid modification alters the binding affinity of the isolated antigen-binding protein for the coronavirus or coronavirus spike (S) protein and/or wherein the at least one amino acid modification alters the neutralization potency of the isolated antigen-binding protein.

22. The isolated antigen-binding protein of claim 21, wherein the at least one amino acid modification occurs at residue 10, 14, 27, 42, 50, 52, 55, 56, 70, 85, 91, 92, and/or 93 of the LCVR sequence.

23. The isolated antigen-binding protein of claim 22, wherein the at least one amino acid modification comprises T10S, S14F, Q27E, K42N, A50G, S52T, Q55E, S56N, E70D, T85S, L91V, N92I, and/or S93D.
24. The isolated antigen-binding protein of claim 23, comprising a LCVR sequence having at least one amino acid modification as compared to a LCVR sequence of SEQ ID NO: 12.
25. The isolated antigen-binding protein of claim 20, wherein the at least one amino acid modification comprises N92I.
26. The isolated antigen-binding protein of any one of claims 1-25, which binds to an epitope on the SARS-CoV-2 receptor binding domain (RBD) comprising any one of residues 319-541.
27. The isolated antigen-binding protein of any one of claims 16-26, wherein the at least one amino acid modification increases the binding affinity of the isolated antigen-binding protein for the SARS-CoV-2 receptor binding domain (RBD) and/or increases the neutralization potency of the isolated antigen-binding protein.
28. The isolated antigen-binding protein of any one of claims 16-27, wherein the at least one amino acid modification occurs at the binding interface between the isolated antigen-binding protein and the coronavirus or the coronavirus spike (S) protein.
29. The isolated antigen-binding protein of claim 27, wherein the binding affinity and/or neutralization potency is increased by about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% or more.
30. The isolated antigen-binding protein of any one of claims 16-29, wherein the isolated antigen-binding protein specifically binds to the SARS-CoV-2 receptor binding domain (RBD) with an affinity of about 0.66 nM.
31. The isolated antigen-binding protein of any one of claims 1-29, comprising:
- (i) a HCVR having an amino acid sequence of SEQ ID NO: 1 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 8;
 - (ii) a HCVR having an amino acid sequence of SEQ ID NO: 2 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 9;

- (iii) a HCVR having an amino acid sequence of SEQ ID NO: 3 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 10;
- (iv) a HCVR having an amino acid sequence of SEQ ID NO: 4 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 11;
- (v) a HCVR having an amino acid sequence of SEQ ID NO: 5 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 12;
- (vi) a HCVR having an amino acid sequence of SEQ ID NO: 6 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 13;
- (vii) a HCVR having an amino acid sequence of SEQ ID NO: 7 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 14;
- (viii) a HCVR having an amino acid sequence of SEQ ID NO: 112 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 128;
- (ix) a HCVR having an amino acid sequence of SEQ ID NO: 113 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 129;
- (x) a HCVR having an amino acid sequence of SEQ ID NO: 314 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 330;
- (xi) a HCVR having an amino acid sequence of SEQ ID NO: 315 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 331; or
- (xii) a HCVR having an amino acid sequence of SEQ ID NO: 316 and a LCVR sequence having an amino acid sequence of SEQ ID NO: 332.

32. The isolated antigen-binding protein of any one of claims 1-31, which is a IGHV3-53/IGKV1-9-derived antibody.

33. The isolated antigen-binding protein of any one of claims 1-32, which is a germline revertant antibody.

34. The isolated antigen-binding protein of claim 33, wherein the germline revertant antibody is C1A-gl or C1A-gl*.

35. The isolated antigen-binding protein of any one of claims 1-34, wherein the antigen-binding protein is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fd, an Fv, a single chain Fv (scFv), a single-domain antibody (sdAb), a diabody, a triabody, a tetrabody, a minibody, or a domain antibody.

36. The isolated antigen-binding protein of any one of claims 1-35, wherein the antigen-binding protein is a human monoclonal antibody or an antigen-binding fragment thereof.

37. The isolated antigen-binding protein of any one of claims 1-36, wherein the antigen-binding protein is a multi-specific antibody.

38. The isolated antigen-binding protein of claim 37, wherein the multi-specific antibody is a bi-specific antibody.

39. The isolated antigen-binding protein of claim 37, wherein the multi-specific antibody is a tri-specific antibody.

40. The isolated antigen-binding protein of any one of claims 1-36, wherein the antigen-binding protein is selected from the group consisting of human monoclonal antibody C2.0, C2.1, C2.2, C2.3, C2.4, C2.5, and C2.6.

41. The isolated antigen-binding protein of any one of claims 1-36, wherein the antigen-binding protein is a human monoclonal C2.1 antibody or an antigen-binding fragment thereof.

42. The isolated antigen-binding protein of any one of claims 1-36, wherein the antigen-binding protein is a human monoclonal C2.2 antibody or an antigen-binding fragment thereof.

43. The isolated antigen-binding protein of any one of claims 1-36, wherein the antigen-binding protein is a human monoclonal C2.4 antibody or an antigen-binding fragment thereof.

44. The isolated antigen-binding protein of any one of claims 1-43, wherein the antigen-binding protein binds specifically to the S1 subunit of the coronavirus spike (S) protein.

45. The isolated antigen-binding protein of claim 44, wherein the antigen-binding protein binds specifically to the receptor binding domain (RBD), N-terminal domain (NTD), or C-terminal domain (CTD) of the spike protein subunit 1 (S1).

46. The isolated antigen-binding protein of any one of claims 1-45, wherein the antigen-binding protein binds specifically to an epitope within a highly conserved region of the coronavirus or coronavirus (S) protein that is not protected by glycosylation and/or conformational masking.

47. The isolated antigen-binding protein of claim 46, wherein the antigen-binding protein binds specifically to the N-terminal domain (NTD) of the spike protein subunit 1 (S1) or to the spike protein subunit 2 (S2) of the coronavirus spike (S) protein.

48. The isolated antigen-binding protein of any one of claims 1-45, wherein the antigen-binding protein binds specifically to the receptor binding domain (RBD) of the spike protein subunit 1 (S1).

49. The isolated antigen-binding protein of any one of claims 1-48, wherein the antigen-binding protein binds specifically to the SARS-CoV-2 spike protein (S; SEQ ID NO: 100).

50. The isolated antigen-binding protein of claim 44, wherein the spike protein subunit 1 (S1) is in the “pre-fusion” conformation (“S2P”).

51. The isolated antigen-binding protein of claim 44, wherein the receptor binding domain (RBD) of the spike protein subunit 1 (S1) is in the “down” or “up” configuration.

52. The isolated antigen-binding protein of claim 51, wherein the receptor binding domain (RBD) of the spike protein subunit 1 (S1) is in the “up” configuration.

53. The isolated antigen-binding protein of any one of claims 1-52, wherein the antigen-binding protein is active against circulating SARS-CoV-2 variants and/or against high-risk bat coronaviruses.

54. The isolated antigen-binding protein of any one of claims 1-53, wherein the antigen-binding protein binds specifically to the coronavirus or coronavirus spike (S) protein at both a physiological pH of about 7.0 and at an acidic/endosomal pH of about 6.5 to about 4.5.

55. The isolated antigen-binding protein of any one of claims 1-54, wherein the antigen-binding protein inhibits coronavirus spike (S) protein binding to angiotensin converting enzyme 2 (ACE2).

56. The isolated antigen-binding protein of claim 55, wherein the antigen-binding protein inhibits the binding of coronavirus spike protein subunit 1 (S1) to ACE2.

57. The isolated antigen-binding protein of claim 55 or 56, wherein the antigen-binding protein competitively inhibits SARS-CoV-2 binding to ACE2.

58. The isolated antigen-binding protein of any one of claims 1-57, wherein the antigen-binding protein is capable of inhibiting viral fusion with and/or viral entry into a cell.

59. The isolated antigen-binding protein of claim 58, wherein the cell is an ACE2-expressing cell.

60. The isolated antigen-binding protein of any one of claims 1-59, which neutralizes SARS-CoV-2 with an IC_{50} of about 62 ng/ml to 440 ng/ml as measured by a plaque reduction neutralization test (PRNT).

61. The isolated antigen-binding protein of any one of claims 1-60, which neutralizes SARS-CoV-2 pseudotype with greater than about 90% reduction in entry at a concentration of $100 \mu\text{g ml}^{-1}$.

62. The isolated antigen-binding protein of any one of claims 1-61, which
5 neutralizes SARS-CoV-2 pseudotype with IC_{50} values ranging from about 0.008 to $0.671 \mu\text{g ml}^{-1}$ in a dose response pseudotype neutralization assay.

63. The isolated antigen-binding protein of any one of claims 1-62, which neutralizes infectious SARS-CoV-2 with an IC_{50} value of less than $1 \mu\text{g ml}^{-1}$.

64. The isolated antigen-binding protein of any one of claims 1-63, which specifically bind the SARS-CoV-2 receptor binding domain (RBD) with an affinity of about 9 nM to about 76 nM.

65. The isolated antigen-binding protein of any one of claims 1-64, which is cross-reactive to 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East

Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and/or variants thereof.

66. A pharmaceutical composition comprising an isolated antigen-binding protein according to any one of claims 1-65 and a pharmaceutically acceptable carrier or diluent.

67. A pharmaceutical composition comprising at least two isolated antigen-binding protein according to any one of claims 1-65 and a pharmaceutically acceptable carrier or diluent.

68. The pharmaceutical composition of claim 67, which comprises three or more, four or more, or five or more isolated antigen-binding protein according to any one of claims 1-65.

69. The pharmaceutical composition of claim 67 or 68, further comprising additional therapeutic agent.

70. The pharmaceutical composition of any one of claims 67-69, wherein the at least two isolated antigen-binding proteins specifically bind to non-competing epitopes on the same or different coronaviruses or coronavirus spike (S) proteins.

71. The pharmaceutical composition of any one of claims 67-70, wherein the at least two isolated antigen-binding proteins independently bind to a neutralizing epitope or a non-neutralizing epitope on the same or different coronaviruses or coronavirus S proteins.

72. The pharmaceutical composition of any one of claims 67-71, wherein the at least two isolated antigen-binding proteins are independently selected from the groups consisting of an isolated antigen-binding protein that

- (i) is cross-reactive to more than one coronaviruses or variant thereof,
- (ii) cross-neutralizes more than one strain of a coronavirus,
- (iii) specifically binds to a coronavirus spike (S) protein,
- (iv) specifically binds to a receptor binding domain (RBD) of the spike protein subunit 1 (S1),
- (v) specifically binds to a N-terminal domain (NTD) of the spike protein subunit 1 (S1),

- (vi) specifically binds to a C-terminal domain (CTD) of the spike protein subunit 1 (S1),
- (vii) specifically binds to a spike protein subunit 2 (S2),
- (viii) destabilizes the prefusion conformation of a coronavirus spike (S) protein,
- (ix) specifically binds a non-receptor binding domain (RBD) neutralizing epitope,
- (x) specifically binds a receptor binding domain (RBD) neutralizing epitope,
- (xi) competes with binding to ACE2, and
- (xii) does not compete with binding to ACE2.

73. The pharmaceutical composition of claim 72, wherein the coronavirus is selected from the group consisting of 229E (alpha coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus), HKU1 (beta coronavirus), MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS), SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS), SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19), and variants thereof.

74. An isolated polynucleotide molecule comprising a polynucleotide sequence that encodes an antigen-binding protein of any one of claims 1-65.

75. A vector comprising the polynucleotide molecule of claim 74.

76. A cell expressing the isolated polynucleotide molecule of claim 74 or the vector of claim 75.

77. A method of treating or preventing a coronavirus infection in a subject, the method comprising administering to the subject an effective amount of an antigen-binding protein of any one of claims 1-65, or the pharmaceutical composition of any one of claims 66-73.

78. A method of preventing SARS-CoV-2 transmission comprising administering to the subject an effective amount of an antigen-binding protein of any one of claims 1-65, or the pharmaceutical composition of any one of claims 66-73, thereby inhibiting viral replication.

79. A method of providing broad spectrum immunity against circulating SARS-CoV-2 variants and high-risk bat coronaviruses comprising administering to the subject an

effective amount of an antigen-binding protein of any one of claims 1-65, or the pharmaceutical composition of any one of claims 66-73.

80. The method of claim 77, wherein the coronavirus infection is an infection by a SARS-CoV-2 virus.

81. The method of any one of claims 77-80, wherein the subject has, or is at risk of having, COVID-19.

82. The method of claim 81, wherein the antigen-binding protein is administered to the subject prior to onset of one or more manifestations of COVID-19.

83. The method of claim 81, wherein the antigen-binding protein is administered to the subject after the subject exhibits one or more manifestations of COVID-19.

84. The method of any one of claims 81-83, wherein the method results in the amelioration of one or more manifestations of COVID-19.

85. The method of any one of claims 82-84, wherein the one or more manifestations of COVID-19 is selected from the group consisting of fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle ache, body ache, headache, new loss of taste, new loss of smell, sore throat, congestion, runny nose, nausea, vomiting, and diarrhea.

86. The method of any one of claims 77-85, wherein the method results in passive immunity to a SARS-CoV-2 infection.

87. The method of claim 86, wherein the passive immunity lasts for at least about 1 week to about 2 weeks, at least about 1 month to about 3 months, at least about 3 months to about 6 months, or at least about 6 months to about 12 months.

88. The method of any one of claims 77-87, wherein the method results in a reduction in the level of viral entry.

89. The method of claim 88, wherein the method results in a reduction of at least about 80%, 85%, 90%, 95%, 99% or 100% as compared to a control level.

90. The method of any one of claims 77-89, wherein the method results in a reduction in the level of viral titer in the subject.

91. The method of claim 90, wherein the method results in a reduction of at least about 80%, 85%, 90%, 95%, 99% or 100% as compared to a control level.

92. The method of any one of claims 77-87, wherein the method results in a reduction in the level of SARS-CoV-2 viral RNA in the subject.

93. The method of claim 92, wherein the method results in a reduction of at least about 80%, 85%, 90%, 95%, 99% or 100% as compared to a control level.

94. The method of any one of claims 77-93, wherein the antigen-binding protein is administered intranasally, intravenously, intramuscularly, or subcutaneously.

95. The method of claim 94, wherein the antigen-binding protein is administered as a transfusion of convalescent plasma.

96. The method of any one of claims 77-95, wherein the antigen-binding protein is administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days after viral shedding is first detected in a sample from the subject.

97. The method of claim 96, wherein the antigen-binding protein is administered after prophylactic and/or therapeutic antibody administration.

98. The method of claim 96 or 97, wherein the antigen-binding protein is administered in combination with an additional therapeutic agent.

99. The method of claim 98, wherein the additional therapeutic agent comprises a small molecule drugs targeting a viral enzyme.

100. The method of claim 99, wherein the viral enzyme comprises a al RNA-dependent RNA polymerase and/or a viral protease

101. The method of any one of claims 77-100, wherein the antigen-binding protein is administered at a dosage of about 10 mg/kg to 150 mg/kg of recipient body weight.

102. The method of any one of claims 77-101, wherein the subject is at higher risk for severe COVID-19.

103. The method of claim 102, wherein:

(i) the subject is 65 years or older;

(ii) the subject is living in a nursing home or a long-term care facility;

(iii) the subject is a first-responder;

(iv) the subject is suffering from an underlying disease or condition selected from the group consisting of chronic lung disease, moderate to severe asthma, serious heart condition, cancer, poorly controlled HIV or AIDS, severe obesity (body mass index [BMI] of 40 or higher), diabetes, chronic kidney disease undergoing dialysis, and liver disease;

(v) the subject is receiving, has recently received, or is about to receive a cancer treatment, a bone marrow or organ transplantation, a corticosteroid, or other immune weakening treatment;

(v) the subject is a smoker; and/or

(iv) the subject is immunocompromised.

104. The method of any one of the preceding claims, wherein the method extends the subject's life span by at least about 30, 60, 90, 120, 180 or 360 days or more.

105. A method of producing antigen-binding protein capable of neutralizing a SARS-CoV-2 virus, the method comprising culturing a cell of claim 76 under conditions that allow production of an antigen-binding protein as set forth in any one of claims 1-65.

106. The method of claim 105, further comprising isolating or purifying the antigen-binding protein.

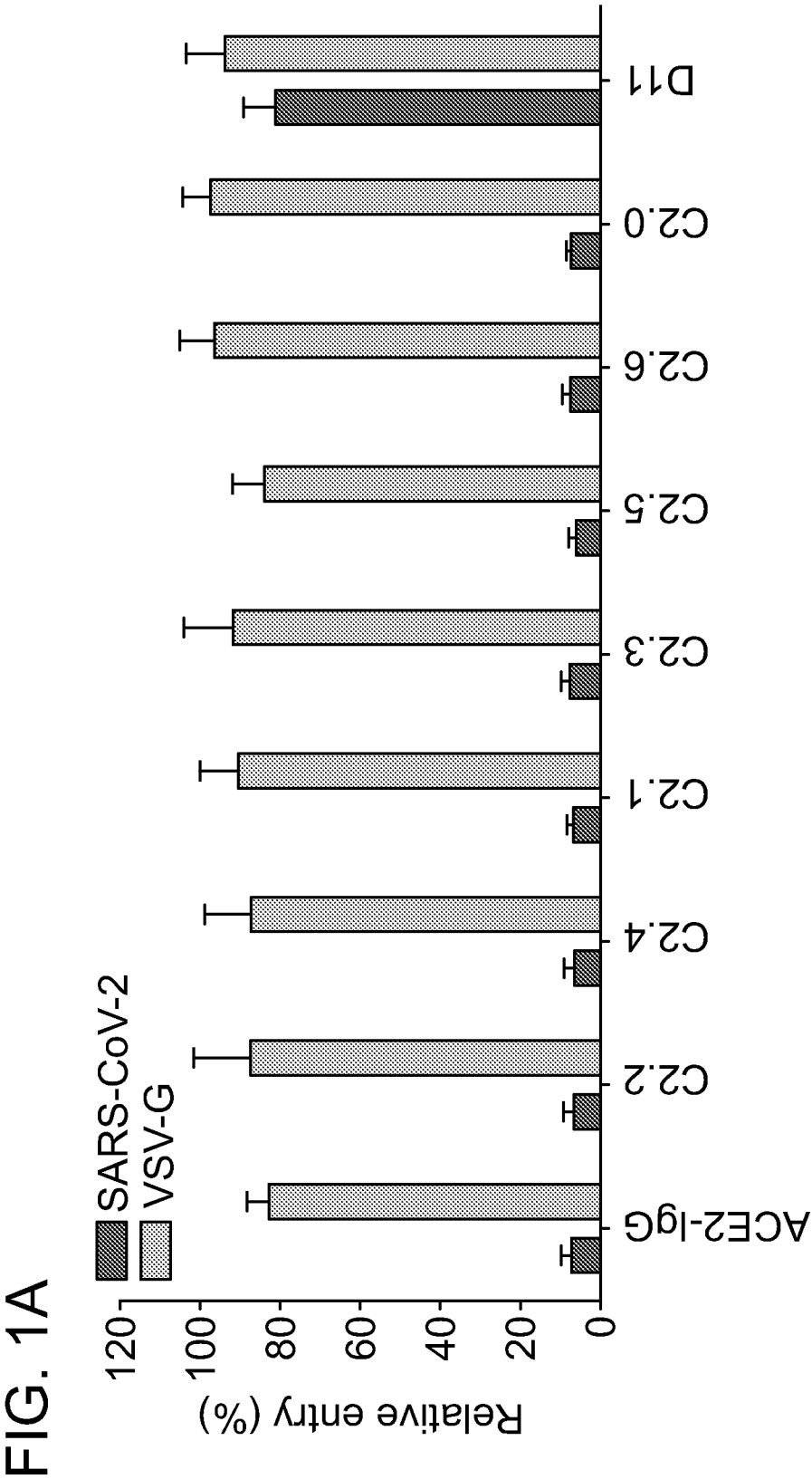


FIG. 1B

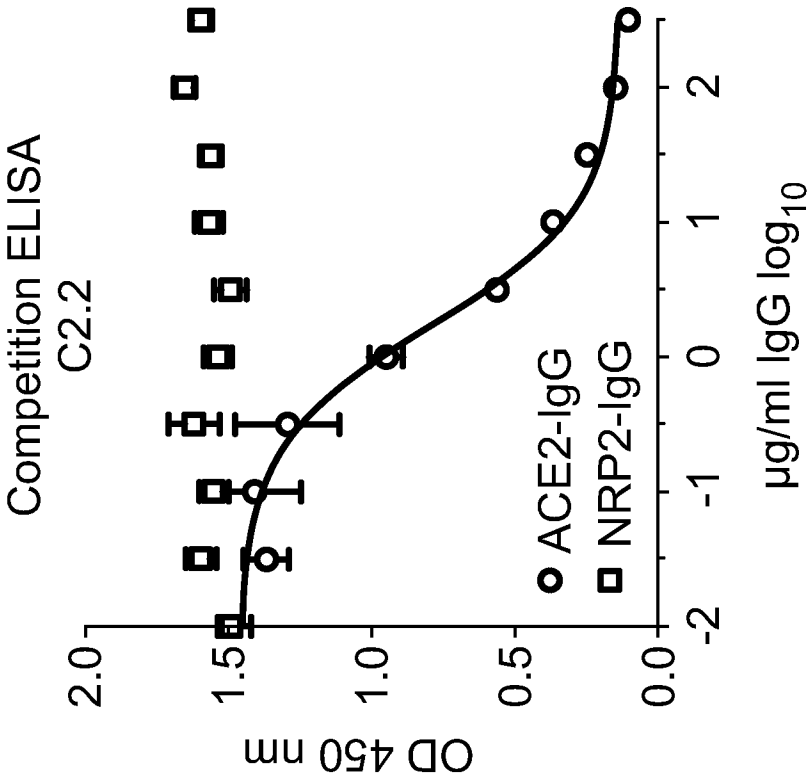
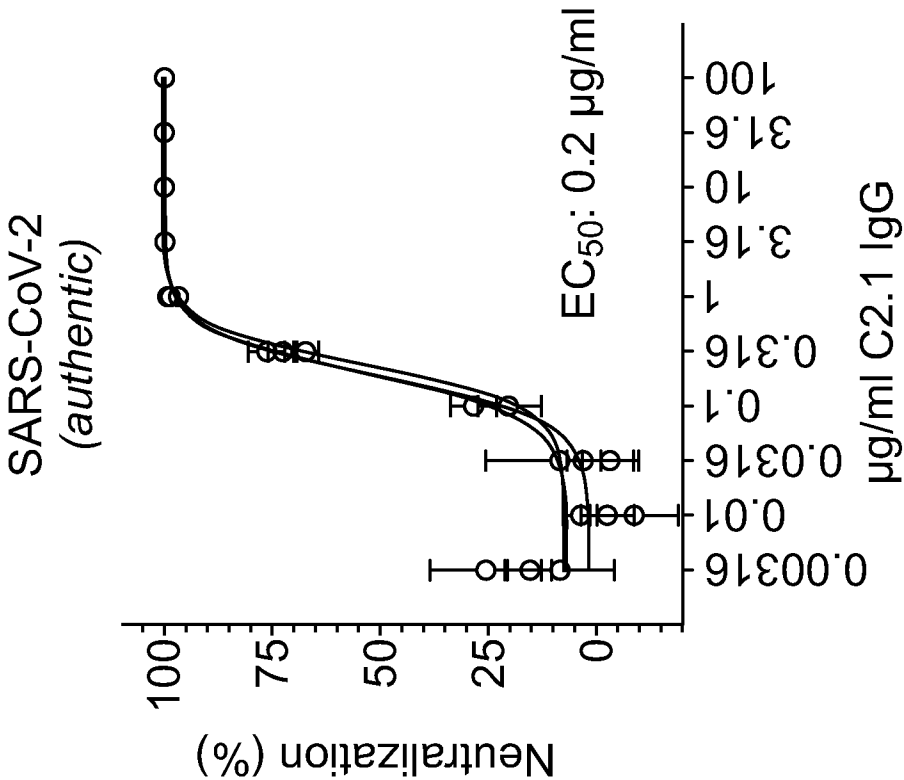


FIG. 1C



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FIG. 2A

Variable Heavy Chains

Input Sequences:

