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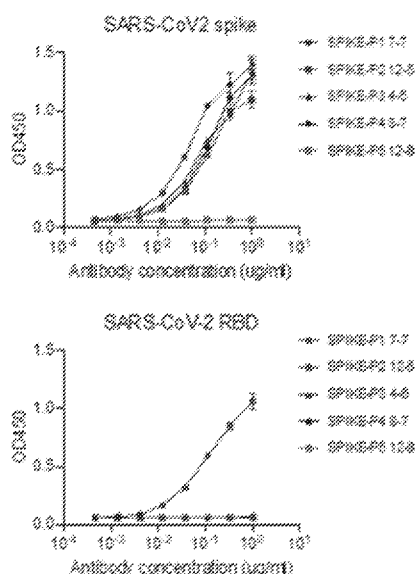
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(54) Title: COMPOSITIONS AND METHODS RELATING TO SARS-COV-2 NEUTRALIZING ANTIBODIES

(57) Abstract: Described herein are antibody reagents that bind therapeutic targets such as SARS-COV2 spike protein, as well as methods of using such antibody reagents.

**Figs. 4D**

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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COMPOSITIONS AND METHODS RELATING TO SARS-COV-2 NEUTRALIZING ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 63/256,384 filed October 15, 2021; 63/305,424 filed February 1, 2022, and 63/346,654 filed May 27, 2022, the contents of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

[0002] The technology described herein relates to anti-SARS-COV-2 antibody reagents and methods of using such antibody reagents.

BACKGROUND

[0003] The ongoing COVID-19 pandemic and the previous SARS outbreak highlight the danger of recurrent human infections by the emergence of novel pathogenic variants of Coronavirus, which may be resistant to pre-existing human immunity and available vaccines or drugs. Accordingly, described herein is the creation of antibodies that specifically bind S proteins from SARS CoV-2 and related viruses in this family. The approach described herein provides antibodies that target conserved regions on S protein from the viral family and broadly neutralize different types of Coronavirus, including potential emergent variants in the future.

SUMMARY

[0004] The technology described herein provides an antibody, antibody reagent, or antigen-binding fragment thereof, that specifically binds a SARS-COV-2 polypeptide, said antibody reagent, or antigen-binding fragment thereof, comprising at least one heavy or light chain complementarity determining region (CDR) selected from Table 1 or Table 3. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment thereof, comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or Table 3 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises light chain CDRs having the amino acid sequences selected from Table 1 or Table 3 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or Table 3 and light chain CDRs having the amino acid sequences selected from Table 1 or Table 3 or a conservative substitution variant of such amino acid sequence.

[0005] In one aspect of any of the embodiments, described herein is an antibody, antibody reagent, antigen-binding fragment thereof, or chimeric antigen receptor (CAR), that specifically binds a SARS-COV-2 polypeptide, said antibody reagent, or antigen-binding fragment thereof comprising at least one, at least three, or six heavy or light chain complementarity determining region (CDR)s selected from:

- a. the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
- b. the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
- c. the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
- d. the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
- e. the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
- f. the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
- g. the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
- h. the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- i. the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j. the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k. the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l. the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- m. the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n. the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- o. the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- p. the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- q. the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;

- r. the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;
- s. the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t. the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u. the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v. the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w. the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x. the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y. the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z. the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa. the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb. the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc. the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- dd. the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- ee. the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ff. the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- gg. the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- hh. the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
- ii. the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;

- jj. the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
- kk. the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
- ll. the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
- mm. the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
- nn. the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
- oo. the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
- a conservative substitution variant thereof.

In some embodiments of any aspects, the antibody, antibody reagent, or antigen-binding fragment thereof comprises VH and/or VL amino acid sequences selected from:

- a. SEQ ID NOs: 1 and 2;
- b. the VH sequences of one of SEQ ID NOs: 1, 322, 324, 326, 328, and 330-348 and the VL sequences of SEQ ID NO: 2 and 349;
- c. SEQ ID NOs: 9 and 10;
- d. SEQ ID NOs: 17 and 18;
- e. SEQ ID NOs: 25 and 26;
- f. SEQ ID NOs: 33 and 34;
- g. SEQ ID NOs: 41 and 42;
- h. SEQ ID NOs: 49 and 50;
- i. SEQ ID NOs: 57 and 58;
- j. SEQ ID NOs: 65 and 66;
- k. SEQ ID NOs: 73 and 74;
- l. SEQ ID NOs: 81 and 82;
- m. SEQ ID NOs: 89 and 90;
- n. SEQ ID NOs: 97 and 98;
- o. SEQ ID NOs: 105 and 106;
- p. SEQ ID NOs: 113 and 114;
- q. SEQ ID NOs: 121 and 122;
- r. SEQ ID NOs: 129 and 130;
- s. SEQ ID NOs: 137 and 138;
- t. SEQ ID NOs: 145 and 146;
- u. SEQ ID NOs: 153 and 154;

- v. SEQ ID NOs: 161 and 162;
- w. SEQ ID NOs: 169 and 170;
- x. SEQ ID NOs: 177 and 178;
- y. SEQ ID NOs: 185 and 186;
- z. SEQ ID NOs: 193 and 194;
- aa. SEQ ID NOs: 201 and 2025;
- bb. SEQ ID NOs: 209 and 210;
- cc. SEQ ID NOs: 217 and 218;
- dd. SEQ ID NOs: 225 and 226;
- ee. SEQ ID NOs: 233 and 234;
- ff. SEQ ID NOs: 241 and 242;
- gg. SEQ ID NOs: 249 and 250;
- hh. SEQ ID NOs: 257 and 258;
- ii. SEQ ID NOs: 265 and 266;
- jj. SEQ ID NOs: 273 and 274;
- kk. SEQ ID NOs: 281 and 282;
- ll. SEQ ID NOs: 289 and 290;
- mm. SEQ ID NOs: 297 and 298;
- nn. SEQ ID NOs: 305 and 306;
- oo. SEQ ID NOs: 313 and 314; or
- a conservative substitution variant thereof.

In some embodiments of any of the aspects, the antibody, antibody reagent, or antigen-binding fragment thereof comprises one or more of:

- a. a heavy chain CDR3 in which the sequence “YGRS” of the heavy chain CDR3 is mutated to comprise “YGSS” or “YGGS”;
- b. a VH in which the sequence “FDVW” of the VH is mutated to comprise “FDLW”;
- c. a VH in which the sequence “WGAG” of the VH is mutated to comprise “WGRG”;
- d. a VH in which the sequence “GTTV” of the VH is mutated to comprise “GTLV”; and
- e. a VL in which the sequence “FGGG” of the VL is mutated to comprise “FGQG.”

[0006] In one embodiment of any aspect, a first antibody, antibody reagent, or antigen-binding fragment that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with a second antibody comprising a heavy chain CDRs as described herein, e.g., having the amino acid sequences selected from Table 1 and light chain CDRs as

described herein, e.g., having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the first antibody, antibody reagent, or antigen-binding fragment comprises heavy chain CDRs as described herein, e.g., as having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises heavy chain CDRs as described herein, e.g., having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises light chain as described herein, e.g., CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises heavy chain CDRs as described herein, e.g., having the amino acid sequences selected from Table 1 and light chain CDRs as described herein, e.g., having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.

[0007] In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises a heavy chain variable region sequence as described herein, e.g., selected from Table 1. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises a light chain variable region sequence as described herein, e.g., selected from Table 1. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises a heavy chain variable region sequence as described herein, e.g., selected from Table 1 and a light chain variable region sequence as described herein, e.g., selected from Table 1. In one embodiment of any aspect, the antibody, antibody reagent, antigen-binding fragment as described herein, e.g., from Table 1, further comprises a conservative substitution in a sequence not comprised by a CDR.

[0008] In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment described herein is fully human or fully humanized. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment described herein is fully humanized except for the CDR sequences. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment described herein is selected from the group consisting of an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody

[0009] In one embodiment of any aspect, a nucleic acid sequence encodes the antibody, antibody reagent, or antigen-binding fragment described herein. In one embodiment of any aspect,

a cell comprises the antibody, antibody reagent, or antigen-binding fragment or the related nucleic acid sequence.

[0010] In one aspect of any of the embodiments, described herein is a pharmaceutical composition comprising at least one antibody, antibody reagent, or antigen-binding fragment and/or a cell comprising the antibody, antibody reagent, or antigen-binding fragment thereof; or a nucleic acid encoding the antibody, antibody reagent, CAR, or antigen-binding fragment thereof, and a pharmaceutically acceptable carrier. In one embodiment of any aspect, the pharmaceutical composition further comprises at least one antibody, antibody reagent, or antigen-binding fragment thereof and at least one of casirivcimab, imdevimab, or sotrovimab.