>B38_VH

EVQLVESGGGLVQPGGSLRLSCAASGFIVSSNYMSWVRQAPGKGLEWVSVIYSGGSTYYA
DSVKGRFTISRHN SKNTLYLQMNSLRAEDTAVYYCAREAYGMDVWGQGTTVTVS

>CC12.1_VH

EVQLVESGGGLIQPGGSLRLSCAASGLTVSSNYMSWVRQAPGKGLEWVSVIYSGGSTFYA
DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDLDVYGLDVWGQGTTVTVS

>CC12.3_VH

QVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGSTFYA
DSVKGRFTISRDN SKNTLYLQMNSLRVEDTAVYYCARDFGDFYFDYWGQGLTVTVS

>C2.0_VH

EVQLVESGGGLIQPGGSLRLSCVASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYKYGLDYWGQGLTVTVS

>C2.1_VH

EVQLVESGGGLIQPGGSLRLSCAVSGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

>C2.2_VH

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

>C2.3_VH

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN SKNLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

>C2.4_VH

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGATYYA
DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

>C2.5_VH

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

>C2.6_VH

EVQLVESGGGLIQPGGSLRLSCAAAGITVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYA
DSVKGRFTISRDN PKNMLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTVS

FIG. 2B **Variable Heavy Chains**

Alignment:

C2.6_VH	EVQLVESGGGLIQPGGSLRLSCAAAGITVSSNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
C2.2_VH	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
C2.1_VH	EVQLVESGGGLIQPGGSLRLSCAVSGFTVSNNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
C2.0_VH	EVQLVESGGGLIQPGGSLRLSCVASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
C2.4_VH	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGATYYA	60
C2.3_VH	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
C2.5_VH	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGTTYA	60
B38_VH	EVQLVESGGGLVQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGSTYYA	60
CC12.1_VH	EVQLVESGGGLIQPGGSLRLSCAASGLTVSSNYMSWVRQAPGKGLEWVSIYSGGSTFYA	60
CC12.3_VH	QVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSIYSGGSTFYA	60
	*****:*****.:*:**.*****:***	
C2.6_VH	DSVKGRFTISRDNPKNMLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.2_VH	DSVKGRFTISRDN SKNTVFLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.1_VH	DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.0_VH	DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.4_VH	DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.3_VH	DSVKGRFTISRDN SKNIIYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
C2.5_VH	DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS	119
B38_VH	DSVKGRFTISRHN SKNTLYLQMNSLRAEDTAVYYCARE-----AYGMDVWGQGTTVTS	114
CC12.1_VH	DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARDL---DVYGLDVWGQGTTVTS	116
CC12.3_VH	DSVKGRFTISRDN SKNTLYLQMNSLRVEDTAVYYCARDF---GDFYFDYWGQGLTVTS	116
	*****.* ** :.*****.*****.*****:***	

FIG. 2C

Percent Identity Matrix:

1: C2.6_VH	100.00	94.96	94.96	94.96	95.80	96.64	96.64	88.60	89.66	87.07
2: C2.2_VH	94.96	100.00	94.96	96.64	97.48	97.48	98.32	90.35	90.52	88.79
3: C2.1_VH	94.96	96.64	100.00	96.64	97.48	97.48	98.32	90.35	90.52	88.79
4: C2.0_VH	94.96	96.64	96.64	100.00	97.48	97.48	98.32	91.23	91.38	89.66
5: C2.4_VH	95.80	97.48	97.48	97.48	100.00	98.32	99.16	92.11	92.24	90.52
6: C2.3_VH	96.64	97.48	97.48	97.48	98.32	100.00	99.16	91.23	91.38	89.66
7: C2.5_VH	96.64	98.32	98.32	98.32	99.16	99.16	100.00	92.11	92.24	90.52
8: B38_VH	88.60	90.35	90.35	91.23	92.11	91.23	92.11	100.00	92.98	88.60
9: cc12.1_VH	89.66	90.52	90.52	91.38	92.24	91.38	92.24	92.98	100.00	90.52
10: cc12.3_VH	87.07	88.79	88.79	89.66	90.52	89.66	90.52	88.60	90.52	100.00

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FIG. 3A

Variable Light Chains

Input Sequences:

>B38

DIVMTQSPSFLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPPTYTFGQGTKLEIK

>CC12.1_VL

DIVMTQSPSFLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTQSGVPS
RFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPKFTFGPGTKVEIK

>CC12.3_VL

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPRTFGQGTKLEIK

>C2.0_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTESGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQLISYPGTFGQGTKLEIK

>C2.1_VL

DIQLTQSPSSLSAFVGDRVITTCRASEGISSYLAWYQQKPGNAPKLLIYAASLTESGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQLNSYPGTFGQGTKLEIK

>C2.2_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQLNSYPGTFGQGTKLEIK

>C2.3_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYGASTLTQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQVNDYPGTFGQGTKLEIK

>C2.4_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQLISYPGTFGQGTKLEIK

>C2.5_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYAASLTENGVP
RFSGSGSGTDFTLTISSLQPEDFATYYCQQLNSYPGTFGQGTKLEIK

>C2.6_VL

DIQLTQSPSSLSASVGDRVITTCRASQGISSYLAWYQQKPGKAPKLLIYGATTLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFASYCQQLNSYPGTFGQGTKLEIK

FIG. 3C

Percent Identity Matrix:

1: CC12.3_VL	100.00	71.96	73.83	73.83	73.83	73.83	73.83	73.83	73.83	73.83	72.64	70.09
2: C2.1_VL	71.96	100.00	93.46	93.46	93.46	93.46	93.46	96.26	96.26	96.26	91.51	88.79
3: C2.6_VL	73.83	93.46	100.00	96.26	96.26	96.26	96.26	95.33	95.33	95.33	92.45	89.72
4: C2.3_VL	73.83	93.46	96.26	100.00	100.00	95.33	97.20	95.33	97.20	95.33	92.45	89.72
5: C2.5_VL	73.83	96.26	95.33	95.33	100.00	100.00	98.13	98.13	98.13	98.13	93.40	90.65
6: C2.2_VL	73.83	96.26	97.20	97.20	98.13	100.00	100.00	98.13	99.07	99.07	95.28	92.52
7: C2.0_VL	73.83	96.26	95.33	95.33	98.13	98.13	98.13	100.00	99.07	99.07	93.40	90.65
8: C2.4_VL	73.83	95.33	96.26	96.26	97.20	99.07	99.07	99.07	100.00	100.00	94.34	91.59
9: B38	72.64	91.51	92.45	92.45	93.40	95.28	95.28	93.40	94.34	100.00	97.22	97.22
10: cc12.1_VL	70.09	88.79	89.72	89.72	90.65	92.52	92.52	90.65	91.59	97.22	100.00	100.00

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FIG. 4A

C2.0C2.0_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCVASGFTVSSNYMSWVRQAPGKGLEWVSYSGGTTYYADSVKGRFTISRD
 NSKNTLYLQMNSLRAEDTAVYYCARGDVSGYKYGLDYWGGQGTLLVTVS

C2.0_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLESQVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQGISYPGTFGQGTKLEIK

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FIG. 4B

C2.1C2.1_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAVSGFTVSNNYMSWVRQAPGKGLEWVSVIYSGGTTYADSVKGRFTISR
 DNSKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGGGTLVTVS

C2.1_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSAFVGDRVTITCRASEGISSYLAWYQQKPGNAPKLLIYAASTLESQVPSRFSGSGSGTDFLTIS
 SLQPEDFATYYCQQQLNSYPGTFGGQGTKLEIK

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FIG. 4C

C2.2C2.2_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGTTYYADSVKGRFTISRDN
 SKNTVFLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGGQGLTVTS

C2.2_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGGTDFTLTI
 SSLQPEDFATYVCQQLSYPGTFGQGTKLEIK

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FIG. 4D

C2.3C2.3_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWWSVYSGGTTYYADSVKGRFTISRDN
 NSKNILYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGGGTLVTVS

C2.3_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYGASTLQSGVPSRFSGSGSGTDFTLT
 SSLQPEDFATYYCQQVNDYPGTFGQGTKLEIK

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FIG. 4E

C2.4C2.4_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWWSVIYSGGTTYYADSVKGRFTISR
 DNSKNTLYQMNSLRAEDTAVYYCCARGDVSGYRYGLDYWGGGTLVTVS

C2.4_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLT
 SSLQPEDFATYVCQQQLISYPGTFGGQGTKLEIK

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FIG. 4F

C2.5C2.5_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWWSVIYSGGTTYYADSVKGRFTISRDN
 SKNTLYLQMNSLRAEDTAVYYCAARGDVSGYRYGLDYWGWQGQGLVTVS

C2.5_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLENGVPSRFSGSGSGTDFTLT
 ISSLQPEDFATYYCAQQLSNYPGTFGQGTKLEIK

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FIG. 4G

C2.6C2.6_VH: (CDRH1 CDRH2 CDRH3)

EVQLVESGGGLIQPGGSLRLSCAAGITVSSNYSWVRQAPGKGLEWVSVYSGGTTYYADSVKGRFTISRD
 NPKNMLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGGQGLTVTS

C2.6_VL: (CDRL1 CDRL2 CDRL3)

DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYGATTLQSGVPSRFSGSGGTDFTLT
 ISSLQPEDFASYCYCQQLNSYPGTFGQGTKLEIK

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FIG. 4H

>C1A-gI VH: (CDRH1 CDRH2 CDRH3)
 EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGSTYYADS
 VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGSYGLDYWGGQGLVTVS

>C1A-gI VL: (CDRL1 CDRL2 CDRL3)
 DIQLTQSPSFLSASVGDRTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSG
 SSGSGTEFTLTISSLQPEDFATYYCQQQLNSYPGTFGGQGTKLEIK

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FIG. 4I

>C1A-gI* VH: (CDRH1 CDRH2 CDRH3)
 EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKGLEWVSVIYSGGSTYYADS
 VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGGQGLVTVS

>C1A-gI* VL: (CDRL1 CDRL2 CDRL3)
 DIQLTQSPSFLSASVGDRVTTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPSRFSG
 SGSGTEFTLTISLQPEDFATYYCQQQLNSYPGTFGQGTKLEIK

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FIG. 4J

>C2.4opt1 VH: (CDRH1 CDRH2 CDRH3)
 EVQLVESGGGLIQPGLSLRLSCAVSGFTVSNNYMSWVRQAPGKGLEWWSVIYSGGTTYYADS
 VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS

>C2.4opt1 VL: (CDRL1 CDRL2 CDRL3)
 DIQLTQSPSSLSASVGDRTITCRASQGISSYLAWYQQKPKGKAPKLLIYAASTLQSGVP
 SRFSGSGGTDFLTITSSLPEDFATYCYCQQLISYPGTFGQGQTKLEIK

>C2.4opt2 VH: (CDRH1 CDRH2 CDRH3)
 EVQLVESGGGLIQPGLSLRLSCAVSGFIVSNNYMSWVRQAPGKGLEWWSVIYSGGTTYYADS
 VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS

>C2.4opt2 VL: (CDRL1 CDRL2 CDRL3)
 DIQLTQSPSSLSASVGDRTITCRASQGISSYLAWYQQKPKGKAPKLLIYAASTLQSGVP
 SRFSGSGGTDFLTITSSLPEDFATYCYCQQLISYPGTFGQGQTKLEIK

>C2.4opt3 VH: (CDRH1 CDRH2 CDRH3)
 EVQLVESGGGLIQPGLSLRLSCAVSGVIVSNNYMSWVRQAPGKGLEWWSVIYSGGTTYYADS
 VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGDVSGYRYGLDYWGQGLTVTS

>C2.4opt3 VL: (CDRL1 CDRL2 CDRL3)
 DIQLTQSPSSLSASVGDRTITCRASQGISSYLAWYQQKPKGKAPKLLIYAASTLQSGVP
 SRFSGSGGTDFLTITSSLPEDFATYCYCQQLISYPGTFGQGQTKLEIK

FIG. 5A

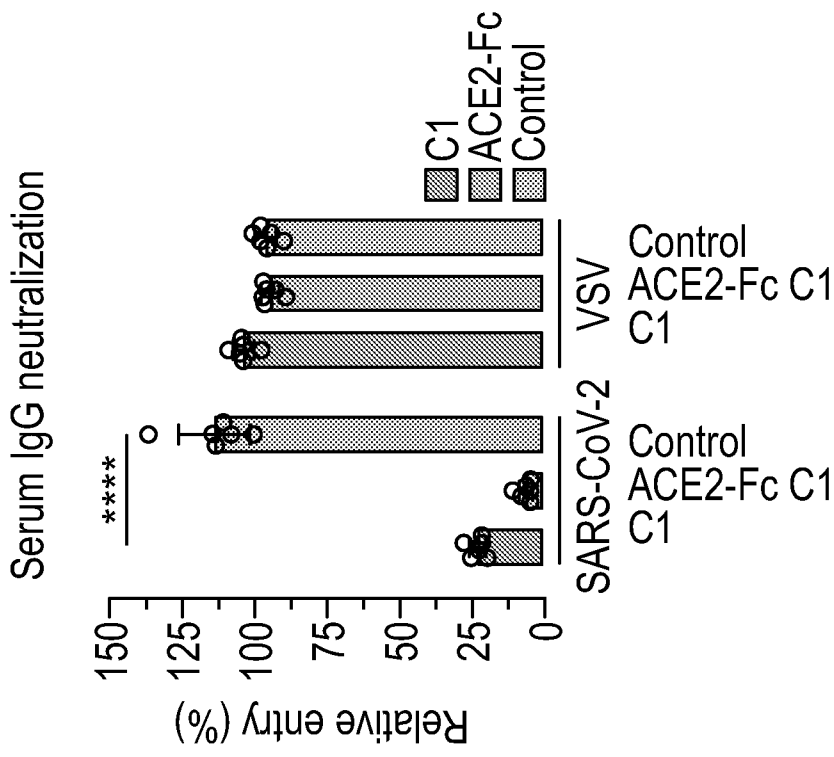


FIG. 5B

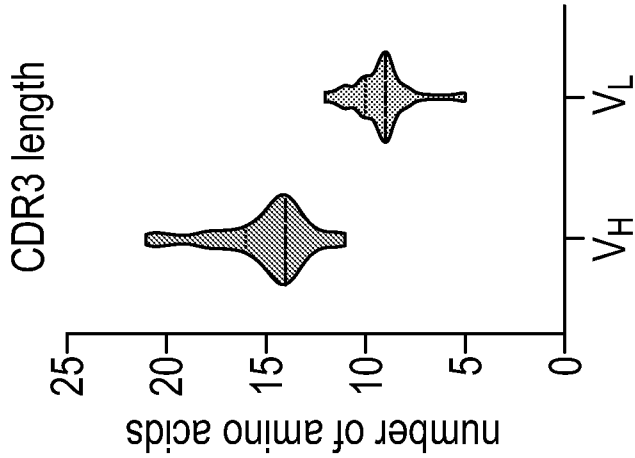


FIG. 5C

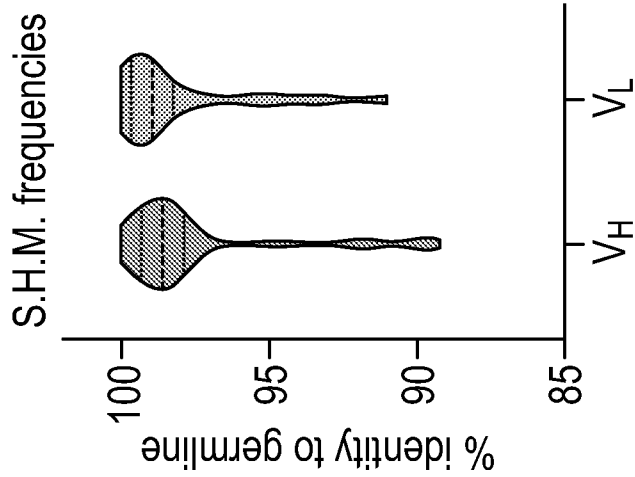


FIG. 5D

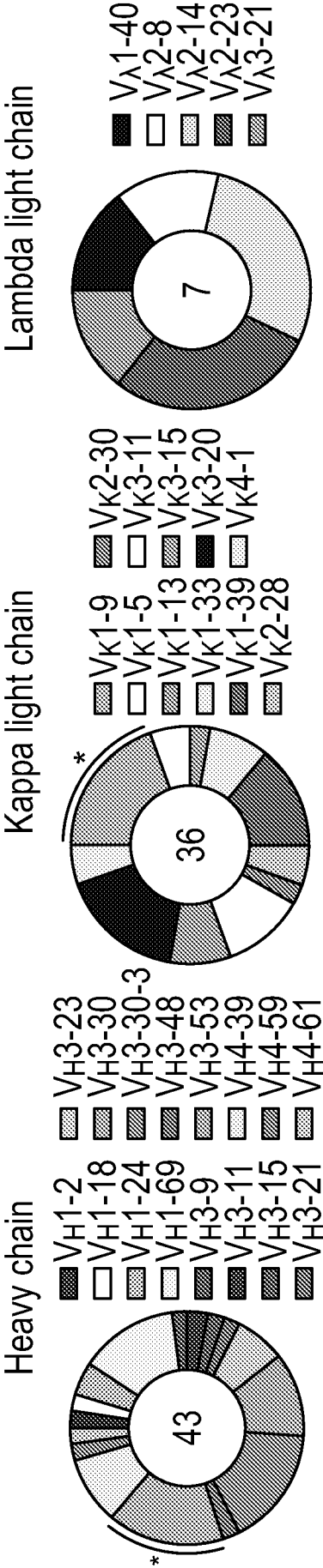


FIG. 5E

Summary of C1A-V_H3-53 antibody binding and neutralization data

Antibody	Number of a.a. changes (V _H , V _L)	Kinetic analysis			SARS-CoV-2 pseudotype	SARS-CoV-2 WA1/2020
		k _a (1/Ms)	k _d (1/S)	K _D (nM)		
C1A-gl	-	4.41E+04	5.58E-03	127	IC ₅₀ µg ml ⁻¹ 0.098*	IC ₅₀ µg ml ⁻¹ 0.126
C1A-gl	-	1.08E+05	4.95E-03	45.7	0.025*	0.102
C1A-B3	3, 2	1.04E+05	7.95E-03	76.3	0.053	0.441
C1A-F10	1, 4	4.52E+04	2.51E-03	55.7	0.008	0.184
C1A-C2	2, 6	1.70E+05	2.39E-03	14.1	0.118	0.132
C1A-H5	5, 5	7.27E+04	6.14E-04	8.5	0.139	0.256
C1A-C4	2, 5	5.23E+04	4.09E-04	7.8	0.046	0.127
C1A-B12	1, 3	8.61E+04	3.63E-04	4.2	0.081	0.062
C1A-H6	2, 4	1.13E+05	1.00E-04	0.9	0.072	0.112
C1A-B12.1	3, 3	1.60E+05	1.07E-04	0.7	0.054*	N.D.
C1A-B12.2	4, 3	1.69E+05	7.51E-05	0.5	0.115*	0.025
C1A-B12.3	5, 3	1.14E+05	6.23E-05	0.5	0.090*	0.032

* Measured with D614Gs lentivirus pseudotypes.
N.D.: Not determined.

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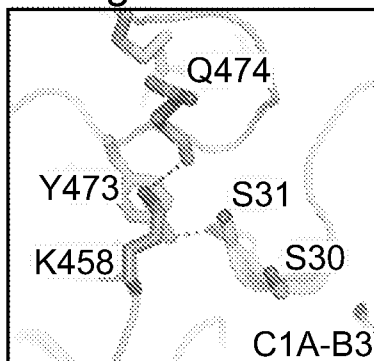
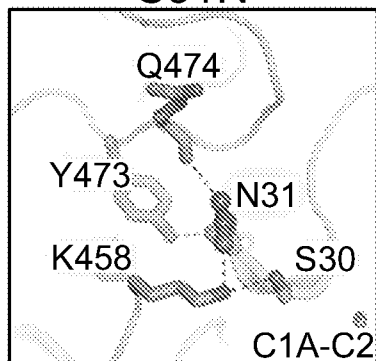
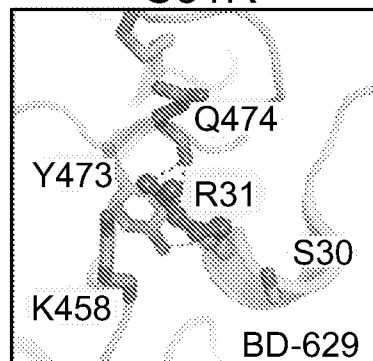
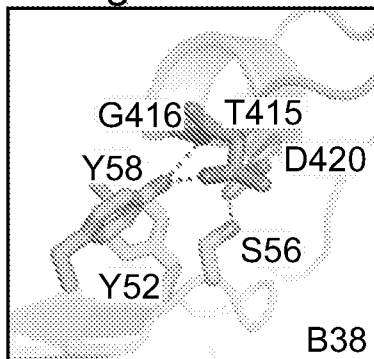
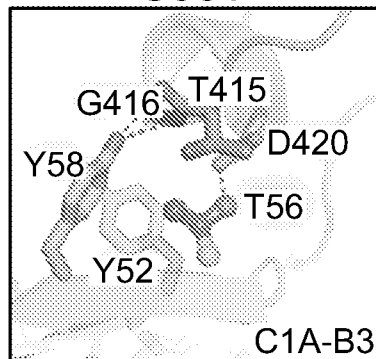
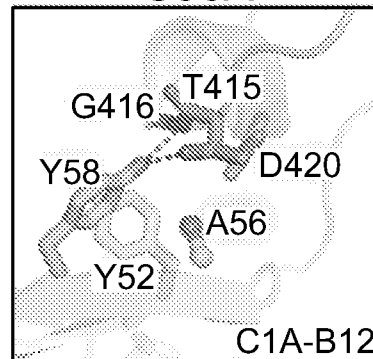
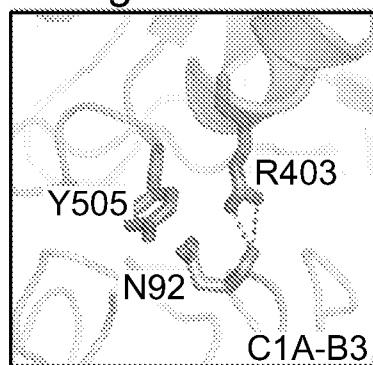
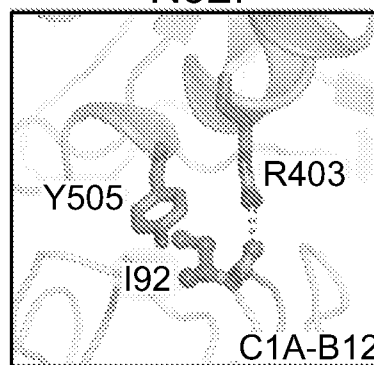
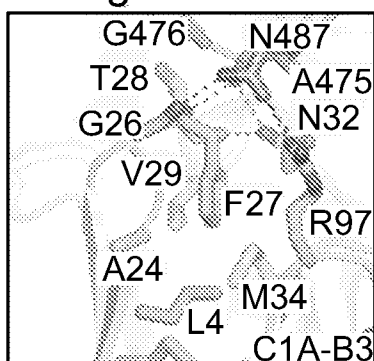
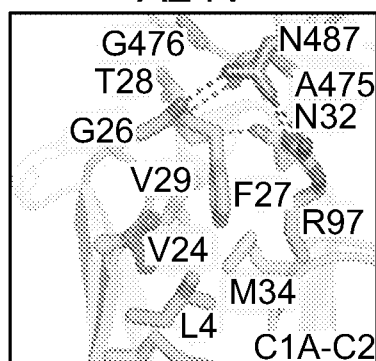
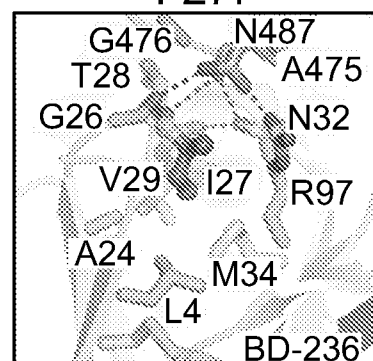
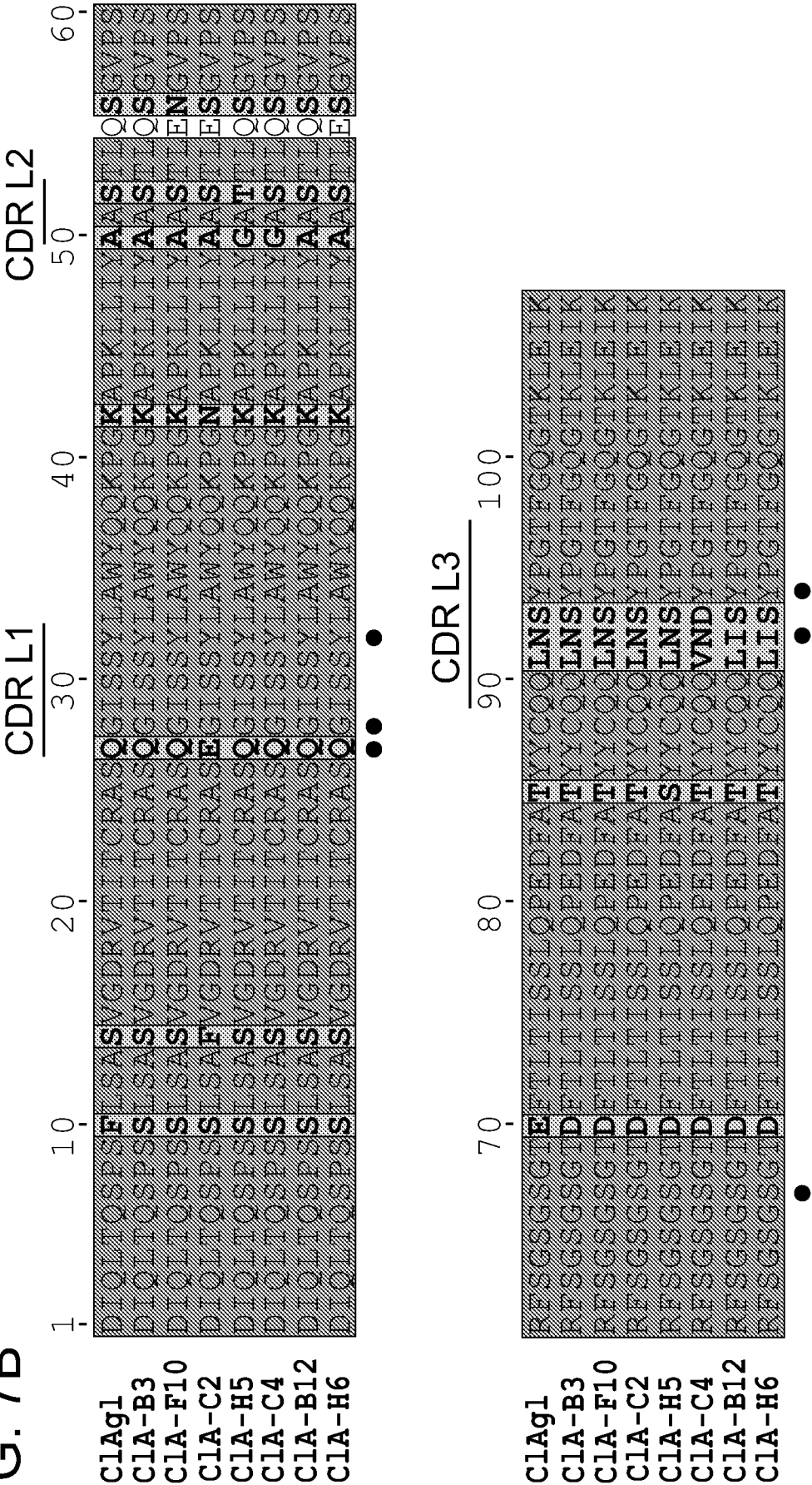
FIG. 6A
germline**FIG. 6B**
S31N**FIG. 6C**
S31R**FIG. 6D**
germline**FIG. 6E**
S56T**FIG. 6F**
S56A**FIG. 6G**
germline**FIG. 6H**
N92I**FIG. 6I**
germline**FIG. 6J**
A24V**FIG. 6K**
F27I

FIG. 7B



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FIG. 7C

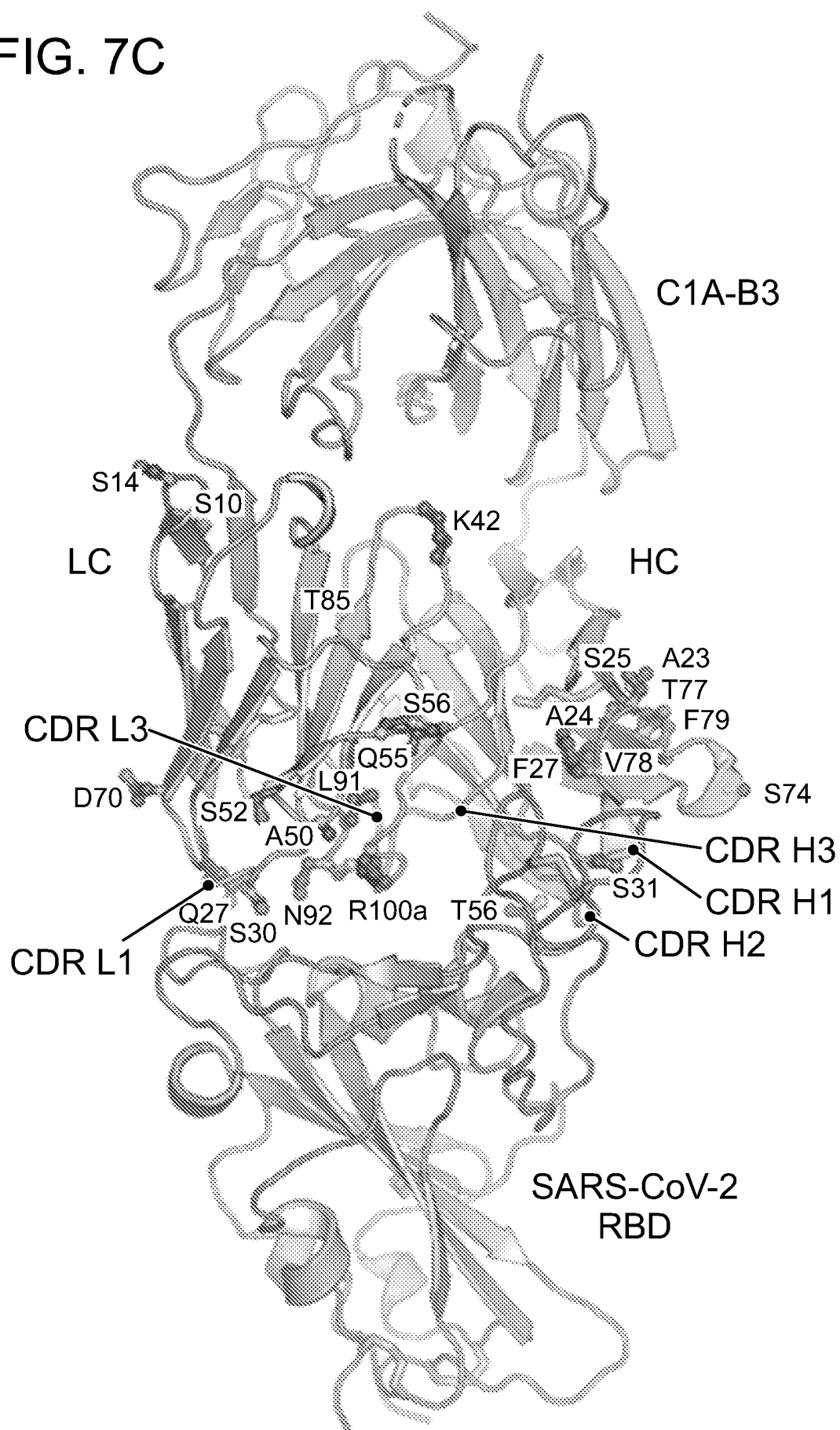
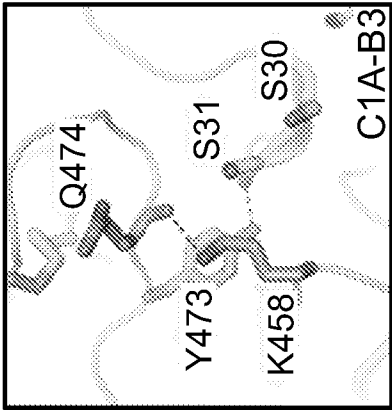
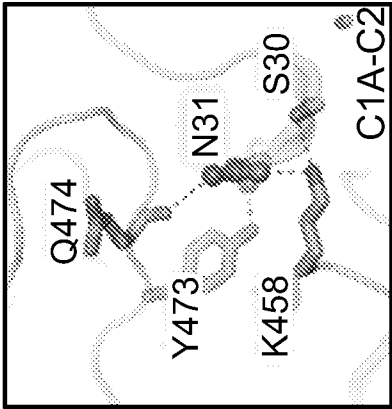


FIG. 7D

germline



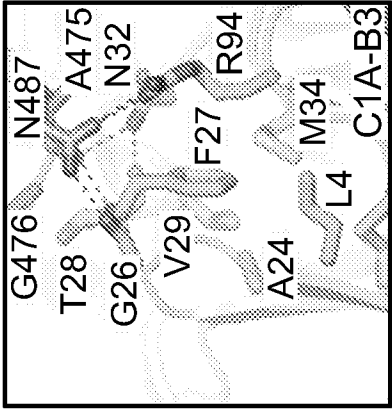
S31N



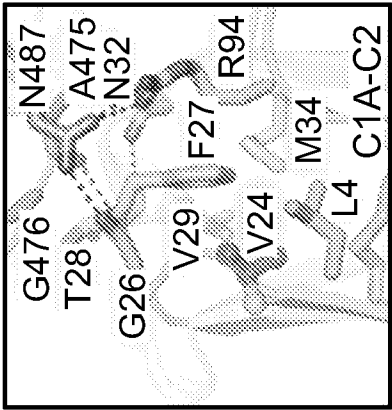
CDR H1

FIG. 7E

germline



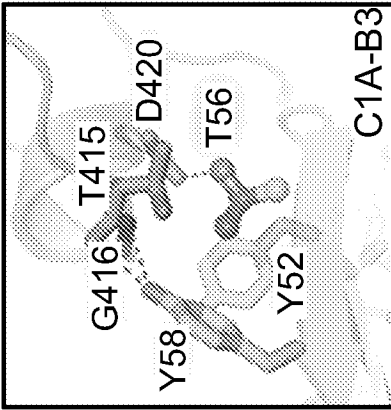
A24V



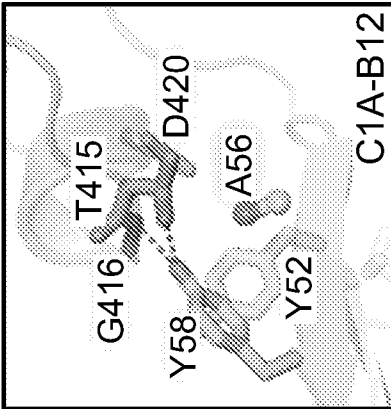
CDR H1/FWR1

FIG. 7F

S56T



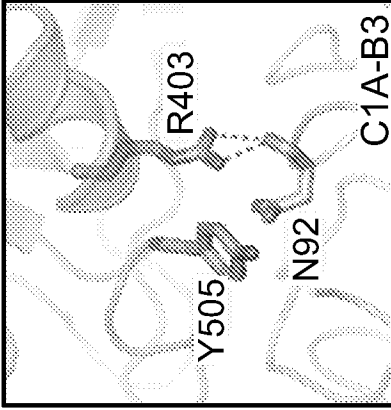
S56A



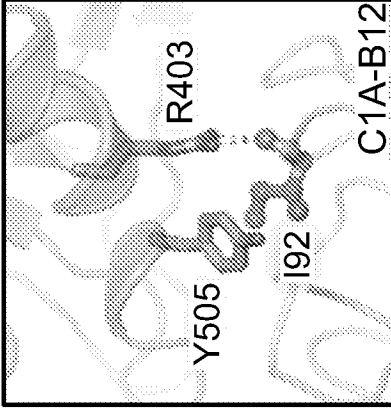
CDR H2

FIG. 7G

germline



N92I



CDR L3

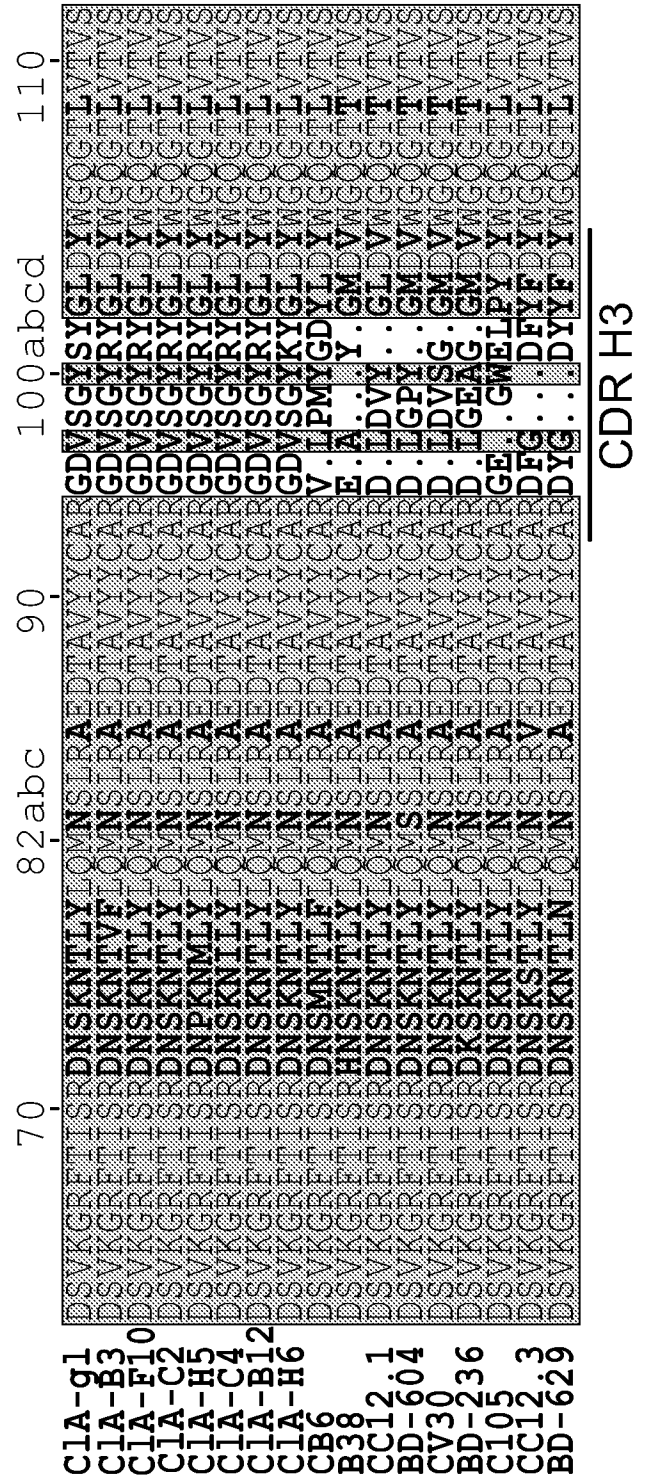
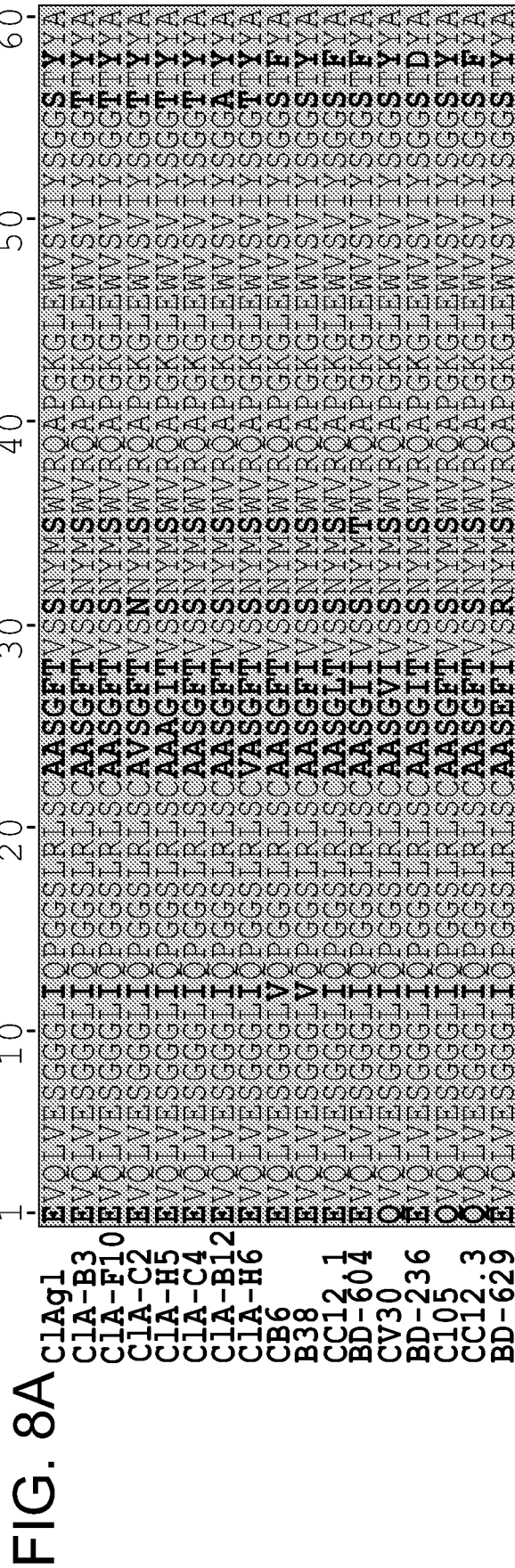


FIG. 8B

ClAg1	D	QL	IQSPSS	FS	ISAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
ClA-B3	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
ClA-F10	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	E	N	G	V	P	S
ClA-C2	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	E	S	G	V	P	S
ClA-H5	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
ClA-C4	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
ClA-B12	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
ClA-H6	D	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	E	S	G	V	P	S
B38	D	VM	IQSPSS	FS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
CC12.1	D	VM	IQSPSS	FS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
BD-604	D	QL	IQSPSS	FS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S
BD-236	A	QL	IQSPSS	SS	LSAS	SVGDRVT	ITCRAS	QGISS	Y	LA	WY	Q	K	LI	Y	A	S	Q	S	G	V	P	S

CDR L2

CDR L1

ClAg1	R	E	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-B3	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-F10	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-C2	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-H5	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-C4	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-B12	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
ClA-H6	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	G	.	.	T	F	G	Q	G	T	K	L	E	I	K	
B38	R	E	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	P	.	Y	T	F	G	Q	G	T	K	L	E	I	K	
CC12.1	R	E	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	P	.	K	F	T	F	G	Q	G	T	K	L	E	I	K
BD-604	R	E	S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	P	.	K	F	T	F	G	Q	G	T	K	L	E	I	K
BD-236	R	E	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	L	N	S	Y	P	P	.	A	T	F	G	Q	G	T	K	L	E	I	K	