[0011] In one aspect of any of the embodiments, described herein is a solid support comprises the antibody, antibody reagent, antigen-binding fragment thereof, or CAR described herein. In one embodiment of any aspect, the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is detectably labeled. In one embodiment of any aspect, the solid support comprises a particle, a bead, a polymer, or a substrate.

[0012] In one aspect of any of the embodiments, described herein is a kit for the detection of a SARS-COV-2 polypeptide in a sample, comprises at least a first antibody, antibody reagent, antigen-binding fragment thereof, or CAR as described herein immobilized on a solid support and comprises a detectable label.

[0013] In one aspect of any of the embodiments, described herein is a molecular complex comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein bound to a SARS-COV-2 polypeptide.

[0014] In one aspect of any of the embodiments, described herein is a method of treating a coronavirus infection in a subject in need thereof, the method comprising administering to the subject:

- i. an antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein;
- ii. a composition comprising an antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein; and/or
- iii. a cell comprising an antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein.

[0015] In one aspect of any of the embodiments, described herein is the antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein; a composition comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein; and/or a cell comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof as described herein; for use in a method of treating a coronavirus infection in a subject in need thereof, the method comprising administering the antibody, antibody reagent, or antigen-binding fragment thereof, composition, and/or cell to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figs. 1A-1C depict the generation and Characterization of the VRC01-rearranging Mouse Models. Fig. 1A: Illustration of genetic modifications in the VRC01-rearranging mouse models. The mouse VH81X was replaced with human VH1-2*02 on the allele lacking IGCR1. The mouse Vk3-2 and Vk3-7 were replaced with the human Vk1-33 and Vk3-20 with a 50bp downstream CBE, respectively. The Cer/sis elements were deleted on the human Vk1-33 alleles. Fig. 1B: HTGTS-rep-seq analysis of Vk usage in wild type 129/Sv mice and Cer/sis deleted mice. The x axis represents Vk locus from the distal to the proximal ends. The histogram displays the percent usage of each Vk of all productive VkJk rearrangements. The deletional Vks were labeled in light blue and dark blue, and the inversional Vks were labeled in orange and red. Fig. 1C: HTGTS-rep-seq analysis of Vk usage in VRC01^{Vk1-33}, VRC01^{Vk1-33-CS} and VRC01^{Vk3-20} mice. The usage of human Vk1-33 and Vk3-20 were labeled in orange and purple, respectively. Data from Figs. 1B and 1C were average of three experimental repeats with error bars representing SDs.

[0017] Figs. 2A-2I demonstrate that human TdT expression further diversified the mouse light chain repertoire. Fig. 2A: Illustration of human TdT gene knocked into mouse Rosa locus. Fig. 2B: Length distribution of N and P nucleotides in Igk LC CDR3s from human, wild-type 129/Sv mouse and hTdT KI mouse naïve B cells. Fig. 2C: The diversity of Igk LC CDR3s. The y axis is the profragment of unique LC CDR3s in total LC CDR3s. All HTGTS-rep-seq libraries were normalized to 10,000 productive junctions for comparison. Fig. 2D: Length distribution of Igk LC CDR3s in human, wild-type mouse and hTdT KI mouse naïve B cells. The statistical comparison for each length was performed between wild-type mouse and hTdT KI mouse CDR L3s. Fig. 2E: Distribution of Microhomology-mediated VkJk Joins in Igk LC CDR3s. Fig. 2F: The diversity of Vk1-33 LC CDR3s in human, wild-type mouse and VRC01^{Vk1-33-CS} mouse naïve B cells. All HTGTS-rep-seq libraries were normalized to 700 productive junctions. Fig. 2G: Length distribution of Vk1-33 LC CDR3s. The profragments of 5-aa LC CDR3s are labelled for comparison. Fig. 2H: The diversity of Vk3-20 LC CDR3s in human, wild-type mouse and VRC01^{Vk3-20} mouse naïve B cells. All HTGTS-rep-seq libraries were normalized to 1000 productive junctions. Fig. 2I: Length distribution of Vk3-20 LC CDR3s. Data from Figs. 2B-2I were average of several experimental repeats (n>=3) with error bars representing SDs. Statistical comparisons were performed using a two-tailed t test. **p* < 0.05, ***p* < 0.01.

[0018] Figs. 3A-3G demonstrate that a strong VRC01-class GC response was induced by eOD-GT8 60mer in VRC01-rearranging mouse models. Fig. 3A: Immunization schema. Fig. 3B: The profragment of VRC01-class GC B cells that express paired V_H1-2 HC and LC with a 5-aa CDR3 among CD4bs-specific GC B cells. Bars represent mean with SD. Fig. 3C: Sequence conservation at each position of 5-aa CDR L3 in naïve repertoires and day 8 GCs induced by eOD-GT8 60mer. The sequences of 5-aa CDR L3s in naïve B cells are derived from HTGTS-rep-seq data shown in Figs.

1C, 8A, and 8C. Fig. 3D: Immunization schema for VRC01^{Vk1-33/Vk3-20/TdT} mice. Fig. 3E: Amino acid mutation frequency in VRC01-class antibodies cloned from both day 8 and day 36 GCs. The median with interquartile range is plotted. Fig. 3F: Amino acid mutations in V_H1-2 HCs of VRC01-class antibodies cloned from day 36 GCs shown in sequence logo profiles. The Q61R mutant was labelled in green. For reference, the intrinsic mutation patterns from non-productive rearrangements are represented in the bottom. Fig. 3G: The binding affinity of the cloned VRC01-class antibodies from day 8 and day 36 GCs to eOD-GT8. Bars represent geometric mean (red). Statistical comparisons in Figs. 3B, 3E, and 3F were performed using a two-tailed t test. **p* < 0.05, ***p* < 0.01, *****p* < 0.0001.

[0019] Figs. 4A-4I demonstrate that novel anti-SARS-CoV2 antibodies were isolated from VRC01 mouse model. Fig. 4A: Illustration of genetic modifications in VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mouse model. Fig. 4B: Immunization schema. Fig. 4C: CDR3 length and sequences of 20 mAbs cloned from spike-specific IgG⁺ B cells. Fig. 4D: Results of ELISAs measuring mAb binding to spike and RBD. Data were average of 3 experimental repeats with error bars representing SDs. Fig. 4E: Results of ELISAs measuring SPIKE-P1 7-7 mAb binding to RBD variants. Fig. 4F: Pseudovirus neutralization assays of SPIKE-P1 7-7 mAb against 7 SARS-CoV2 variants. Fig. 4G: PRNT live virus neutralization assays of SPIKE-P1 7-7 against 4 SARS-CoV2 variants. Fig. 4H: Negative stain 3D reconstructions of SARS-CoV2 spike and SPIKE-P1 7-7 Fab complex. Fig. 4I: Footprint and angle of approach of SPIKE-P1 7-7 mAb.

The nomenclature used in Fig. 4C-4D corresponds to the nomenclature of Table 1 as follows:

Name in Figs. 4C-4D	Name in Table 1
SPIKE-P1	SPIKE-BC1
SPIKE-P2	SPIKE-BC2
SPIKE-P3	SPIKE-BC3
SPIKE-P4	SPIKE-BC4
SPIKE-P5	No correlating antibody present in Table 1
SPIKE-P1 7-7	SPIKE-BC1-7-7
SPIKE-P2 12-5	SPIKE-BC2-12-5
SPIKE-P3 4-6	SPIKE-BC3-4-6
SPIKE-P4 8-7	SPIKE-BC4-8-7
SPIKE-P5 12-8	No correlating antibody present in Table 1

[0020] Figs. 5A-5D depict V_H1-2-rearranging HCs with diverse CDR3s, related to Figs. 1A-1C. Fig. 5A, HTGTS-rep-seq analysis of V_H nonproductive rearrangements in VRC01^{Vk1-33-CS} splenic B cells lacking IGCR element on IgH locus. The mouse V_H81X was replaced by human V_H1-2. The x axis represents V_H locus from the distal to the D_H-proximal ends. The histogram displays the percent of nonproductive rearrangements of each V_H among all V_H nonproductive rearrangements. The frequencies of V_H nonproductive rearrangements represent the V_H usages in primary V(D)J

rearrangements. Fig. 5B, HTGTS-rep-seq analysis of V_H usage in VRC01^{Vk1-33-CS} splenic B cells. Fig. 5C, Length distribution of V_H1-2 HC CDR3 in VRC01^{Vk1-33-CS} and VRC01^{Vk3-20} splenic B cells. Fig. 5D, Venn diagram showed immensely HC CDR3 complexity in VRC01^{Vk1-33-CS} and VRC01^{Vk3-20} mouse models, since there is little overlap in HC CDR3 sequences in three mice. Data from Figs. 5A-5C were average of three experimental repeats with error bars representing SDs.