CDR L3

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FIG. 8C

F27V/T28I

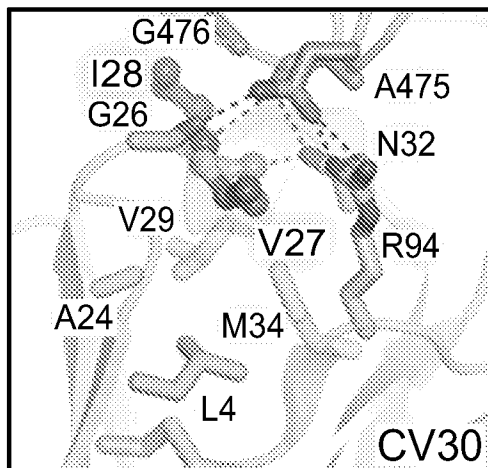


FIG. 8D

T28I

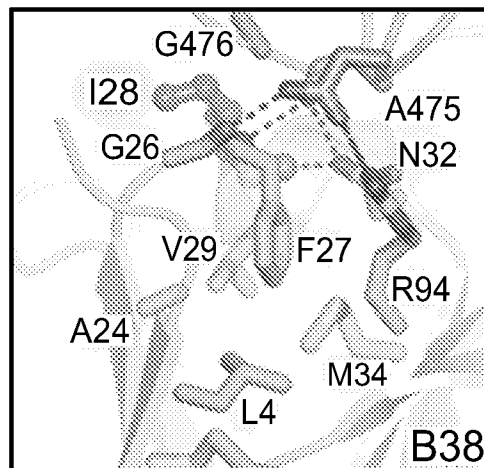


FIG. 8E

G26E/T28I

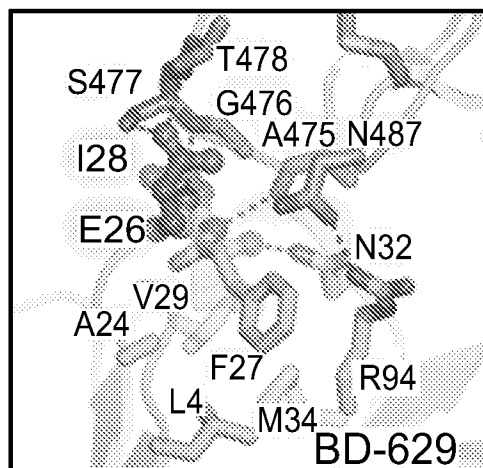


FIG. 8F

germline

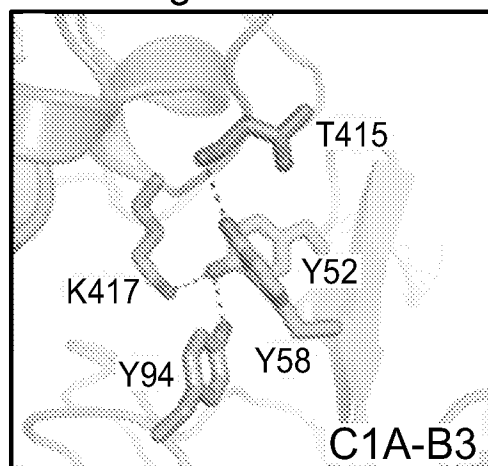


FIG. 8G

Y58F

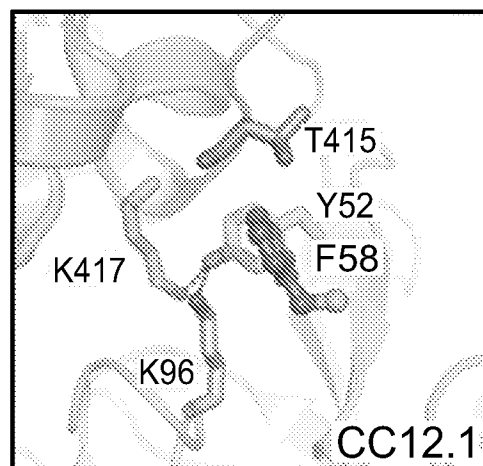


FIG. 9A

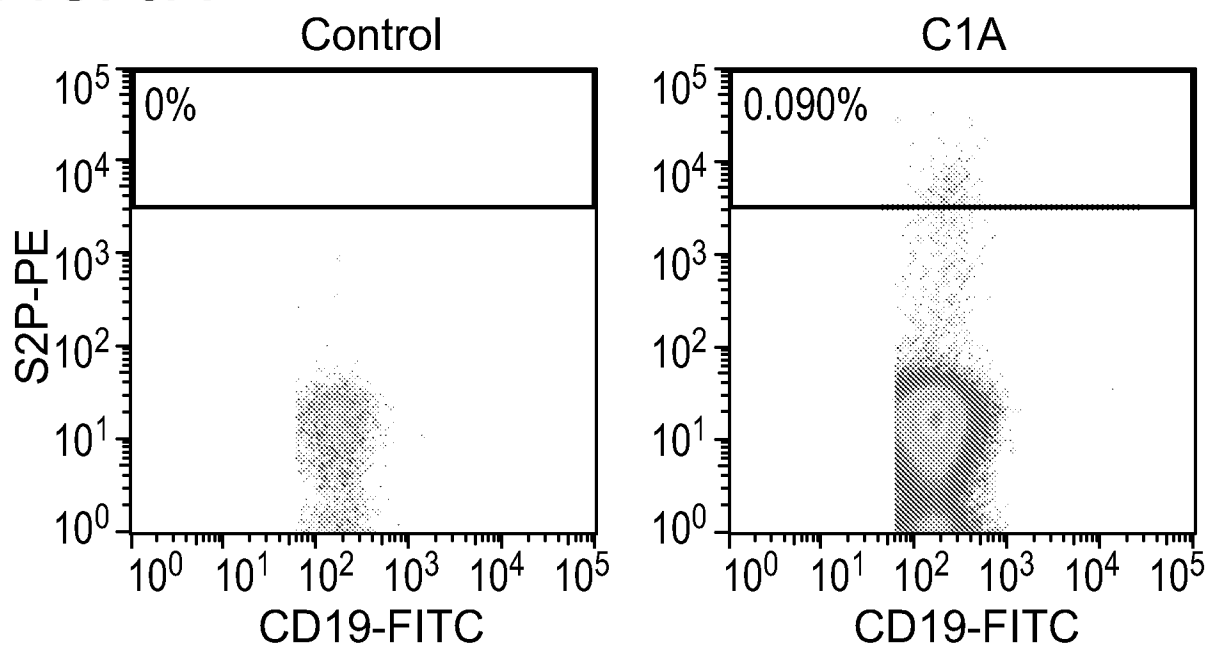
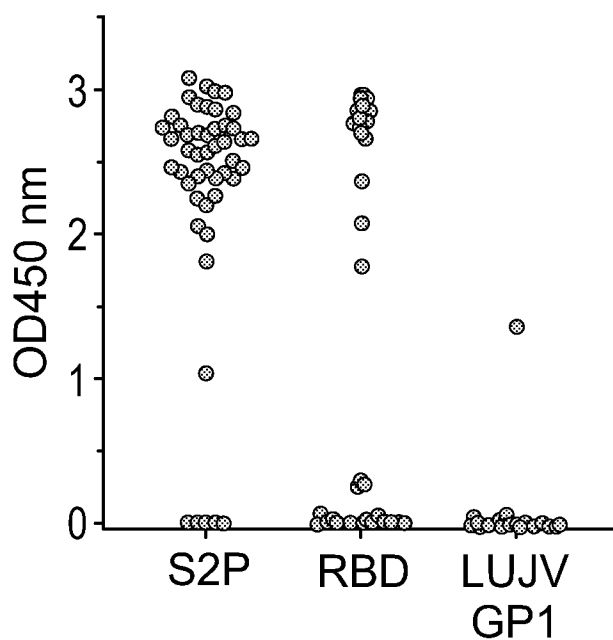
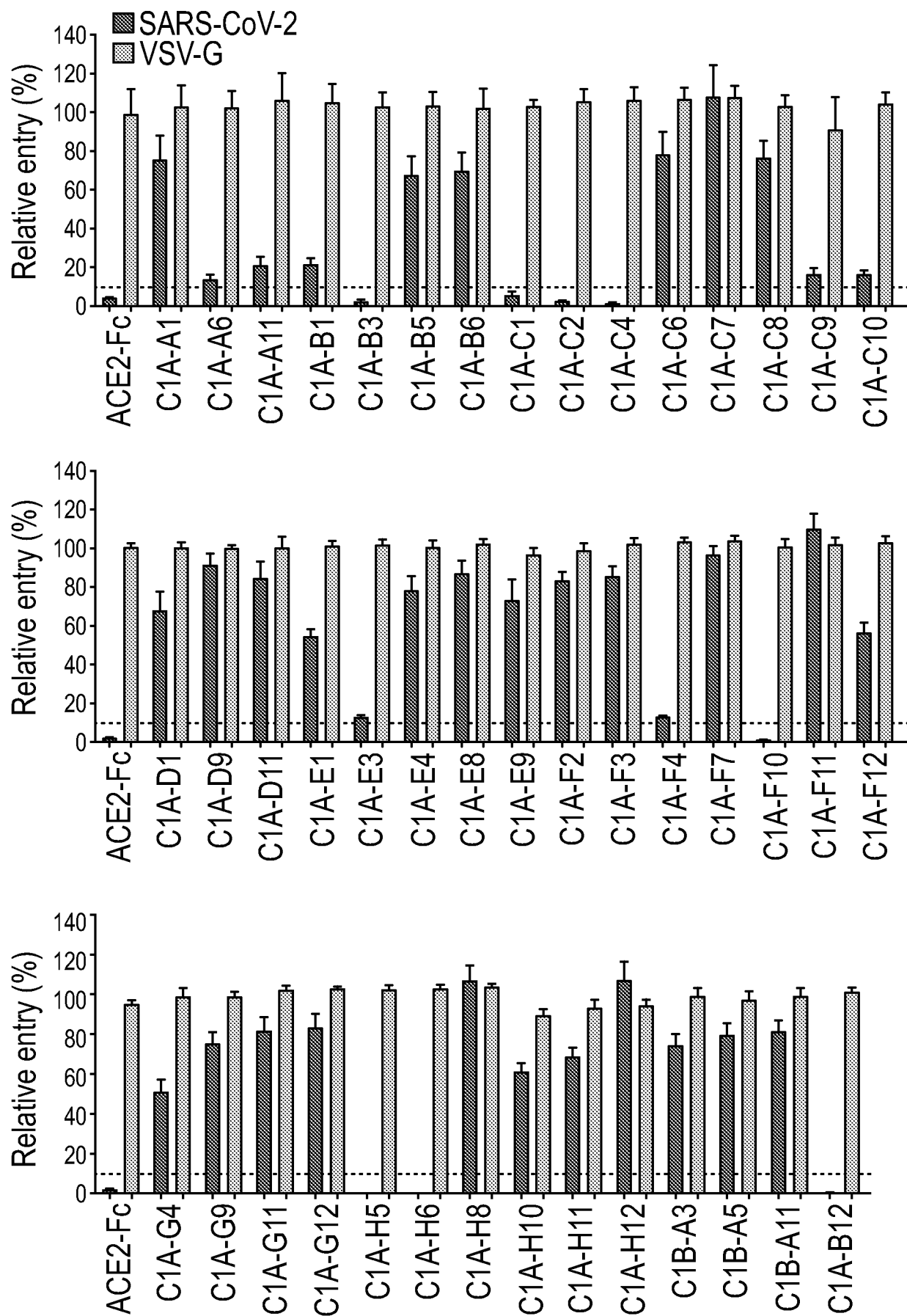


FIG. 9B



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FIG. 9C



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FIG. 10

Antibody	V _H gene	CDR H3 (a.a.)	Identity (%)	V _L gene	CDR L3 (a.a.)	Identity (%)	ELISA (OD 450 nm)			
							Neut.	S2P	RBD	Ctrl
C1A-A1	IGHV1-69	15	99.31	IGKV3-11	11	99.64	-	2.46	0.26	0.00
C1A-A6	IGHV3-11	15	98.96	IGKV1-13	9	98.92	+	2.55	2.81	0.00
C1A-A11	IGHV3-30	20	99.31	IGKV1-33	9	99.28	+	2.87	2.85	-0.01
C1A-B1	IGHV1-2*02	18	97.57	IGKV3-20	10	98.58	+	2.44	0.04	0.00
C1A-B3	IGHV3-53	14	97.89	IGKV1-9	9	98.92	++	2.66	2.94	0.00
C1A-B5	IGHV4-59*11	11	94.74	IGKV4-1*01	9	95.96	-	2.73	0.04	0.00
C1A-B6	IGHV1-24	14	98.61	IGLV2-8	10	99.31	-	3.08	0.00	0.00
C1A-B12	IGHV3-53	14	98.6	IGKV1-9	9	98.57	++	2.69	2.97	-0.01
C1A-C1	IGHV3-23	14	99.31	IGKV1-39	10	100	++	2.95	2.97	-0.01
C1A-C2	IGHV3-53	14	98.6	IGKV1-9	9	97.49	++	2.81	2.86	-0.01
C1A-C4	IGHV3-53	14	98.25	IGKV1-9	9	97.13	++	2.89	2.85	0.00
C1A-C6	IGHV1-69	15	98.96	IGKV3-11	11	98.92	-	2.58	0.02	0.01
C1A-C7	IGHV4-39	12	99.31	IGLV2-14	10	94.79	-	1.04	2.08	1.37
C1A-C8	IGHV1-24	16	99.65	IGLV3-21	12	98.57	-	3.02	0.00	-0.01
C1A-C9	IGHV1-69	14	97.92	IGKV1-39	8	98.92	+	2.99	2.93	0.00
C1A-C10	IGHV3-23	17	98.61	IGLV2-23	12	99.65	+	2.75	0.04	0.01
C1A-D1	IGHV4-39	18	91.75	IGKV1-33	9	93.19	-	2.06	0.30	0.05
C1A-D9	IGHV3-30	20	98.61	IGKV2-30	10	100	-	2.66	1.78	0.02
C1A-D11	IGHV1-69	14	98.26	IGKV3-11	11	99.28	-	2.39	0.00	-0.01
C1A-E1	IGHV3-9*01	13	89.58	IGKV1-39	10	91.04	-	2.70	2.82	-0.01
C1A-E3	IGHV1-18	16	98.61	IGKV1-39	5	98.92	+	2.74	2.86	0.00
C1A-E4	IGHV3-30	14	97.92	IGKV3-20	9	100	-	2.43	0.03	0.00
C1A-E8	IGHV3-48	21	97.22	IGKV2-28	9	100	-	2.73	0.02	0.01
C1A-E9	IGHV3-30	14	100	IGKV3-20	9	100	-	1.81	0.00	0.00
C1A-F2	IGHV3-30-3	15	99.65	IGKV2-28	8	99.66	-	2.25	0.01	0.00
C1A-F3	IGHV4-61	13	98.63	IGKV1-39	10	99.28	-	2.40	0.07	0.01
C1A-F4	IGHV3-30-3	14	99.65	IGKV1-5	9	99.64	+	2.84	2.77	0.00
C1A-F7	IGHV3-15	11	91.84	IGKV4-1	9	93.6	-	2.42	0.01	0.00
C1A-F10	IGHV3-53	14	97.89	IGKV1-9	9	98.21	++	2.98	2.69	0.00
C1A-F11	IGHV1-69	21	89.24	IGKV1-33	8	94.98	-	2.39	2.37	0.00
C1A-F12	IGHV4-39	15	90.03	IGLV1-40	11	95.49	-	2.62	2.78	0.01
C1A-G4	IGHV3-23	17	99.65	IGLV2-23	11	99.65	-	2.57	0.00	0.00
C1A-G9	IGHV3-30-3	14	98.61	IGKV3-20	8	100	-	2.46	0.01	0.00
C1A-G11	IGHV1-69	12	97.92	IGKV1-5	9	99.28	-	2.75	0.01	0.00
C1A-G12	IGHV3-30-3	14	98.26	IGKV3-15	8	98.92	-	2.51	0.07	0.07
C1A-H5	IGHV3-53	14	97.54	IGKV1-9	9	97.49	++	2.69	2.67	0.00
C1A-H6	IGHV3-53	14	98.95	IGKV1-9	9	98.21	++	2.90	2.74	0.04
C1A-H10	IGHV3-21	18	99.31	IGKV3-15	10	100	-	2.64	0.03	0.02
C1A-H11	IGHV3-30	16	98.61	IGLV2-14	10	99.31	-	2.27	0.00	0.00
C1A-H12	IGHV4-39	15	98.28	IGKV3-15	9	99.28	-	2.66	0.01	0.01
C1B-A3	IGHV3-30-3	14	99.65	IGKV3-11	9	100	-	2.00	0.02	0.01
C1B-A5	IGHV3-30-3	14	99.65	IGKV3-20	9	98.94	-	2.20	0.01	0.01
C1B-A11	IGHV3-30-3	14	98.26	IGKV3-20	9	99.29	-	2.35	0.28	0.00

FIG. 11A

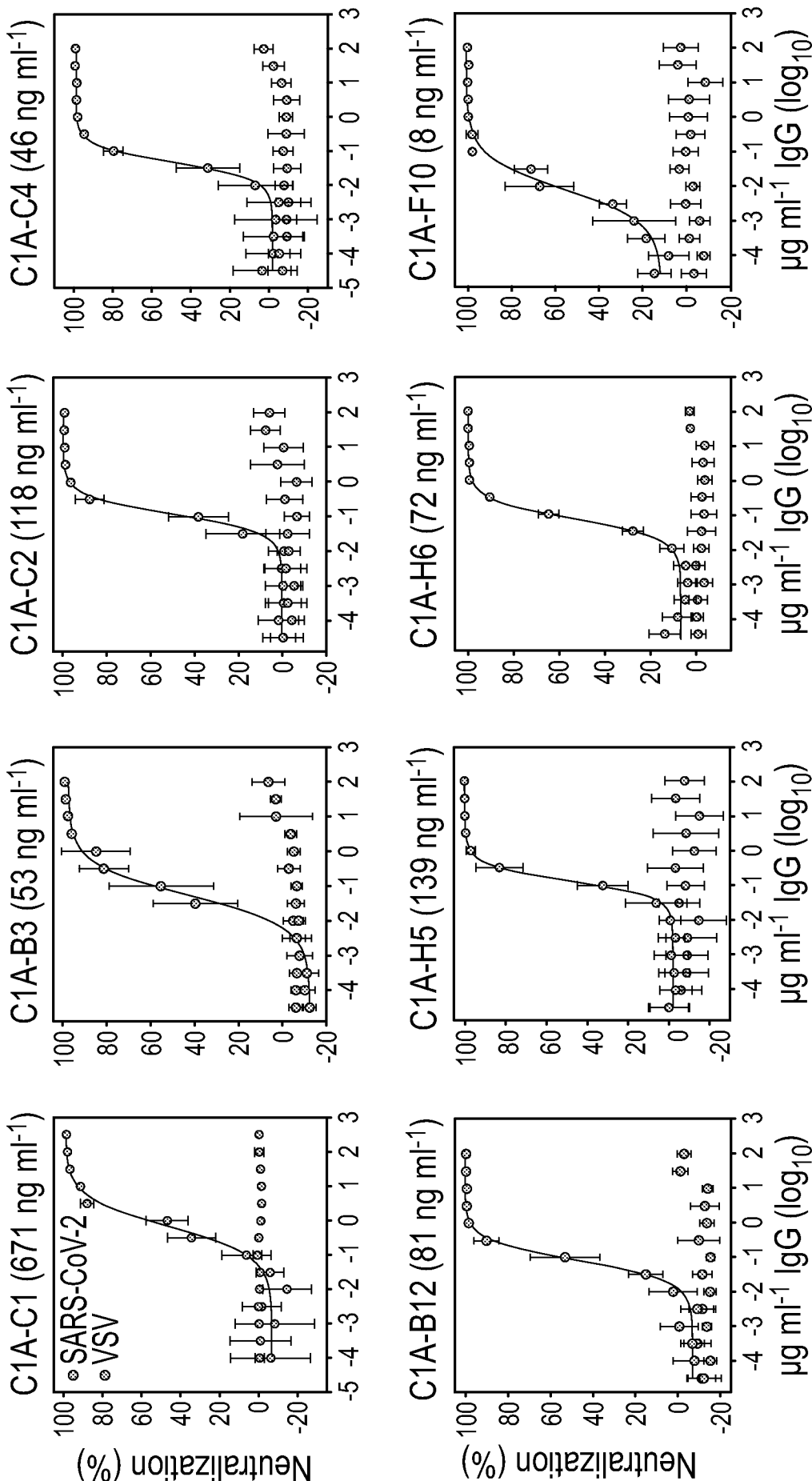
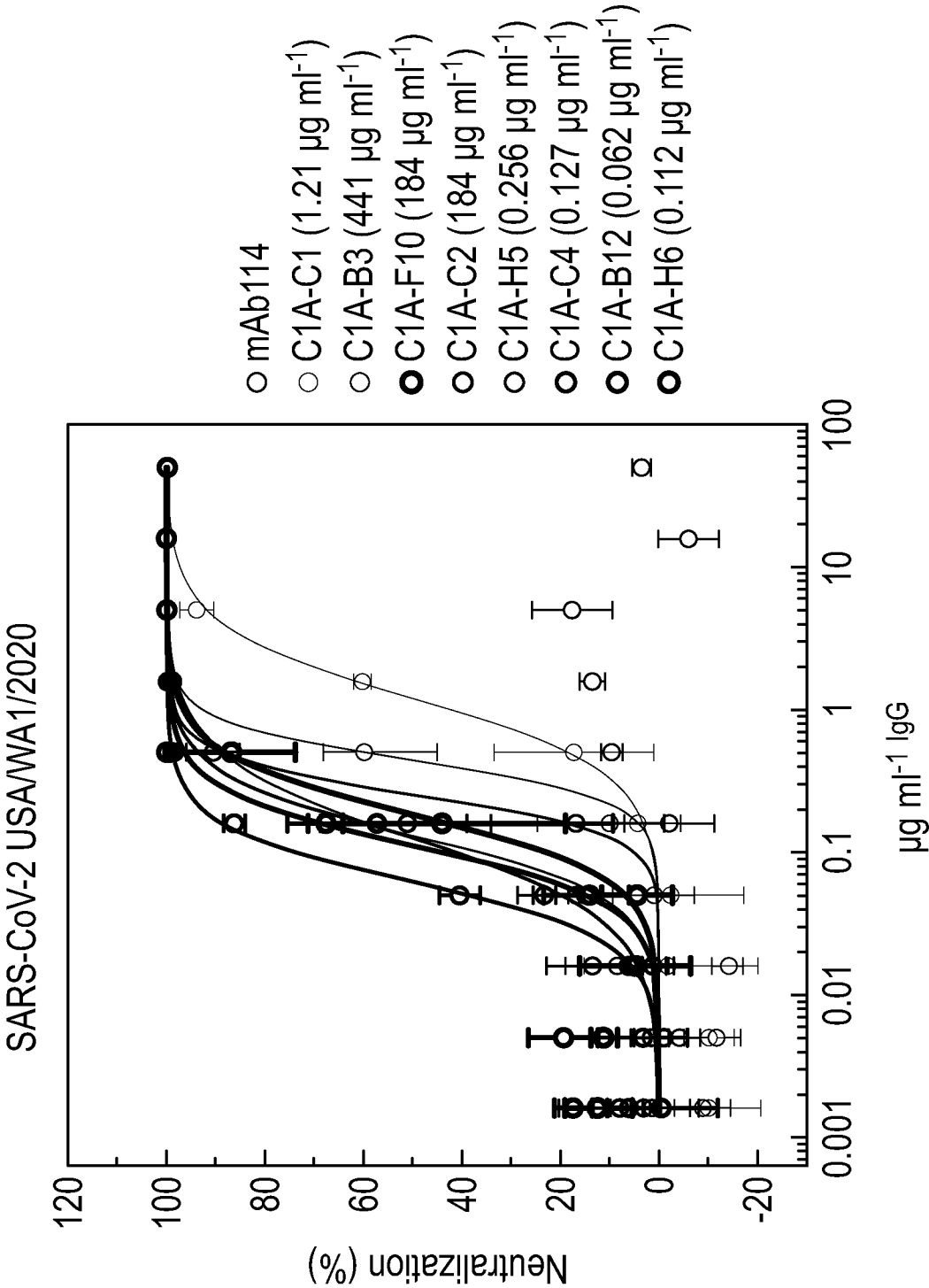
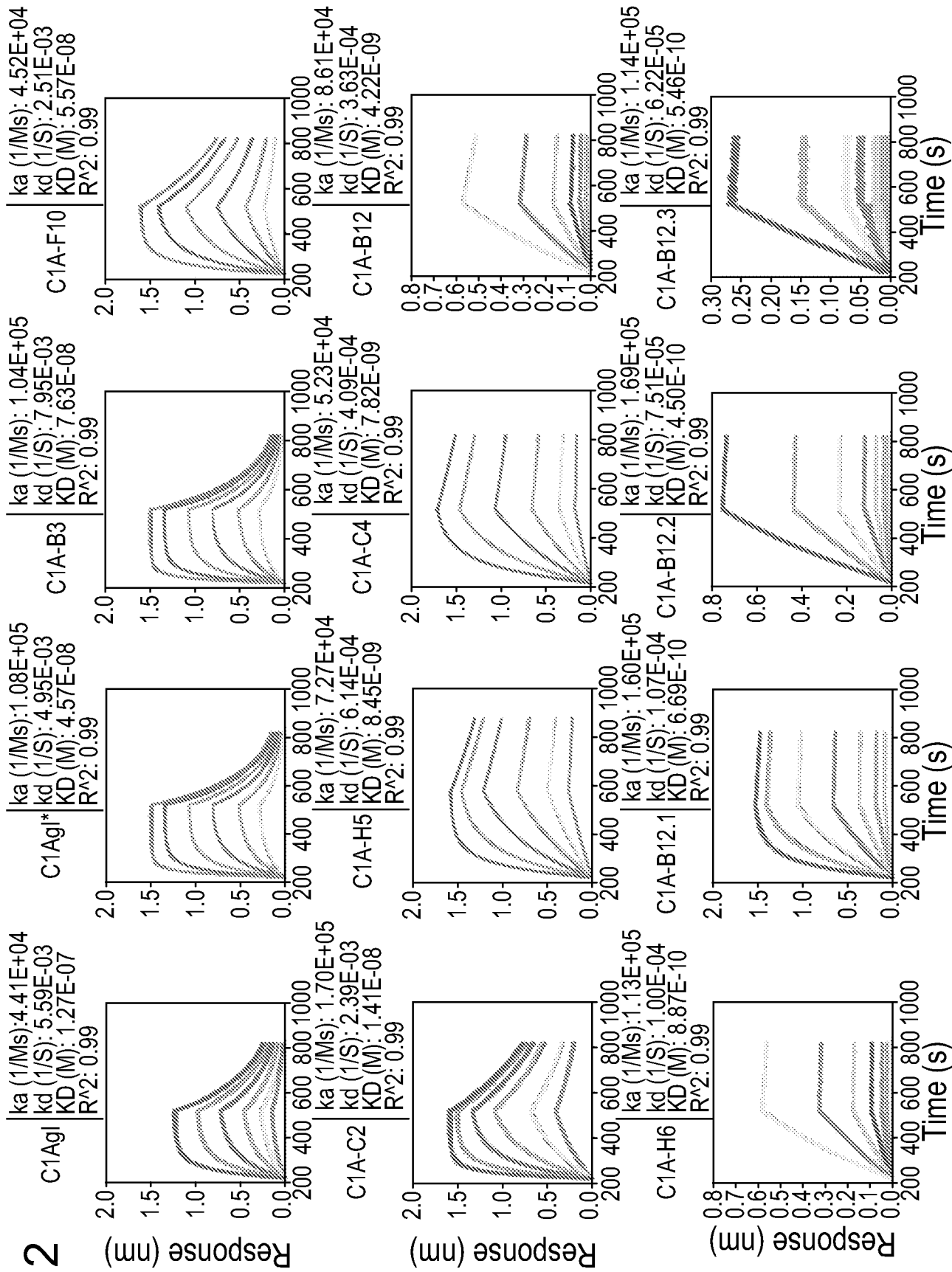