[0021] Figs. 6A-6C demonstrate that Cer/sis deletion increased the utilization of several proximal deletional Vk segments, related to Figs. 1A-1C. Fig. 6A, The diagram illustrates the deleted region of Cer/sis regulatory element targeted by two gRNAs and PCR primers that were used to identify the deleted clones. Fig. 6B, PCR analysis of ES clones with Cer/sis deletion. Fig. 6C, HTGTS-rep-seq analysis of Vk nonproductive rearrangements in wild type 129/Sv and Cer/sis deleted splenic B cells. The x axis represents Vk locus from the distal to the proximal ends. The histogram displays the percent of nonproductive rearrangements of each Vk among all nonproductive VkJk rearrangements. The deletional Vks were labeled in light blue and dark blue, and the inversional Vks were labeled in orange and red. The percentage of Vk segments in nonproductive rearrangements represents the V usage in primary V(D)J recombination. Data were average of three experimental repeats with error bars representing SDs.

[0022] Figs. 7A-7G depict the generation of the VRC01-rearranging LCs, Related to Figs. 1A-1c. Fig. 7A, The diagram, not drawn to scale, illustrates the restriction digests and Southern probe that were used to differentiate the region before (GL) and after Vk1-33 replacement. Fig. 7B, Southern analysis of positive ES clones that showed in (Fig. 7A). Fig. 7C, HTGTS-rep-seq analysis of Vk nonproductive rearrangements in VRC01^{Vk1-33} (up) and VRC01^{Vk1-33-CS} (bottom) splenic B cells. The x axis represents Vk locus from the distal to the proximal ends. The histogram displays the percent of nonproductive rearrangements of each Vk among all nonproductive VkJk rearrangements. The Vk1-33 was labeled in orange. The percentage of Vk segments in nonproductive rearrangements represents the V usage in primary V(D)J recombination. Fig. 7D, The diagram, not drawn to scale, illustrates the restriction digests and Southern probe that were used to differentiate the region before (GL) and after Vk3-20 replacement. Fig. 7E, Southern analysis of positive ES clones that showed in (Fig. 7D). Fig. 7F, HTGTS-rep-seq analysis of Vk nonproductive rearrangements in VRC01^{Vk3-20} splenic B cells. The Vk3-20 was labeled in purple. Fig. 7G, FACS analysis of splenic B cells from wild-type 129/Sv, VRC01^{Vk1-33-CS} and VRC01^{Vk3-20} mice. For each staining, 3 mice were used and displayed similar results.

[0023] Figs. 8A-8G demonstrate that TdT expression diversifies the mouse light chain repertoire, Related to Figs. 2A-2I. Fig. 8A, Frequency of 5-aa LC CDR3s in all mouse Igk LCs, Vk1-33 LCs and Vk3-20 LCs. Fig. 8B, Distribution of N or P nucleotides in CDR3s of human Igk LCs. The proportion of N or P nucleotides in 5-aa CDR3s is labeled in red. Fig. 8C, The diagram illustrates the restriction digest and Southern probe that were used to differentiate the region before and after TdT knock-in. Fig. 8D, Southern analysis of ES clone with TdT knock-in. Fig. 8E, Western

Blot of TdT expression in mouse pre-B cells before and after TdT knock-in. The TdT antibody can detect both human and mouse TdT. Fig. 8G, Distribution of N nucleotides in Igk LCs CDR3s with TdT expression. Fig. 8G, Length distribution HC CDR3s in mouse models with and without TdT expression. Data from Figs. 8A, 8B, 8F, and 8G were average of three experimental repeats with error bars representing SDs. Statistical comparisons were performed using a two-tailed t test. * $p < 0.05$, ** $p < 0.01$.

[0024] Figs. 9A-9D demonstrate the impact of TdT expression on the usages human Vk1-33 and Vk3-20 segments, Related to Figs. 2A-2I. Fig. 9A, HTGTS-rep-seq analysis of Vk usage in VRC01^{Vk1-33-CS/TdT} mice. The x axis represents Vk locus from the distal to the proximal ends. The histogram displays the percent usage of each Vk of all productive VkJk rearrangements. The usage of human Vk1-33 is labeled in orange. Fig. 9B, HTGTS-rep-seq analysis of non-productive Vk rearrangements in VRC01^{Vk1-33-CS} and VRC01^{Vk1-33-CS/TdT} mice. Fig. 9C, HTGTS-rep-seq analysis of Vk usage in VRC01^{Vk3-20/TdT} mice. The usage of human Vk3-20 is labeled in purple. Fig. 9D, HTGTS-rep-seq analysis of non-productive Vk rearrangements in VRC01^{Vk3-20} and VRC01^{Vk3-20/TdT} mice. Data from Figs. 9A-9D were average of three experimental repeats with error bars representing SDs.

[0025] Figs. 10A-10H demonstrate strong VRC01-class GC responses in both VRC01^{Vk1-33-CS} and VRC01^{Vk3-20} mouse models, Related to Figs. 3A-3G. Fig. 10A, Gating strategy for single cell sorting of eOD-GT8-specific germinal center B cells after eOD-GT8 60mer immunization. Fig. 10B, Proportion of GC B cells. Fig. 10C, Proportion of CD4bs-specific GC B cells. Figs. 10D-10E, Pie charts illustrate the proportion of V_H1-2 heavy chain and Igk light chain with 5-aa CDR L3 in the cloned antibodies from CD4bs-specific GC B cells. Each pie chart represents the data from one mouse. The amount of cloned antibodies is labelled in the center of pie chart. Figs. 10F, 10G, and 10H, The frequency of E residue in the 4th position of CDR L3s before (naïve) and after eOD-GT8 60mer immunization. The 5-aa CDR L3s of naïve repertoire is derived from the HTGTS-rep-seq data showed in Fig. 1c, Fig. 9a and 9c. The 5-aa CDR L3s of GC is derived from data in Fig. 10D and 10E. Data from Figs. 10B, 10C, 10F, 10G, and 10H were average of more than three experimental repeats with error bars representing SDs. Statistical comparisons were performed using a two-tailed t test. * $p < 0.05$, ** $p < 0.01$.

[0026] Figs. 11A-11B depict the immunization of VRC01^{Vk1-33-CS/Vk3-20/TdT} Mouse Model with eOD-GT8 60mer, Related to Figs. 3A-3G. Fig. 11A, HTGTS-rep-seq analysis of Vk usage in VRC01^{Vk1-33-CS/Vk3-20/TdT} mouse splenic B cells. The usage of human Vk1-33 is labeled in orange, and the usage of human Vk3-20 is labeled in purple. Data were average of three experimental repeats with error bars representing SDs. Fig. 11B, Table showing the VRC01-class B cells elicited by eOD-GT8 60mer in VRC01^{Vk1-33-CS/Vk3-20/TdT} mouse model. 48 CD4bs-specific GC B cells were sorted from each mice on day 8 GCs post-immunization. The VRC01-class BCRs were identified by single cell RT-PCR following sanger sequencing.

[0027] Figs. 12A-12J demonstrate that VRC01-class antibodies develop SHM and affinity maturation in VRC01^{Vk1-33/Vk3-20/TdT} mice after eOD-GT8 60mer immunizations, Related to Figs. 3A-3G. Fig. 12A, HTGTS-rep-seq analysis of Vk usage in VRC01^{Vk1-33/Vk3-20/TdT} mouse splenic B cells. The usage of human Vk1-33 is labeled in orange, and the usage of human Vk3-20 is labeled in purple. Fig. 12B, FACS analyses of GC B cells on both day 8 and day 36 post-immunization with eOD-GT8. The boxed CD4bs-specific GC B cells were sorted for single cell RT-PCR. Fig. 12C, The Proportion of GC B cells. Fig. 12D, Proportion of CD4bs-specific GC B cells. Fig. 12E, Pie charts illustrate the proportion of V_H1-2 HCs and Igk LCs with 5-aa CDR3s in the cloned antibodies from CD4bs-specific GC B cells. Each pie chart represents the data from one mouse. The amount of cloned antibodies is labelled in the center of pie chart. Fig. 12F, Nucleotide mutation frequency in human VRC01-class antibodies cloned from both day 8 and day 36 CD4bs-specific GC B cells. Each dot represents one heavy chain or one light chain. The median with interquartile range is plotted. Fig. 12G, the frequency of Q61R mutant in D36 V_H1-2 HC compared to that in intrinsic mutation patterns. Fig. 12H, Length distribution of HC CDR3s in naïve repertoire, VRC01/Vk1-33 and VRC01/Vk3-20 antibodies that cloned from GCs induced by eOD-GT8. Fig. 12I, 5-aa LC CDR3 sequences in naïve repertoire and VRC01-class antibodies cloned from GCs induced by eOD-GT8. Fig. 12J, Amino acid mutations in all Vk1-33 and Vk3-20 LCs of VRC01-class antibodies that cloned from D36 GCs shown in sequence logo profiles. For reference, the intrinsic mutation patterns from non-productive rearrangements are represented below. Data from Figs. 12A, 12C, 12D, 12F, and 12G were average of several experimental repeats (n>=3) with error bars representing SDs. Statistical comparisons were performed using a two-tailed t test. **p* < 0.05, ***p* < 0.01