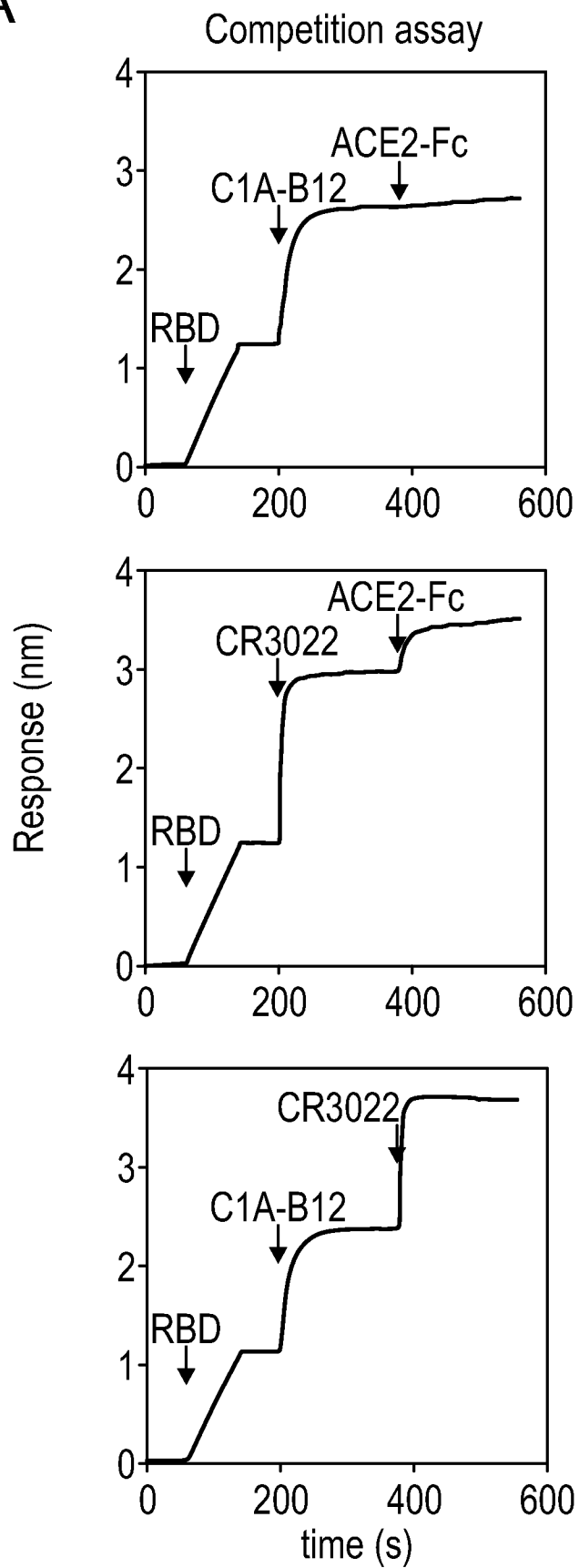
FIG. 11B





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FIG. 13A



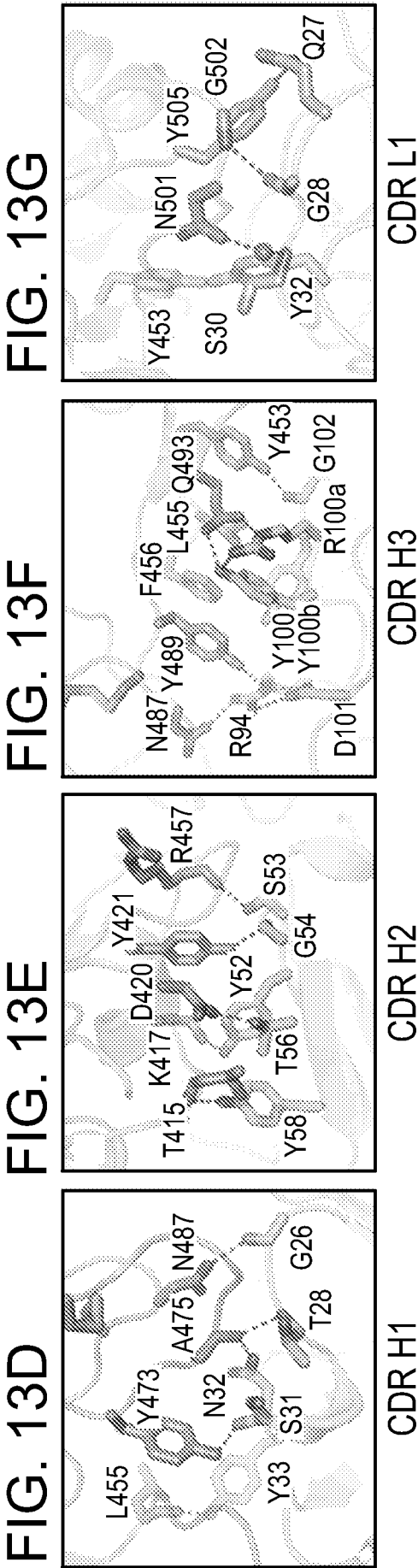
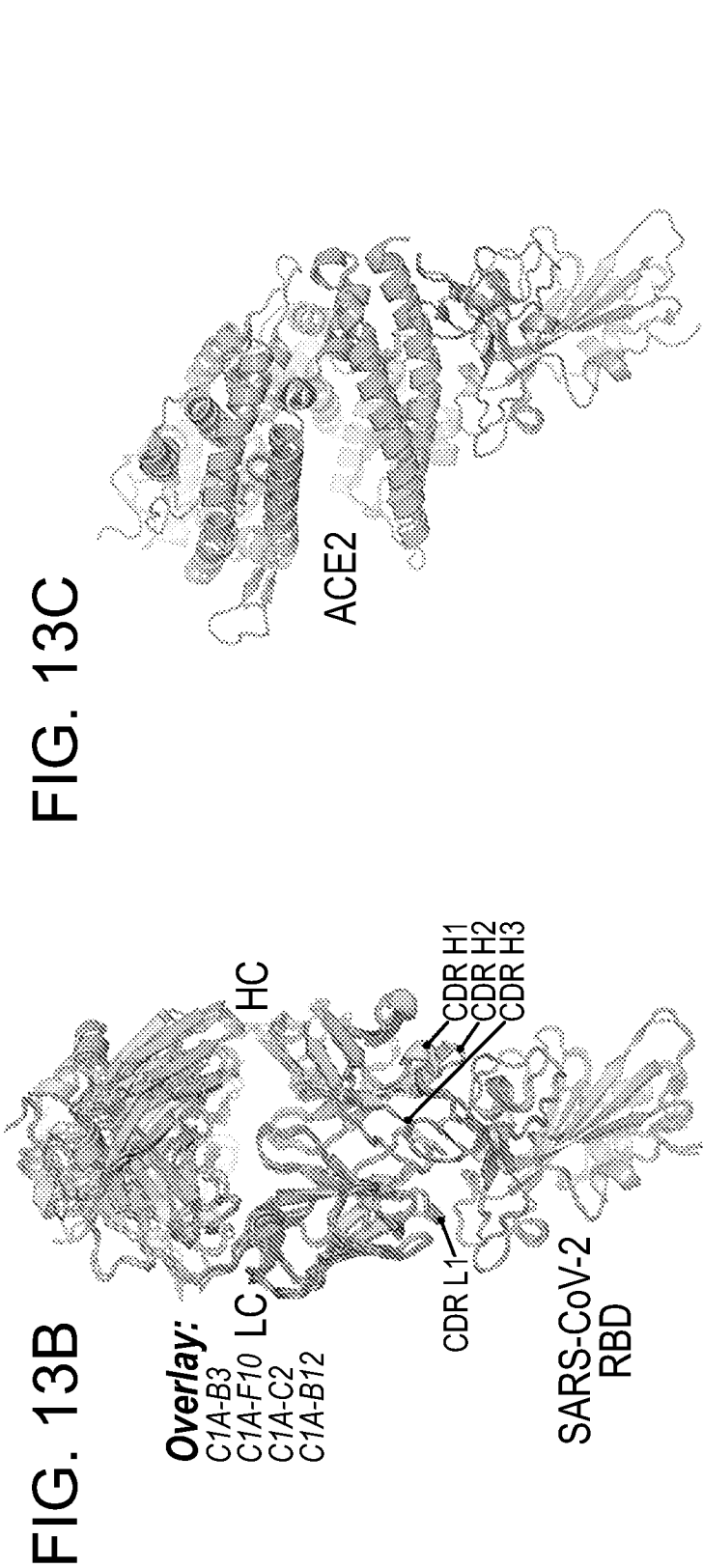


FIG. 14A

FIG. 14B

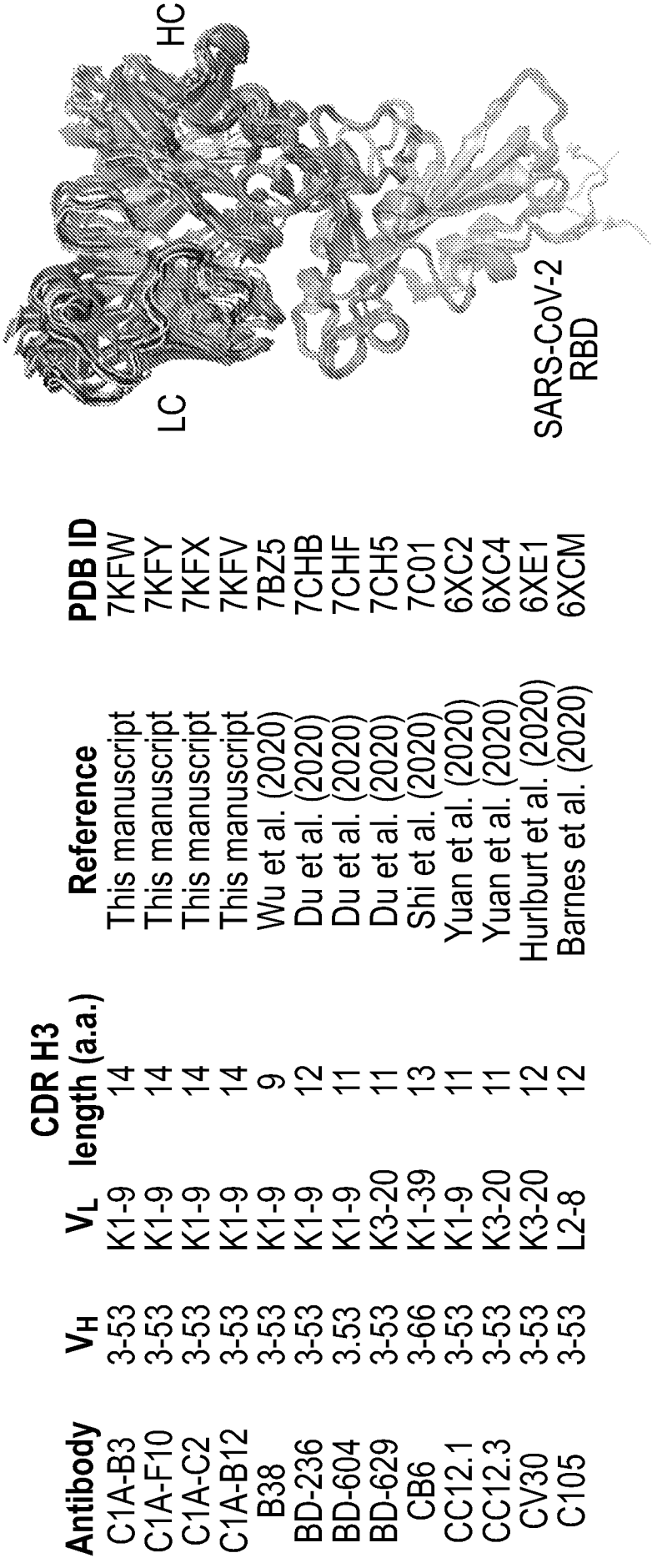


FIG. 14C

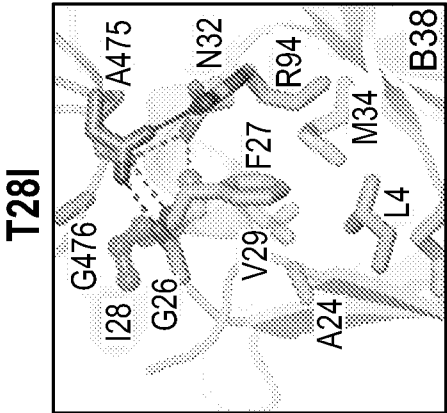


FIG. 14D

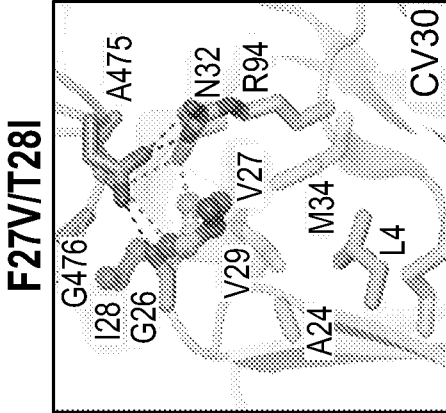


FIG. 14E

	V _H 3-53							V _K 1-9
	24	27	28	31	56			
C1A-gl	A	F	T	S	S			N
C1A-B3	•	•	•	•	T			•
C1A-F10	•	•	•	•	T			•
C1A-C2	V	•	•	N	T			•
C1A-H5	•	I	•	•	T			•
C1A-C4	•	•	•	•	T			I
C1A-B12	•	•	•	•	A			I
C1A-H6	•	•	•	•	T			I
C1A-B12.1	V	•	•	N	T			I
C1A-B12.2	V	•	I	N	T			I
C1A-B12.3	V	V	I	N	T			I

FIG. 14F

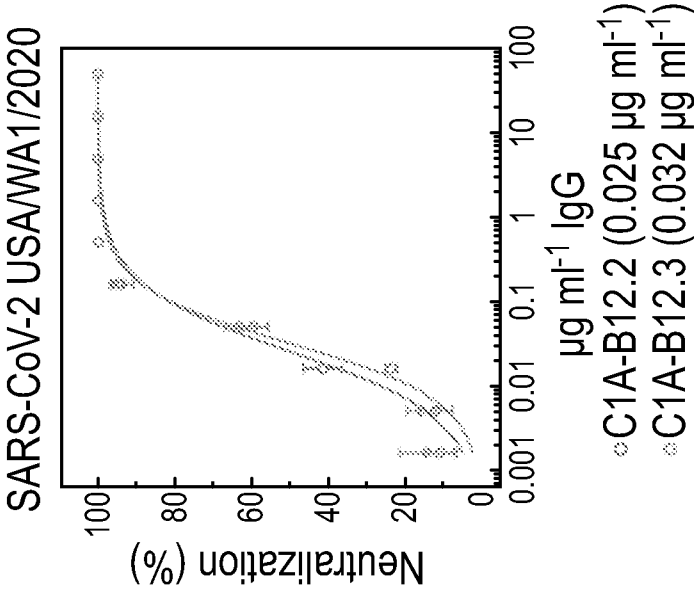


FIG. 15A

D5-18*01									
99	100	100a	100b	100c					
germline	GGA	TAC	AGC	TAT	GGT				
C1A-B3	GGA	TAC	AGA	TAT	GGT				
C1A-B12	GGA	TAC	AGA	TAT	GGT				
C1A-C2	GGA	TAC	AGG	TAT	GGT				
C1A-C4	GGA	TAC	AGG	TAT	GGT				
C1A-F10	GGA	TAC	AGA	TAT	GGT				
C1A-H5	GGA	TAC	AGG	TAT	GGT				
C1A-H6	GGA	TAC	AGA	TAT	GGT				
					■ S ■ R ■ K				

FIG. 15B

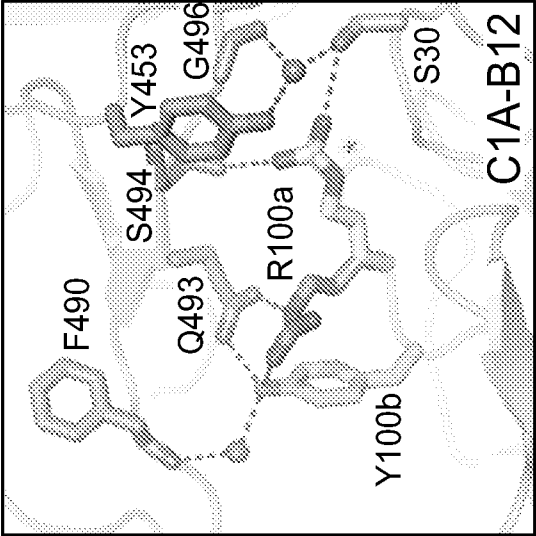


FIG. 15E

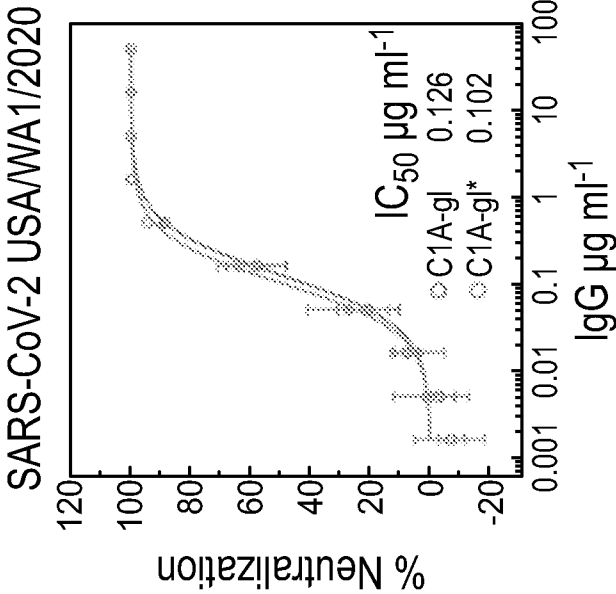


FIG. 15C

CDR H3		C1A-gl	ARGDVSGY	100a	102
				SYGLDY	
CDR H3		C1A-gl*	ARGDVSGY	100a	102
				RYGLDY	

FIG. 15D

Kinetic analysis				
Antibody	k_a (1/Ms)	k_d (1/S)	K_D (nM)	
C1A-gl	4.41E+04	5.58E-03	127	
C1A-gl*	1.08E+05	4.95E-03	46	

FIG. 16A

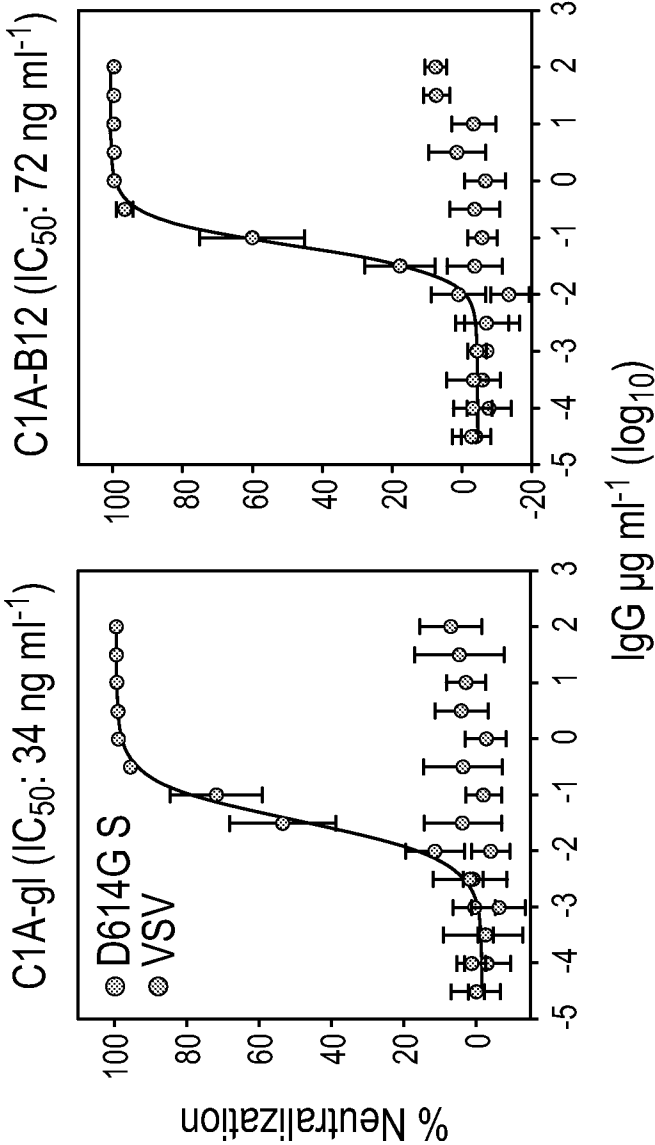
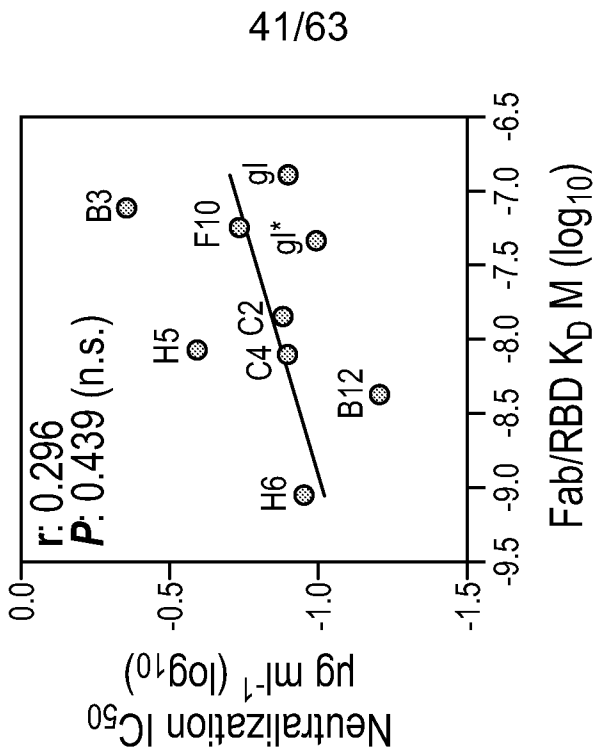


FIG. 16B



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FIG. 17

	C1A-B3/RBD ^a (PDB 7KFV)	C1A-B12/RBD ^a (PDB 7KFW)	C1A-C2/RBD ^a (PDB 7KFX)	C1A-F10/RBD ^a (PDB 7KFY)
Data collection				
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>C</i> 222 ₁	<i>C</i> 222 ₁
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	84.8, 113.3, 268.89	84.8, 113.3, 268.9	83.5, 149.2, 146.1	85.7, 146.8, 144.6
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	200-2.77 (2.94- 2.77) ^b	200-2.10 (2.23- 2.10) ^b	200-2.22 (2.36- 2.22) ^b	200-2.16 (2.29- 2.16) ^b
<i>R</i> _{sym}	0.38 (3.17) ^b	0.238 (2.01) ^b	0.136 (2.23) ^b	0.118 (1.13) ^b
<i>R</i> _{pim}	0.11 (1.03) ^{b,c}	0.095 (0.85) ^{b,c}	0.062 (1.01) ^{b,c}	0.076 (0.61) ^{b,c}
<i>I</i> / σ	5.4 (0.7) ^b	9.0 (1.4) ^b	6.9 (0.6) ^b	6.2 (0.7) ^b
Completeness (%)	98.1 (88.6) ^b	98.9 (94.7) ^b	99.1 (97.1) ^b	94.0 (89.4) ^b
Redundancy	6.9 (6.5) ^b	7.0 (6.4) ^b	3.6 (3.6) ^b	2.1 (2.0) ^b
Refinement				
Resolution (Å)	133.92-2.79	134.44-2.10	74.60-2.23	74.04-2.16
No. reflections	63645	149225	44541	48373
<i>R</i> _{work} / <i>R</i> _{free}	0.19/0.23	0.18/0.22	0.19/0.23	0.19/0.22
No. atoms				
Protein	14414	14511	4861	4855
Ligand/ion	42	42	14	14
Water	400	1642	325	532
<i>B</i> -factors (Å ²)				
Protein	88	47	63	52
Ligand/ion	96	99	103	101
Water	66	53	65	55
R.m.s. deviations				
Bond lengths (Å)	0.008	0.008	0.008	0.008
Bond angles (°)	0.980	0.970	1.000	1.000

FIG. 18A

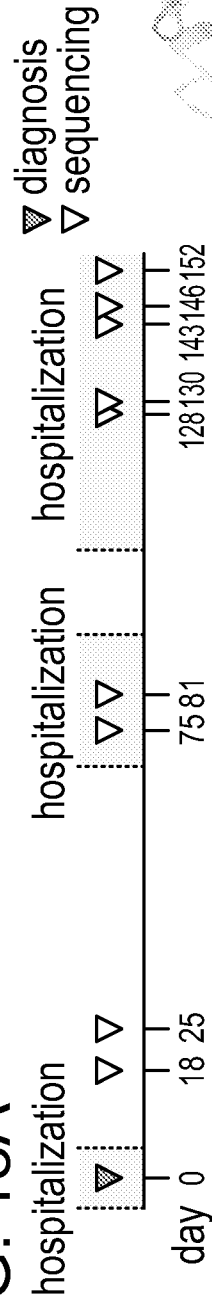


FIG. 18B

SARS-CoV-2 RBD positions														
Sequence	417	440	478	484	486	489	493	494	501					
Day 18	K	N	T	E	F	Y	Q	S	N					
Day 25	-	-	K	K	-	-	-	-	-					
Day 75	-	-	K	K	-	-	-	P	-					
Day 81	-	-	K	K	-	-	-	P	-					
Day 128	-	-	K	K	-	H	K	P	-					
Day 130	-	-	K	-	-	-	K	P	-					
Day 143	-	-	K	-	-	-	-	P	-					
Day 146	-	D	K	-	-	-	K	P	-					
Day 152	-	-	K	A	I	-	-	P	-					
Day 146*	-	D	K	-	-	H	K	P	-					
Day 152*	-	-	K	A	I	-	-	P	-					
UK (B.1.1.7)	-	-	-	-	-	-	-	-	-					
SA (B.1.351)	N	-	-	K	-	-	-	-	-					
BR (P.1)	T	-	-	K	-	-	-	-	-					
USA (B.1.1.7)	-	-	-	-	-	-	K	-	-					
UK (B.1.1.7)	-	-	-	-	-	-	R	-	-					
USA (B.1.1.7)	-	-	-	-	-	H	-	-	-					