[0028] Figs. 13A-13D depict the GC response in VRC01^{Vk1-33/Vk3-20/TdT} mouse models immunized by 426c-degly3-Ferritin, Related to Figs. 3A-3G. Fig. 13A, FACS analyses of GC B cells on day 8 post-immunization with 426c-degly3-Ferritin. The boxed CD4bs-specific GC B cells were sorted for single cell RT-PCR. Fig. 13B, The Proportion of GC B cells. Fig. 13C, Proportion of CD4bs-specific GC B cells. Fig. 13D, Table shown the VRC01-class B cells elicited by 426c-degly3-Ferritin in 5 VRC01^{Vk1-33/Vk3-20/TdT} mice. 96 CD4bs-specific GC B cells were sorted from each mouse on day 8 GCs post-immunization. The VRC01-class BCRs were identified by single cell RT-PCR following sanger sequencing. Data from Figs. 13B and 13C were average of 5 mice with error bars representing SDs.

[0029] Figs. 14A-14J demonstrate that novel anti-SARS-CoV2 antibodies isolated from VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mice, Related to Figs. 4A-4J. Fig. 14A, Length distribution of V_H1-2 HC CDR3 in VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} splenic B cells. Fig. 14B, Venn diagram showed immensely HC CDR3 complexity in VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mouse models, since there is little overlap in HC CDR3 sequences in three mice. Fig. 14C, ELISA results shown the IgM (up) and IgG (bottom) titer in peripheral blood of VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mice compared to wild-type 129/Sv mice. Fig. 14D, FACS analyses of splenic B and T cell populations in VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mice

compared to wild-type 129/Sv mice. Fig. 14E, Results of ELISAs measuring serums on week 2 and week 6 post-immunizations with SARS-CoV2 spike binding to SARS-CoV2 spike, RBD, NTD and SARS-CoV1 spike. Gating strategy for single cell sorting of SARS-CoV2 spike-specific IgG⁺ B cells after SARS-CoV2 spike immunization. The upper panel showed the mice immunized with SARS-CoV2 spike and poly I:C adjuvant. The bottom panel showed the mice immunized with poly I:C only. Figs. 14F-14H, Results of ELISAs measuring mAbs binding to SARS-CoV2 spike, RBD and NTD. Figs. 14I-14J, Results of ELISAs measuring the cross-reactivity of mAbs binding to several coronavirus strains. Data from Figs. 14A, 14C, and 14E were average of three experimental repeats with error bars representing SDs.

[0030] The nomenclature used in Figs. 14F-14H corresponds to the nomenclature of Table 1 as follows:

Name in Figs. 14F-14H	Name in Table 1
SPIKE-P1 1-3	SPIKE-BC1-1-3
SPIKE-P1 7-7	SPIKE-BC1-7-7
SPIKE-P1 6-2	SPIKE-BC1-6-2
SPIKE-P1 9-6	SPIKE-BC1-9-6
SPIKE-P1 7-3	SPIKE-BC1-7-3
SPIKE-P2 1-6	SPIKE-BC2-1-6
SPIKE-P2 4-6	SPIKE-BC2-4-6
SPIKE-P2 12-2	SPIKE-BC2-12-2
SPIKE-P2 12-5	SPIKE-BC2-12-5
SPIKE-P3 4-6	SPIKE-BC3-4-6
SPIKE-P3 4-2	SPIKE-BC3-4-2
SPIKE-P3 9-8	SPIKE-BC3-9-8
SPIKE-P3 10-1	SPIKE-BC3-10-1
SPIKE-P4 1-5	SPIKE-BC4-1-5
SPIKE-P4 8-7	SPIKE-BC4-8-7
SPIKE-P4 6-5	SPIKE-BC4-6-5
SPIKE-P4 7-3	SPIKE-BC4-7-3
SPIKE-P4 10-2	SPIKE-BC4-10-2
SPIKE-P4 11-3	SPIKE-BC4-11-3
SPIKE-P5 12-8	