○ Predicted effects on C1A-V_H3-53 mAbs | ● introduces clash | ○ removes/alters contact

FIG. 18C

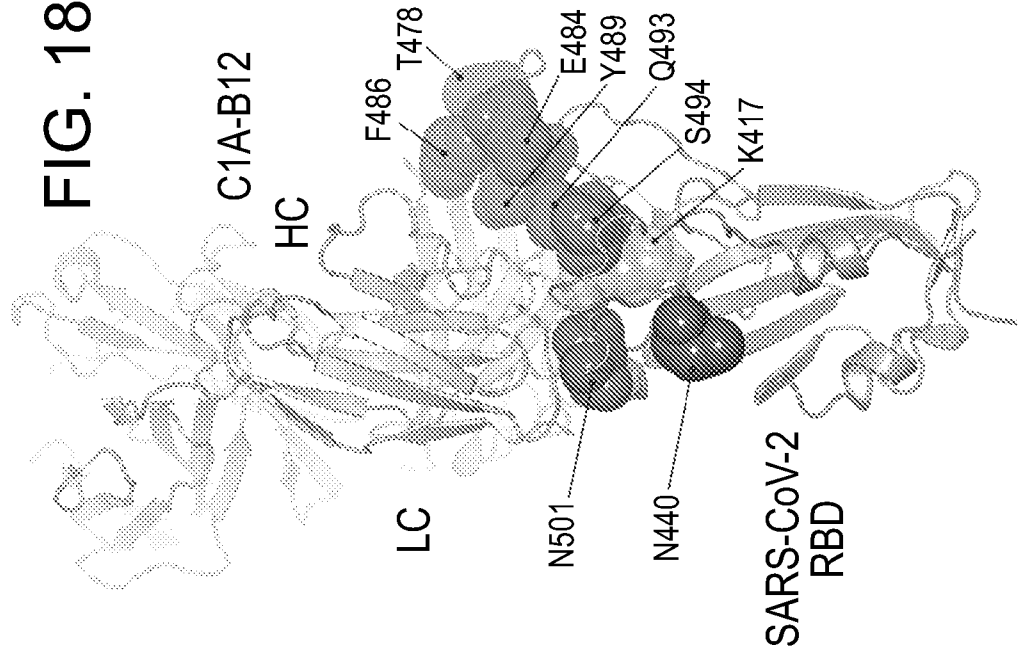


FIG. 18D

● Q493K

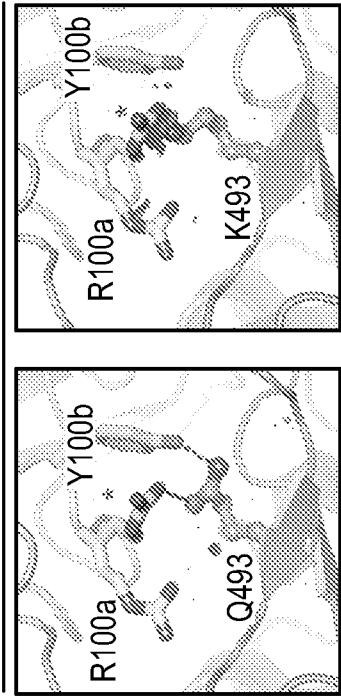


FIG. 18E

● N501Y

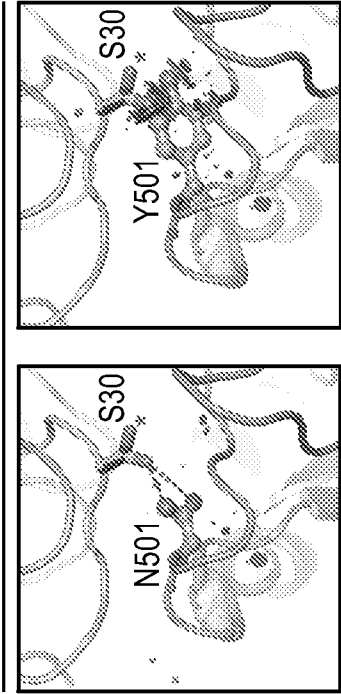


FIG. 18F

○ E484K

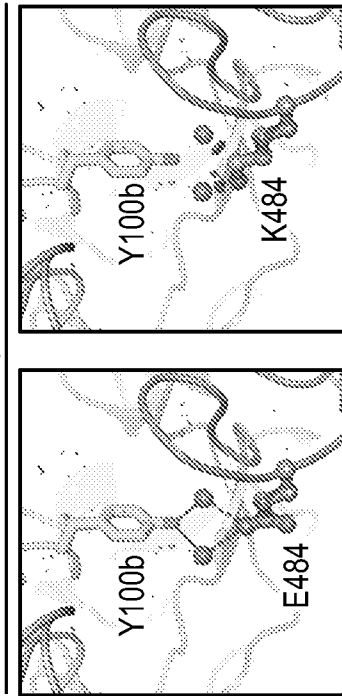


FIG. 18G

○ F486I

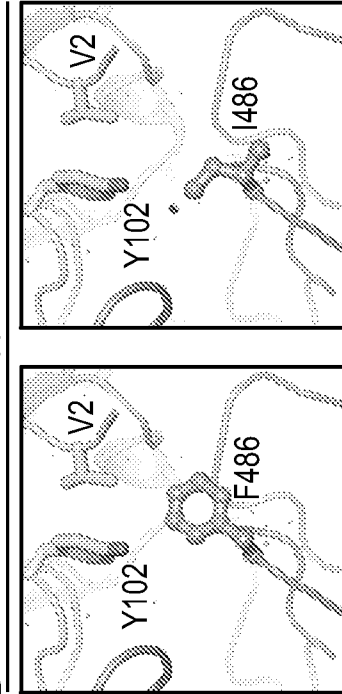


FIG. 18H

○ Y489H

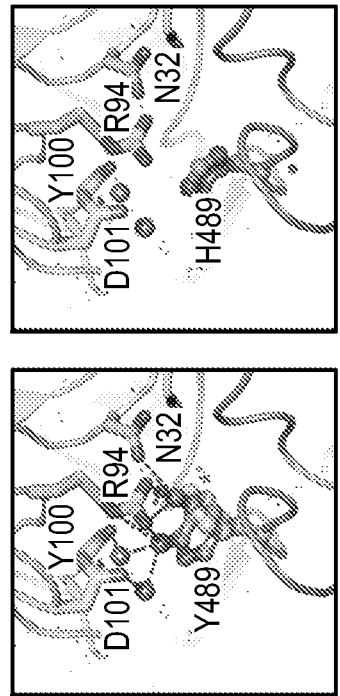


FIG. 18I

○ K417N

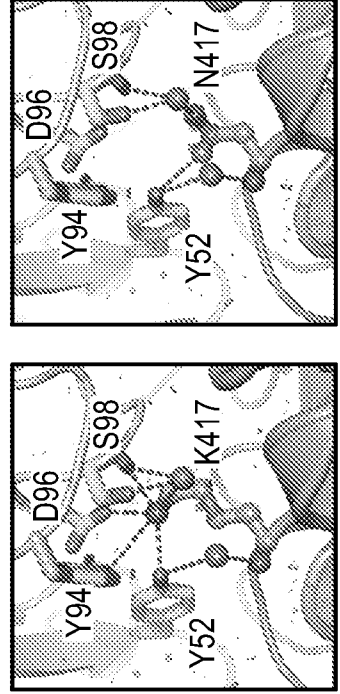


FIG. 18J
Human derived SARS-CoV-2 S sequences containing mutations of interest.

Virus name	PANGO lineage	Accession No.	S mutations of interest	Location	Collection date	Donor	Originating laboratory	Submitting laboratory	Authors
hCoV-19/USA/FL-CDC-STM-0000013-F04/2021	B.1.1.7	EPI_ISL_884605	D614G, N501Y, Q493K, Y114del	Florida, United States	01/14/2021	Unknown	Respiratory Viruses Branch, Centers for Disease Control and Prevention	Respiratory Viruses Branch, Centers for Disease Control and Prevention	Cook, P.W., Batra, D., Rambo-Martin, B.L., de Feo, E., Antico, J., Iran C., Tolentino, M., Wickline, S., Gietzen, K., Sickler, B., P., Liu, J., Allen, E., Febbo, P., Galloway, S., Washington, N.L., White, S., Levan, G., Barret, K.S., Cirulli, E., Bolze, A., Ascencio, A., Rivera-Garcia, C., Cho, R., Nguyen, J., Wang, S., Ramirez, J., Cassens, T., Sandoval, E., Isaksson, M., Lee, W., Becker, D., Laurent, M., Lu, J., Paden, C.R., Tong, S., MacCannell, D.
hCoV-19/Israel/CVL-618ngs/2020	B.1.362	EPI_ISL_889024	D614G, Q493K	Israel	12/29/2020	Unknown	Israel Central Virology laboratory	Israel National Consortium for SARS-CoV-2 sequencing	Neta Zuckerman, Efrat Dahan Bucris, Michal Mandelboim, Dana Bar-Ilan, Oran Erster, Tzvia Mann, Omer Murik, David A. Zeevi, Assaf Rokney, Joseph Jaffe, Eva Nachum, Maya Davidovich Cohen, Ephraim Fass, Gal Zizelski, Valenci, Mor Rubinstein, Efrat Roman, Israel Nissan, Efrat Glick-Saar, Omri Nayschool, Gideon Rechavi, Ella Mendelson, Orna Mor
hCoV-19/Switzerland/B_S42473265/2020	B.1.160	EPI_ISL_830854	D614G, Q493K	Basel, Switzerland	10/07/2020	Male (unknown)	University Hospital Basel, Clinical Virology	University Hospital Basel, Clinical Bacteriology	Tim Roloff, Madlen Stange, Helena MB Seth-Smith, Alfredo Mari, Karoline Leuzinger, Julia Bleilicki,

FIG. 18K

hCoV-19/Switzerland/B S-42471446/2020	B.1.160	EPI_ISL_830843	D614G, Q493R, Q493K	Basel, Switzerland	10/06/2020	Female (unknown)	University Hospital Basel, Clinical Virology	University Hospital Basel, Clinical Bacteriology	Manuel Battegay, Hans Hirsch, Adrian Egli
hCoV-19/England/MILK- 11C2FCD/2021	B.1.1.7	EPI_ISL_1006449	D614G, Q493R, N501Y, Y144del	United Kingdom, England	01/28/2021	Unknown (81)	Lighthouse Lab in Milton Keynes	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	The Lighthouse Lab in Milton Keynes and Alex Alderton, Roberto Amato, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
hCoV-19/USA/CDC- STM- A100413/2021	B.1.1.7	EPI_ISL_850699	D614G, N501Y, Y489H, Y144del	California, United States	01/07/2021	Female (21)	Helix / Illumina	Genomics and Discovery, Respiratory Viruses Branch, Division of Viral Diseases, Centers for Disease Control and Prevention	Peter W. Cook, Dhvani Batra, Ben L. Rambo-Martin Eileen de Feo, Jan Antico, Christine Tran, Matthew Tolentino, Shannon Wickline, Kim Gietzen, Brad Sickler, Jingtao Liu, Eric Allen, Phil Febbo, Summer Galloway, Nicole L. Washington, Simon White, Geraint Levan, Kelly Schiabor Barrett, Elizabeth Cirulli, Alexandre Bolze, Ary Ascencio, Charlotte Rivera-Garcia, Ryan Cho, Jason Nguyen, Sherry Wang, Jimmy Ramirez, Tyler Cassens, Efrén Sandoval, Magnus Isaksson, William Lee, David Becker, Marc Laurent, James Lu, Clinton R. Paden, Suxiang Tong, Duncan MacCannell

FIG. 18L

hCoV-19/England/MILK-99469D/2020	B.1.1.7	EPI_ISL_552392	D614G, Y489H	United Kingdom, England	08/25/2020	unknown	Lighthouse Lab in Milton Keynes	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	The Lighthouse Lab in Milton Keynes and Alex Alderton, Roberto Amato, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team (http://www.sanger.ac.uk/covid-team)
hCoV-19/England/205261299/2020	B.1.1.7	EPI_ISL_754289	D614G, N501Y, Y144del	United Kingdom, England	12/20/2020	Male (78)	Respiratory Virus Unit, National Infection Service, Public Health England	COVID-19 Genomics UK (COG-UK) Consortium	PHE Covid Sequencing Team
hCoV-19/South Africa/Tygerberg-461/2020	B.1.351	EPI_ISL_745186	D614G, E484K, K417N, N501Y, L242del, A243del, L244del	South Africa, Western Cape	12/07/2020	Female (34)	Wallacedene Clinic wc WAL	National Health Laboratory Service (NHLS), Tygerberg	Susan Engelbrecht, Kayla Delaney, Bronwyn Klennans, Hourivah Tegally, Edean Wilkindon, Geert van Zyl, Wolfgang Preiser, Tullio de Oliveira
hCoV-19/Brazil/AM-20143138FN-R2/2020	P.1	EPI_ISL_811149	D614G, K417T, E484K, N501Y	Brazil, Amazonas, Manaus	12/30/2020	Female (29)	Laboratorio de Ecologia de Doencas Transmissíveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazonia	Laboratorio de Ecologia de Doencas Transmissíveis na Amazonia, Instituto Leonidas e Maria Deane - Fiocruz Amazoni	Valdineite Nascimento, Victor Souza, André Corado, Fernanda Nascimento, George Silva, Agatha Costa, Debora Duarte, Luciana Goncalves, Matilde Mejia, Karina Pessoa, Maria Julia Brandão, Michele Jesus, Felipe Naveca
hCoV-19/England/MILK-F9DBDB/2021	B.1.429	EPI_ISL_852237	D614G, E484K, N501Y, S494P	United Kingdom, England	01/08/2021	Unknown	Lighthouse Lab in Milton Keynes	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	The Lighthouse Lab in Milton Keynes and Alex Alderton, Roberto Amato, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team

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FIG. 18M

hCoV-19/Mozambique/NS-K008170/2021	B.1.351	EPI_ISL_964941	D614G, K417N, E484K, T478K, N501Y, L242del, A243del, L244del	Africa, Mozambique	01/14/2021	Female (25)	Instituto Nacional de Saude (INS), Mozambique	KRISP, KZN Research Innovation and Sequencing Platform	Nalia Ismael, Nadia Siteo, Paulo Arnaldo, Nedio Mabunda, Giandhari J, Pillay S, Emmanuel S, Tegally H, Wilkinson E, de Oliveira T
hCoV-19/Denmark/DCG C-5481/2020	B.1.1.298	EPI_ISL_620806	D614G, N440D, Y453F	Denmark, Nordjylland	09/28/2021	Unknown	Department of Virus and Microbiological Special Diagnostics, Statens Serum Institut, Denmark	Albertsen lab, Department of Chemistry and Bioscience, Aalborg University, Denmark	Danish Covid-19 Genome Consortia
hCoV-19/England/MILK-11C2FCD/2021	B.1.1.7	EPI_ISL_1006449	D614G, N501Y, Q493R, Y144del	United Kingdom, England	01/28/2021	Unknown	Lighthouse Lab in Milton Keynes	Wellcome Sanger Institute for the COVID-19 Genomics UK (COG-UK) Consortium	The Lighthouse Lab in Milton Keynes and Alex Alderton, Roberto Amato, Sonia Goncalves, Ewan Harrison, David K. Jackson, Ian Johnston, Dominic Kwiatkowski, Cordelia Langford, John Sillitoe on behalf of the Wellcome Sanger Institute COVID-19 Surveillance Team
hCoV-19/England/LON D-12E726E/2021	B.1.1.7	EPI_ISL_997803	D614G, F486L, N501Y, Y144del	United Kingdom, England	02/10/2021	Unknown	University College London, Great Ormond Street Hospital for Children NHS Foundation Trust, Imperial College Healthcare NHS Trust	COVID-19 Genomics UK (COG-UK) Consortium	Sergi Castellano, Rachel Williams, Mark Kristiansen, Paola Resende Silva, Sunando Roy, Tony Brooks, Helena Tutil, Paola Niola, Patricia Dyal, Charlotte Williams, Leysa Forrest, Yasmin Panchbhaya, Jacqueline Findlay, Samuel Weeks, Julianne Brown, Kathryn Harris, Paul Randell, James Price, Alison Holmes, Judith Breuer

FIG. 19A

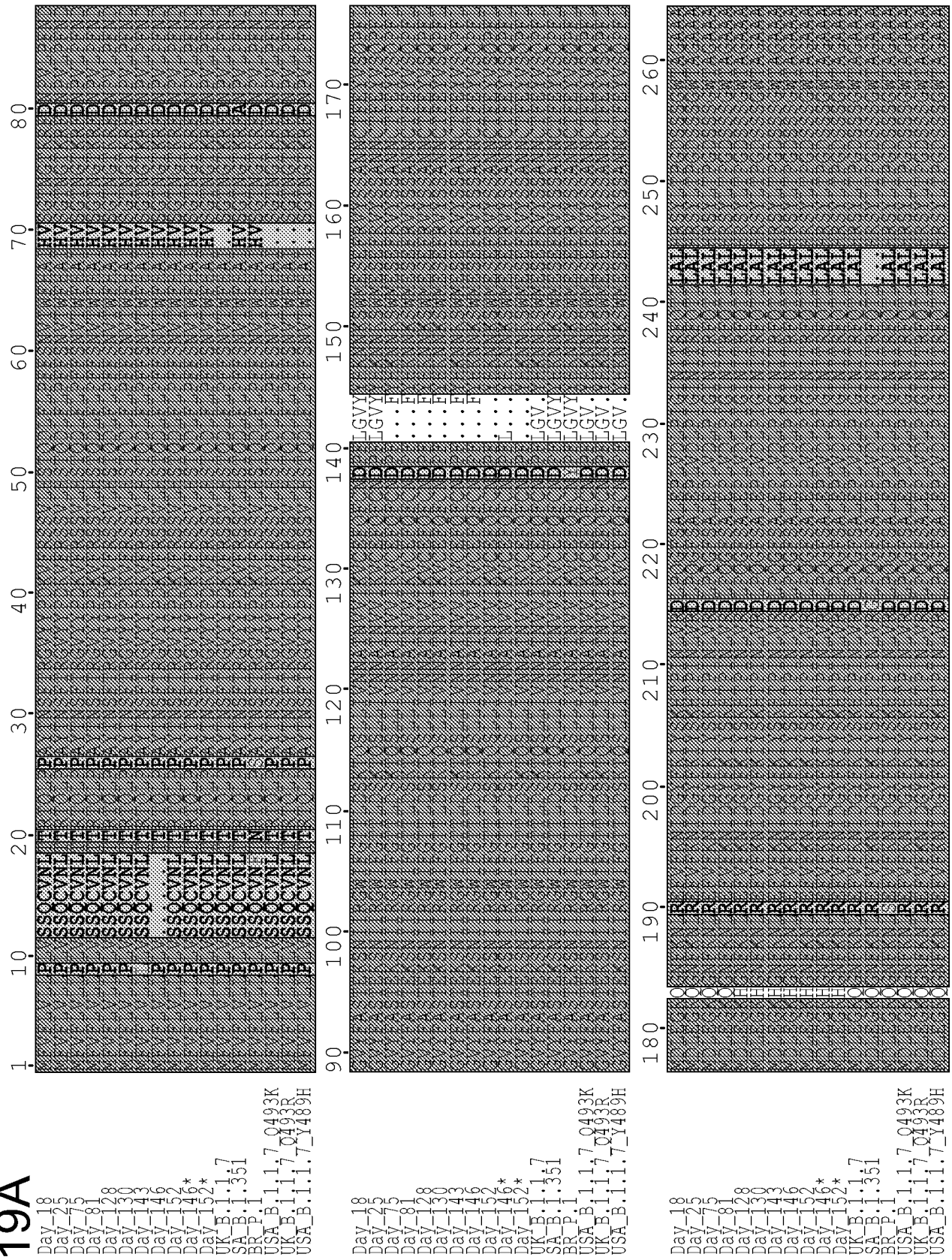


FIG. 19B

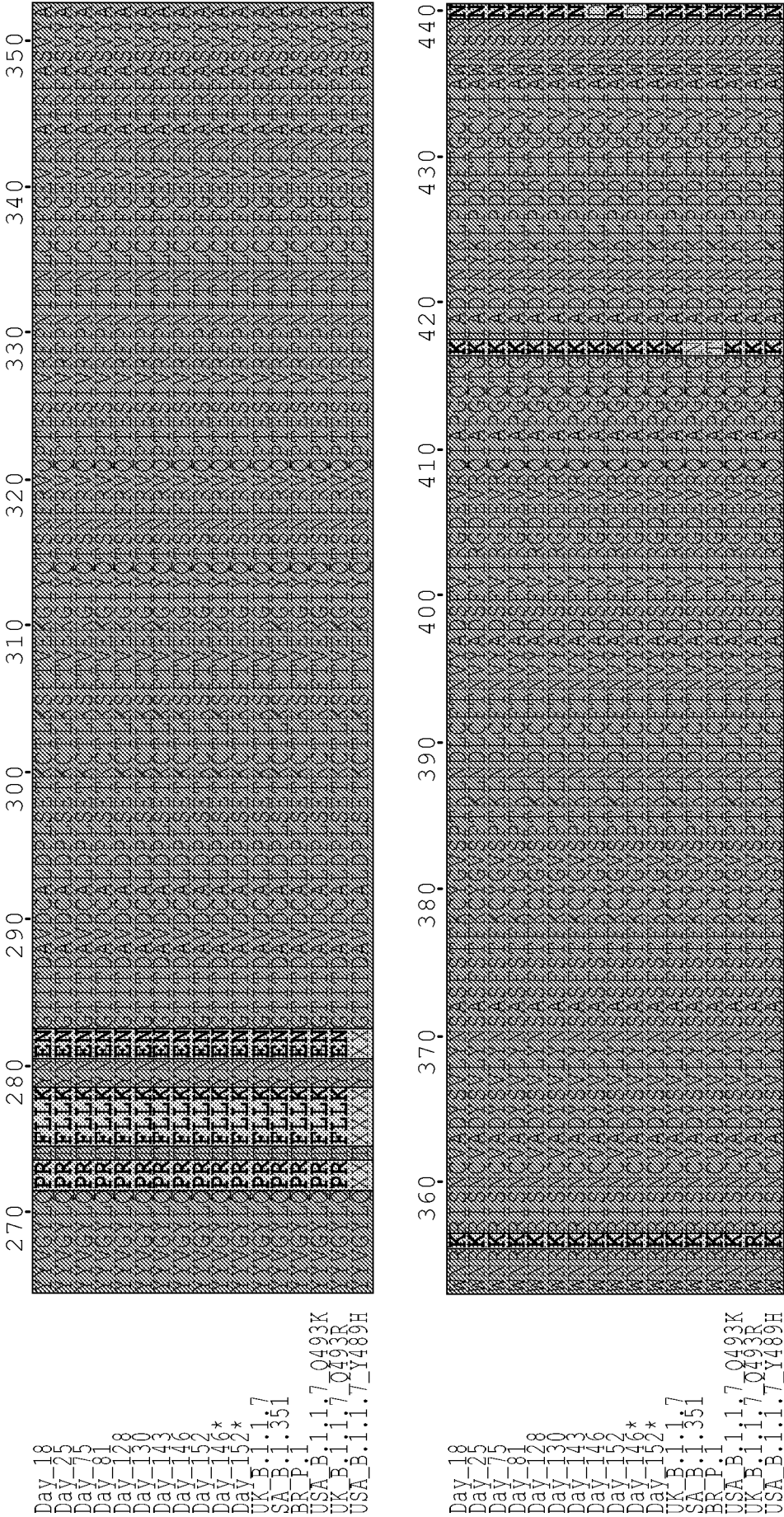


FIG. 19C

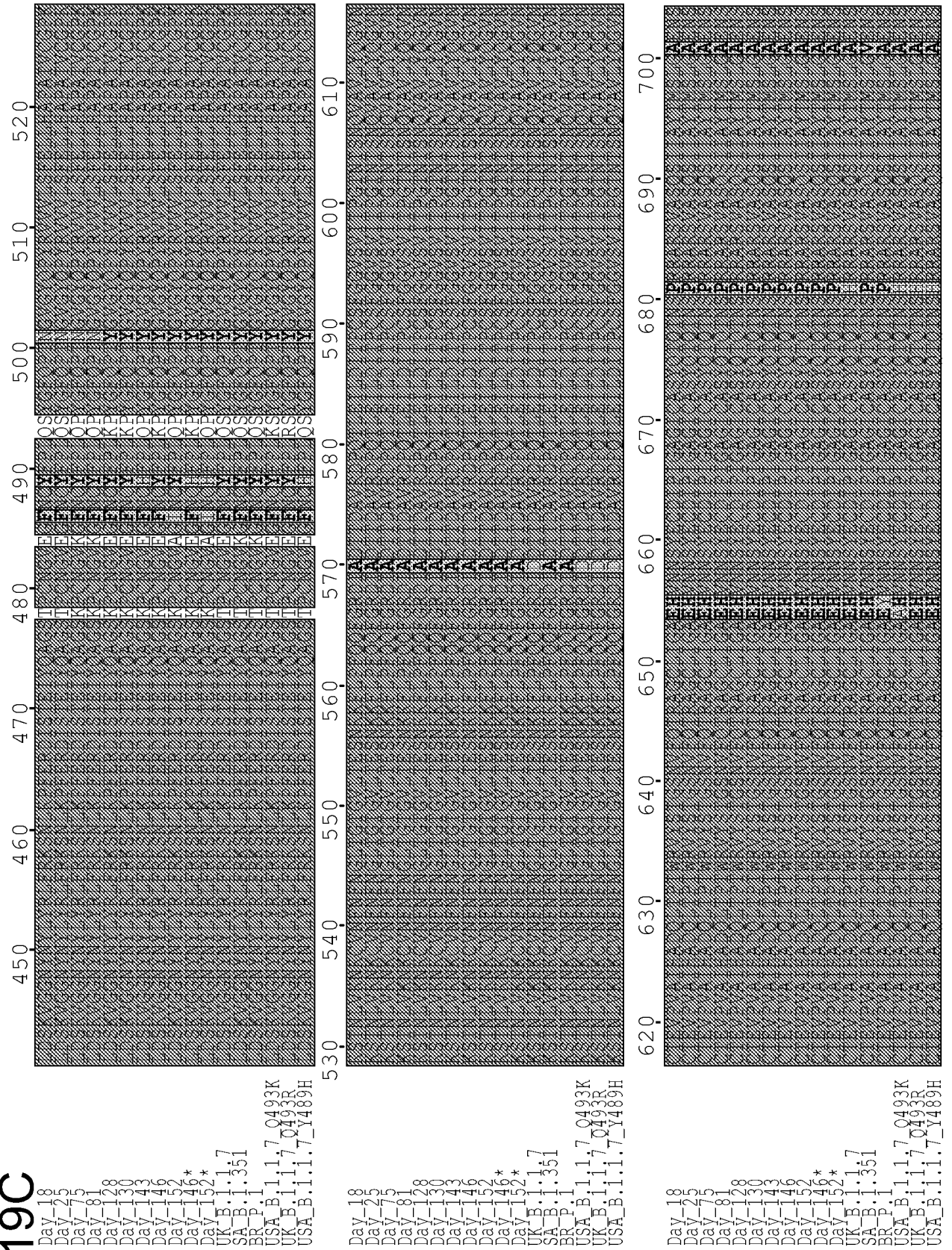


FIG. 19E

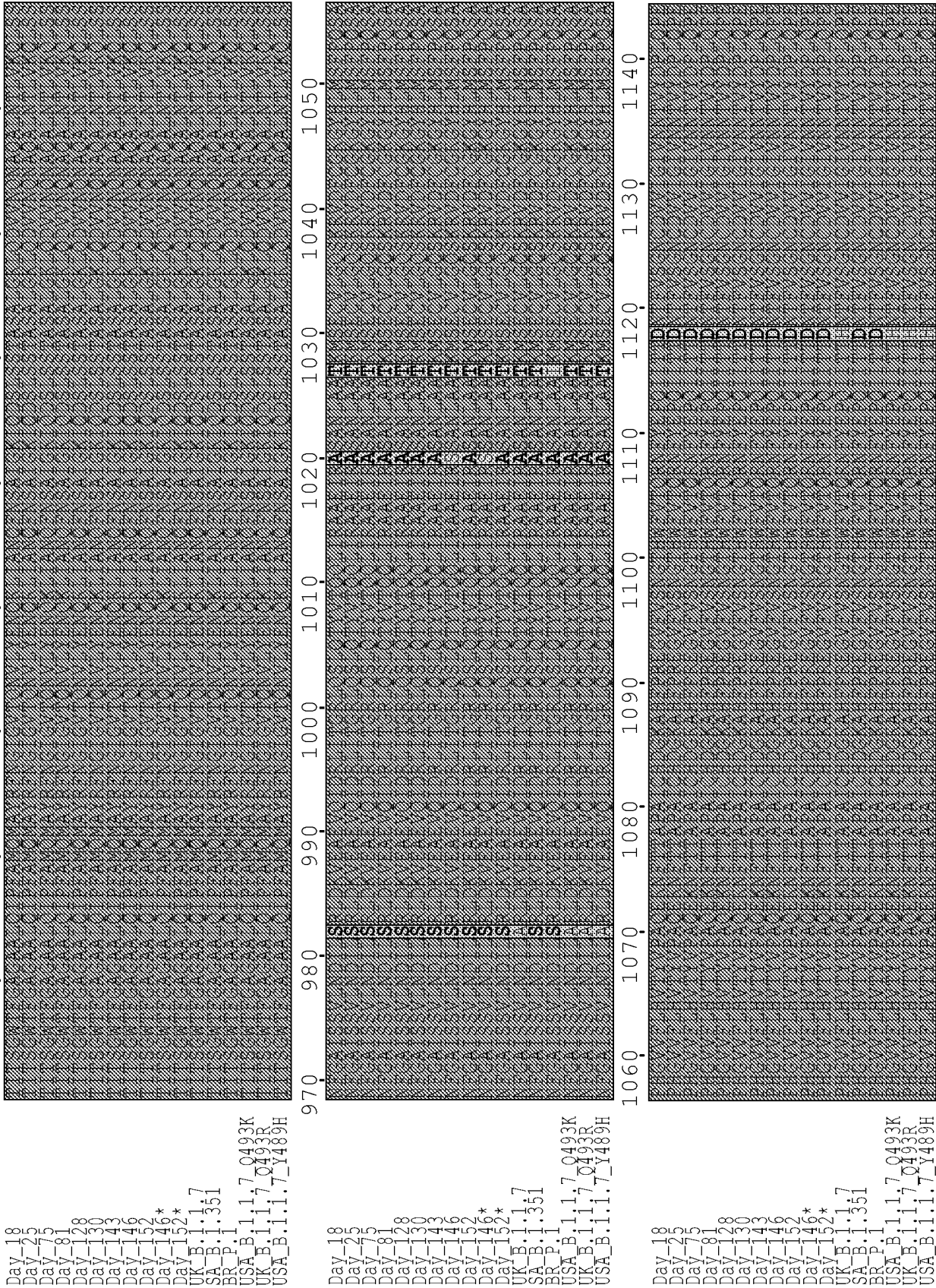


FIG. 19F

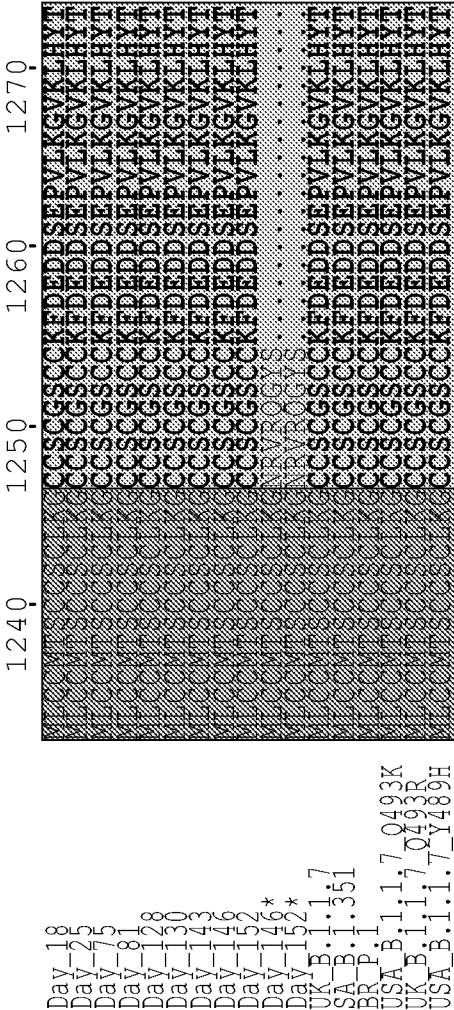
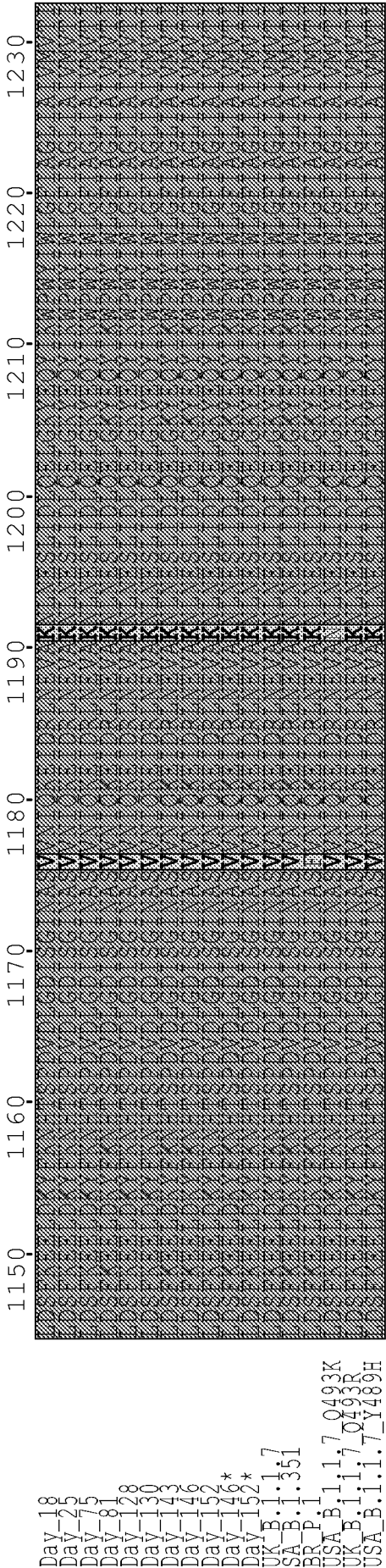
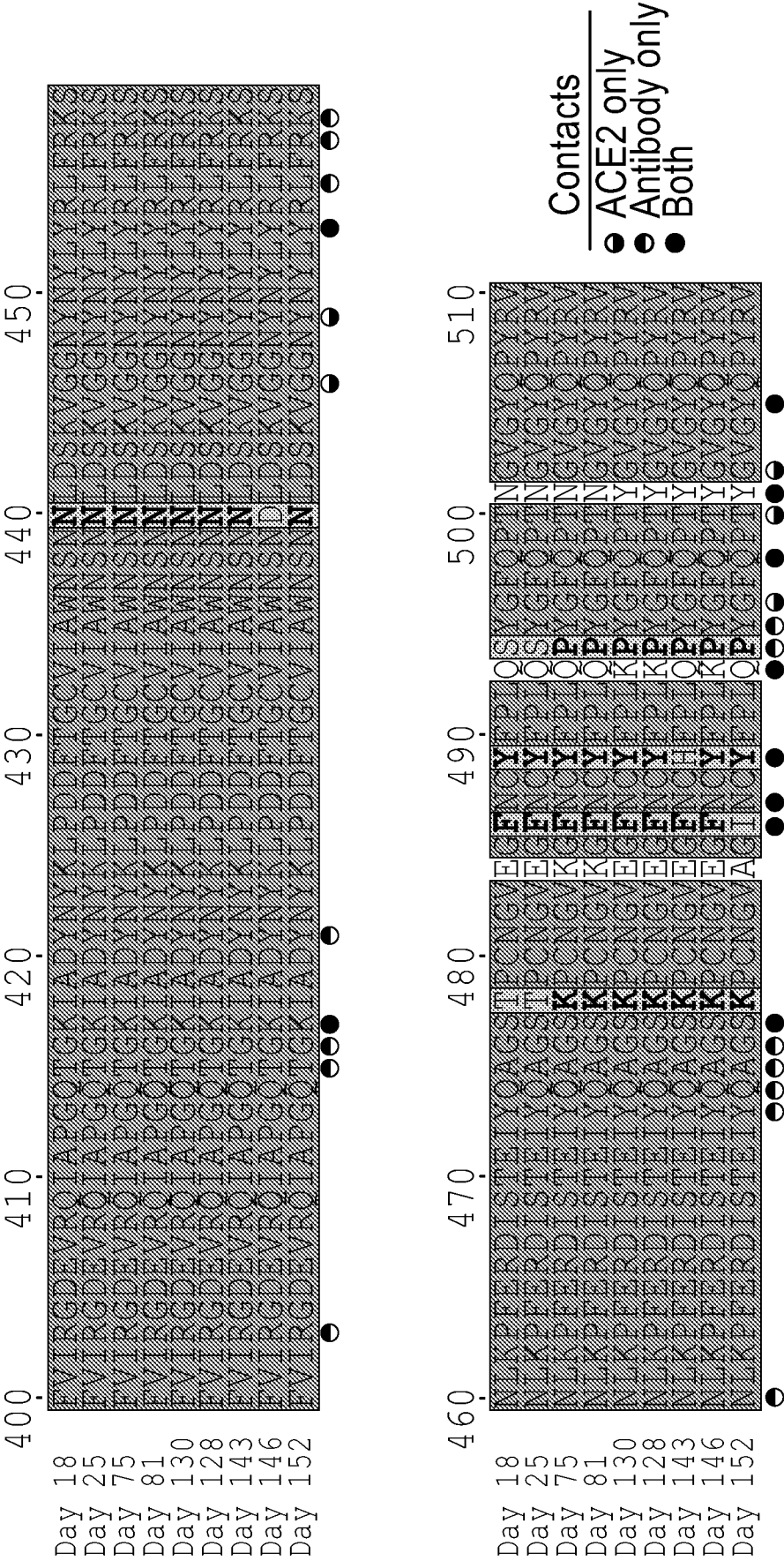
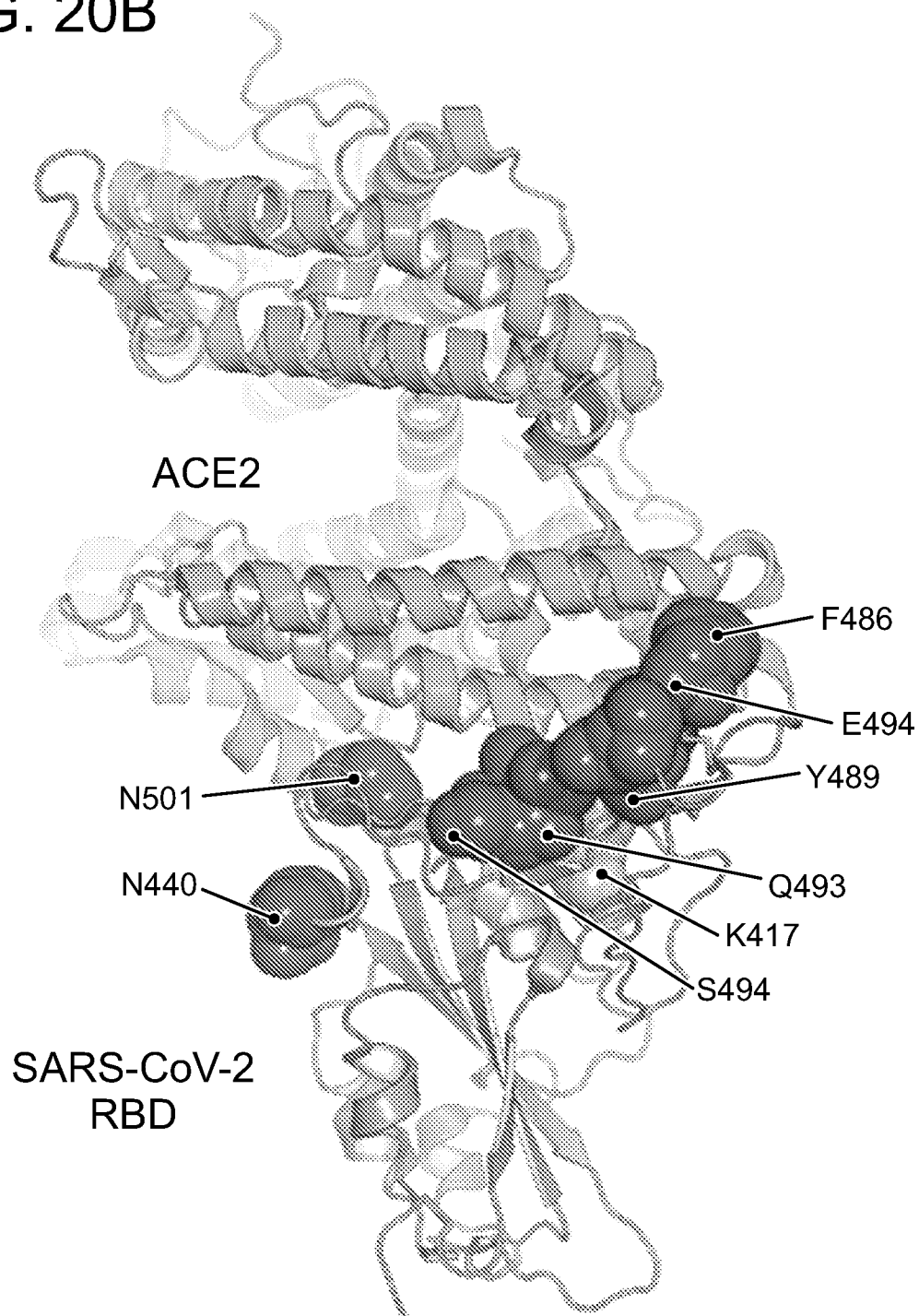


FIG. 20A



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FIG. 20B



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FIG. 20C

Q493

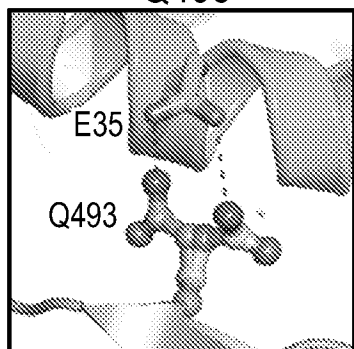


FIG. 20D

N501

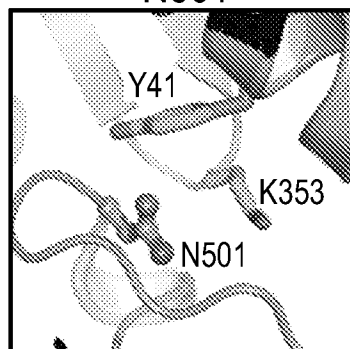


FIG. 20E

E484

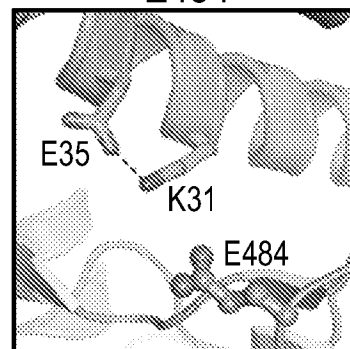


FIG. 20F

F486

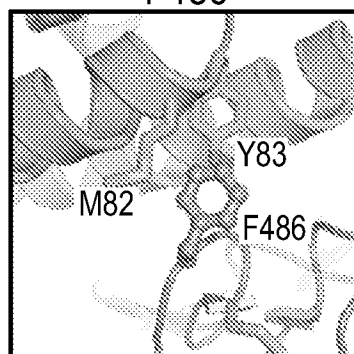


FIG. 20G

Y489

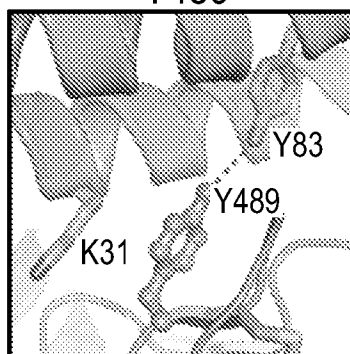


FIG. 20H

K417

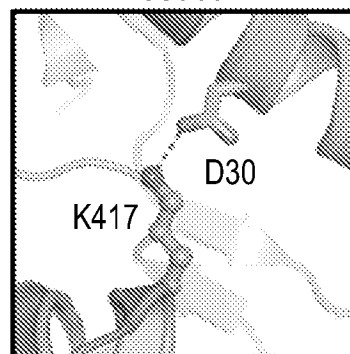


FIG. 20I

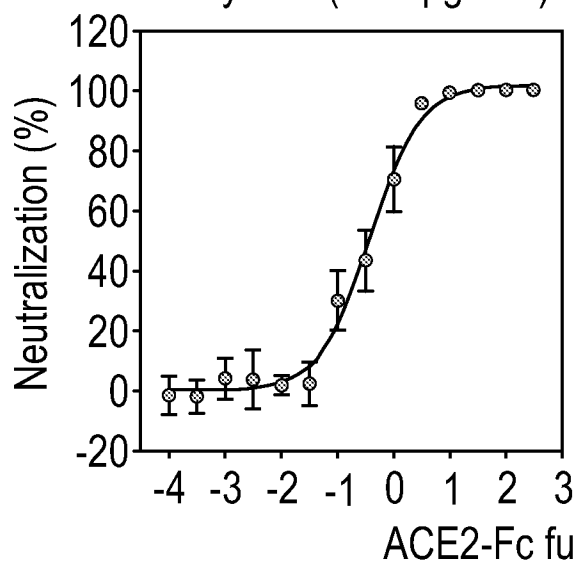
Day 146*(0.38 $\mu\text{g ml}^{-1}$)

FIG. 20J

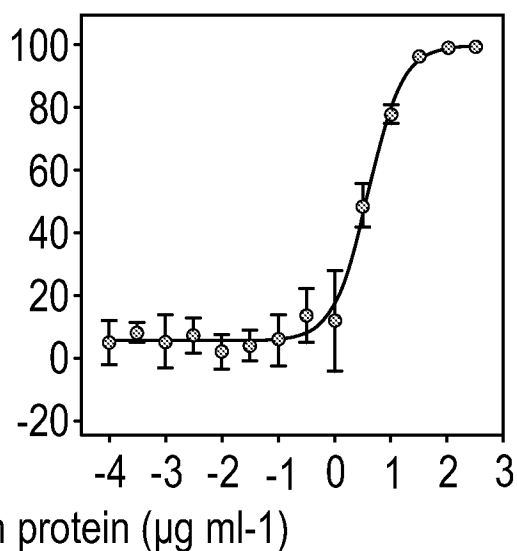
Day 152 (3.9 $\mu\text{g ml}^{-1}$)

FIG. 21A

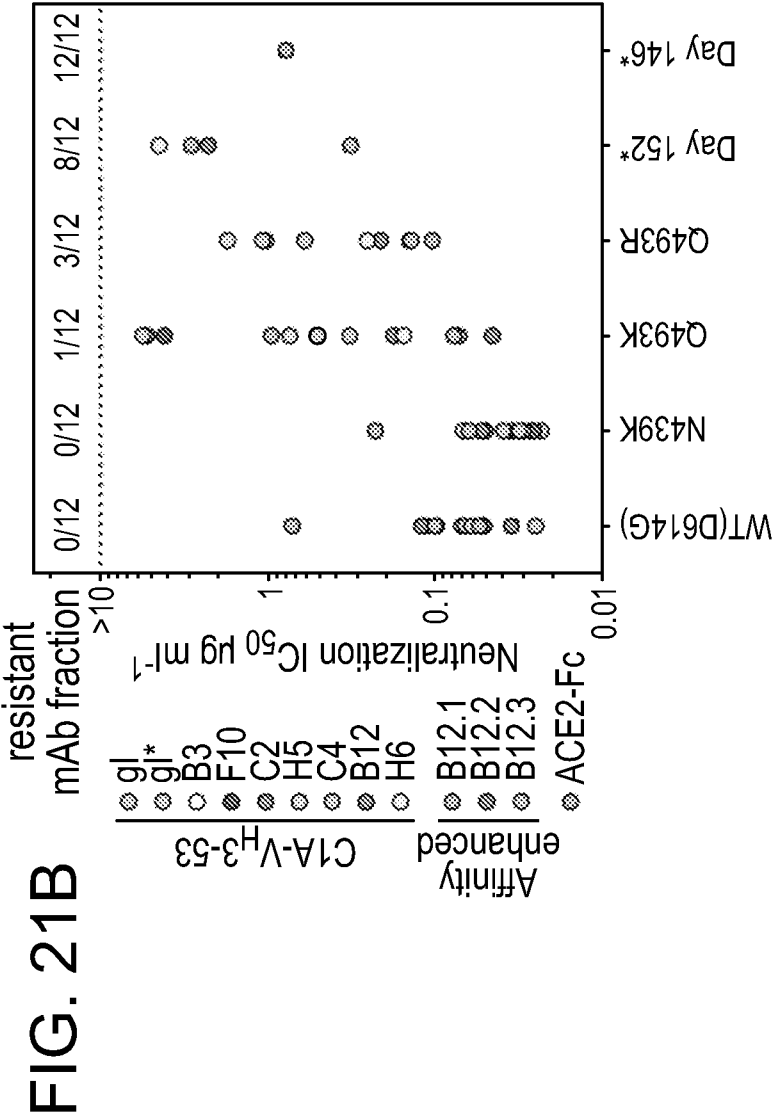
	C1A-V _H 3-53										Affinity enhanced		
	ACE2-Fc	gl	gl*	B3	F10	C2	H5	C4	B12	H6	B12.1	B12.2	B12.3
WT (D614G)	0.725	0.098	0.025	0.069	0.051	0.052	0.060	0.066	0.035	0.114	0.054	0.115	0.090
N439K	0.226	0.039	0.031	0.052	0.026	0.035	0.030	0.023	0.027	0.068	0.050	0.052	0.062
Q493K	0.965	0.513	5.63	>10	4.18	5.32	0.749	0.321	0.176	0.153	0.072	0.045	0.077
Q493R	1.04	1.09	>10	>10	7.98	>10	1.76	0.610	0.211	0.252	0.104	0.141	0.139
Day 152*	>10	>10	>10	>10	>10	>10	>10	>10	>10	4.48	>10	>10	>10
Day 146*	0.789	>10	>10	>10	>10	>10	>10	>10	>10	>10	2.88	2.30	0.318

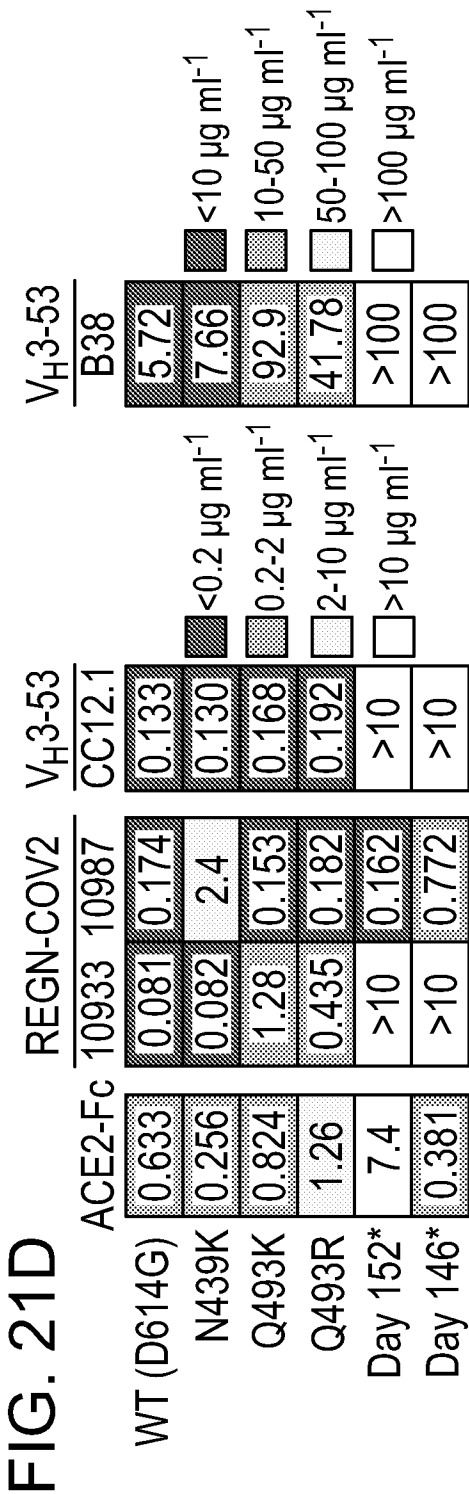
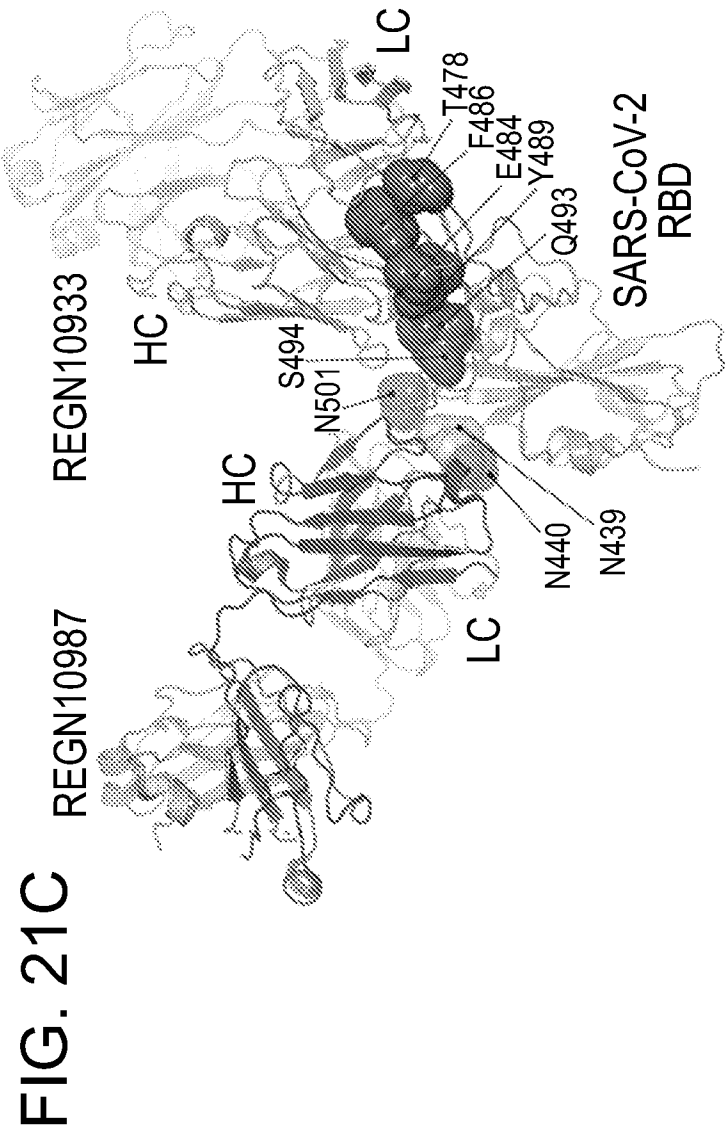
<0.2 µg ml⁻¹

0.2-2 µg ml⁻¹

2-10 µg ml⁻¹

>10 µg ml⁻¹





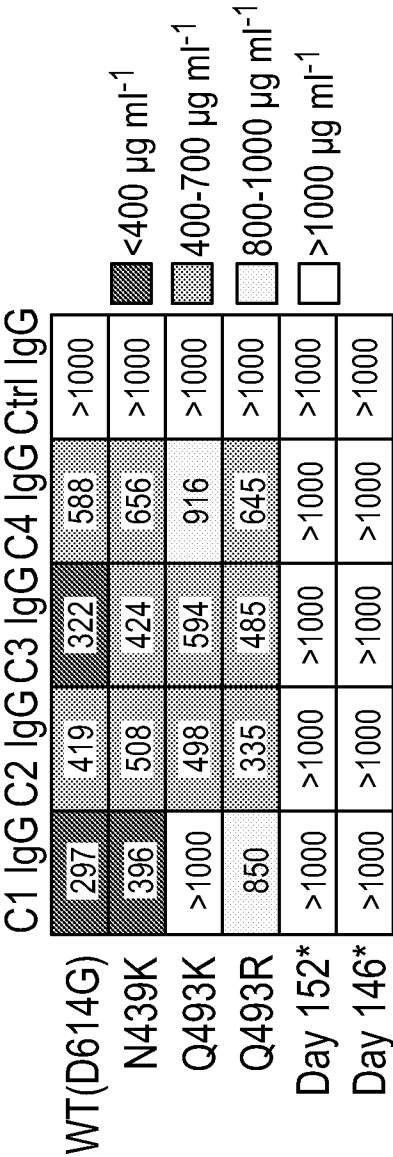
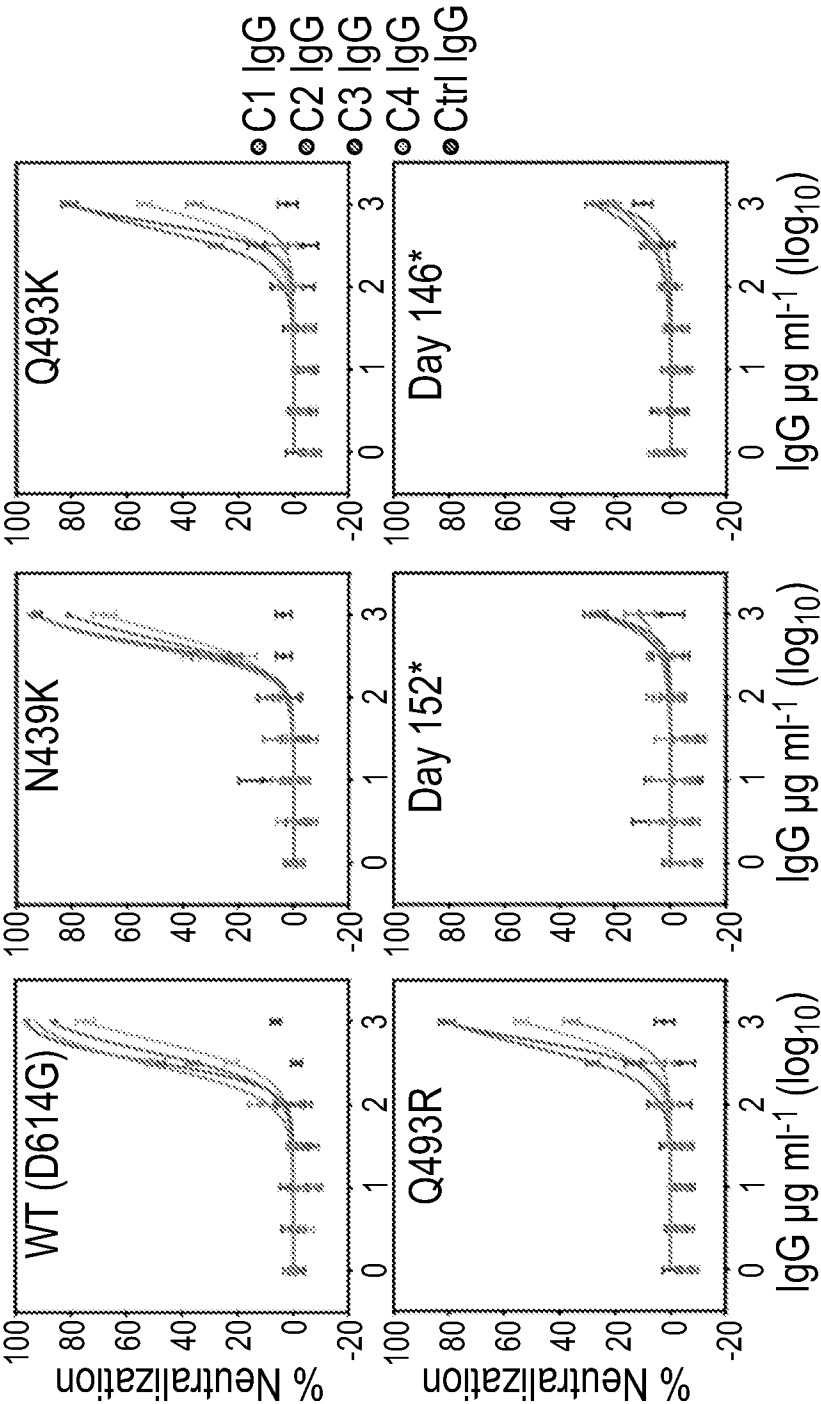
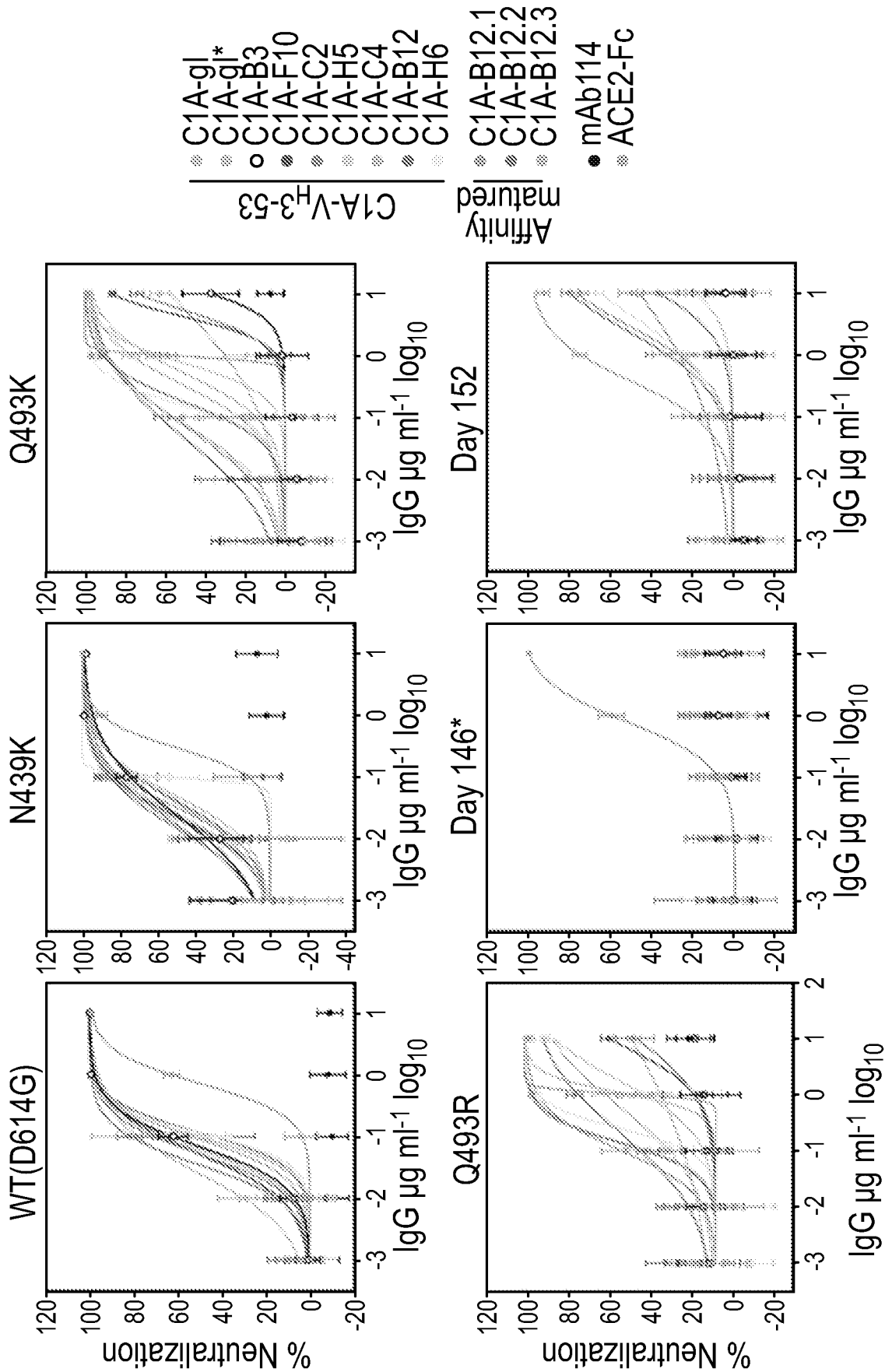


FIG. 22A



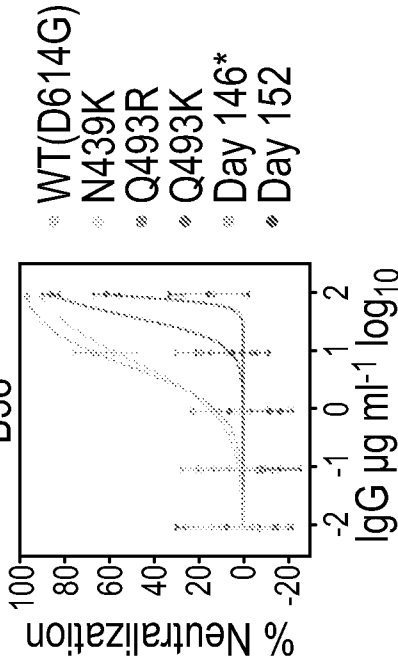
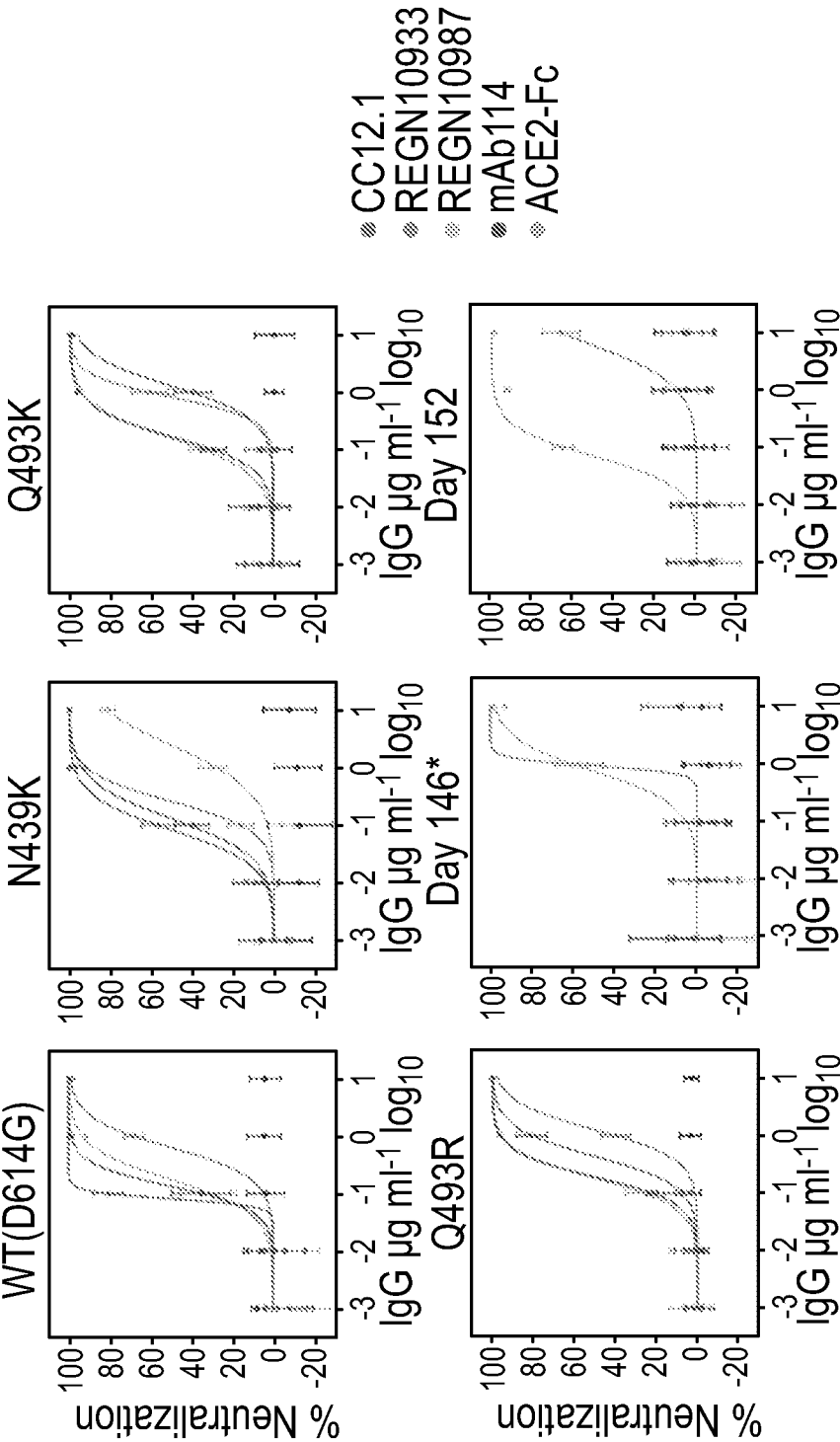


FIG. 22B

FIG. 22C

FIG. 23B

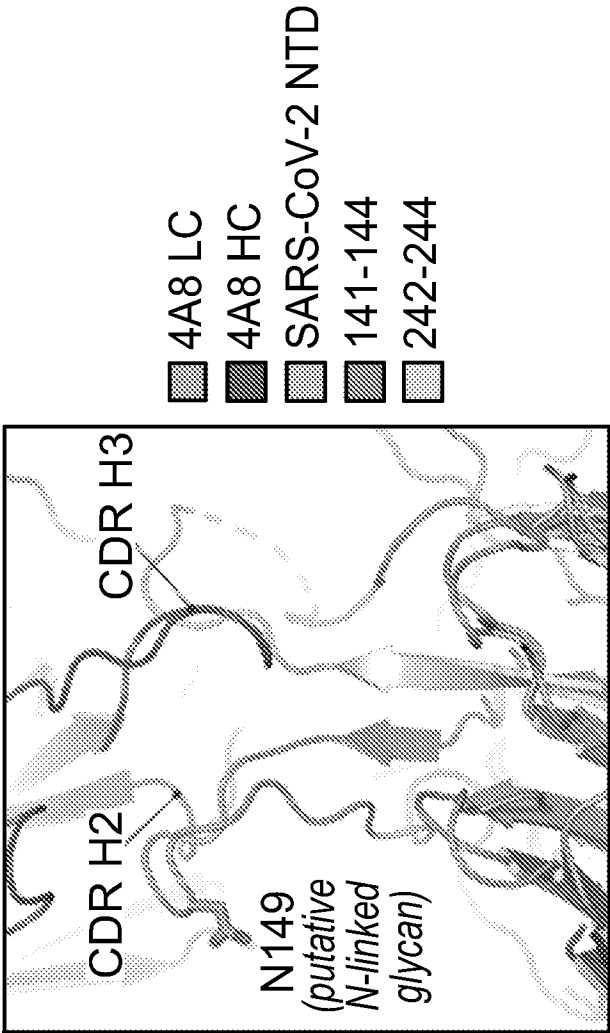


FIG. 23A

Variant	NTD Δ
Day 18	-
Day 25	-
Day 75	141-143
Day 81	141-143
Day 128	141-143
Day 130	141-143
Day 143	141-143
Day 146	141-143
Day 152	141-144
UK (B.1.1.7)	144
SA (B.1.351)	242-244