[0031] Figs. 15A-15E demonstrate that novel anti-RBD antibodies isolated from VRC01^{VH1-2} ONLY/Vk1-33-CS/TdT mice, Related to Figs. 4A-4I. Fig. 15A, RBD immunogens used to immunize VRC01^{VH1-2} ONLY/Vk1-33-CS/TdT mice. Fig. 15B, Results of ELISAs measuring serums on week 2 and week 6 post-immunizations with 3 RBD immunogens to SARS-CoV2 RBD. Fig. 15C, FACS analyses of splenic IgG⁺ B cells. The RBD-specific IgG⁺ B cells were sorted to clone anti-RBD antibodies. Fig. 15D, CDR3 length and sequences of 37 mAbs cloned from RBD-specific IgG⁺ B

cells. Fig. 15E, Results of ELISAs measuring mAbs binding to SARS-CoV2 spike, RBD. Data from Figs. 15B and 15E were average of three experimental repeats with error bars representing SDs.

[0032] The nomenclature used in Fig. 15D corresponds to the nomenclature of Table 2 as follows:

Name in Fig. 15D	Name in Table 2
SPIKE-P1	SPIKE-BC1
RBDNP-9	RBDNP-BC9
RBDNP-P11	RBDNP-BC11
VHH7-P3	VHH7-BC3
VHH7-P5	VHH7-BC5
VHH7-P7	VHH7-BC7
VHH7-P8	VHH7-BC8
VHH7-P10	VHH7-BC10

[0033] The nomenclature used in Fig. 15E corresponds to the nomenclature of Table 1 as follows:

Name in Fig. 15E	Name in Table 1
SPIKE-P1	SPIKE-BC1-7-7
RBDNP-P9-78	RBDNP-BC9-78
RBDNP-P11—34	RBDNP-BC11-34
VHH7-P3-66	VHH7-BC3-66
VHH7-P5-82	VHH7-BC5-82
VHH7-P7-53	VHH7-BC7-53
VHH7-P8-59	VHH7-BC8-59
VHH7-P10-19	VHH7-BC10-19

[0034] Fig. 16 depicts the amino acid sequences of V_H1-2 heavy chain of anti-SARS-CoV-2 monoclonal antibodies isolated from VRC01^{VH1-2 ONLY/Vk1-33-CS/TdT} mouse model.

[0035] The nomenclature used in Fig. 16 refers to the heavy chain sequences of antibodies, which correspond to the heavy chains of the antibodies of Table 1 as follows:

Name in Fig. 16	Name in Table 1
SPIKE-P1 37-1-3	SPIKE-BC1-1-3
SPIKE-P1 37-7-7	SPIKE-BC1-7-7
SPIKE-P1 37-6-2	SPIKE-BC1-6-2
SPIKE-P1 37-9-6	SPIKE-BC1-9-6
SPIKE-P1 37-7-3	SPIKE-BC1-7-3
RBDNP-P9-83-HC78	RBDNP-BC9-78
RBDNP-P11-HC-9	RBDNP-BC11-9
RBDNP-P11-HC-23	RBDNP-BC11-23

RBDNP-P11-HC-31	RBDNP-BC11-31
RBDNP-P11-HC-34	RBDNP-BC11-34
RBDNP-P11-HC-47	RBDNP-BC11-47
RBDNP-P11-HC-60	RBDNP-BC11-60
RBDNP-P11-HC-78	RBDNP-BC11-78
VHH7-3-HC-37	VHH7-BC3-37
VHH7-3-HC-66	VHH7-BC3-6
VHH7-5-HC-29	VHH7-BC5-29
VHH7-5-HC-75	VHH7-BC5-75
VHH7-5-HC-82	VHH7-BC5-82
VHH7-7-HC-53	VHH7-BC7-53
VHH7-7-HC-76	VHH7-BC7-76
VHH7-7-HC-79	VHH7-BC7-79
VHH7-7-HC-85	VHH7-BC7-85
VHH7-8-HC-5	VHH7-BC8-5
VHH7-8-HC-7	VHH7-BC8-7
VHH7-8-HC-59	VHH7-BC8-59
VHH7-8-HC-62	VHH7-BC8-62
VHH7-10-HC-19	VHH7-BC10-19

[0036] Fig. 17 depicts amino acid sequences of V κ 1-33 light chain of anti-SARS-CoV-2 monoclonal antibodies isolated from VRC01^{VH1-2 ONLY/V κ 1-33-CS/Td τ} mouse model. The nomenclature used in Fig. 17 refers to the light chain sequences of antibodies, which correspond to the light chains of the antibodies of Table 1 as follows:

Name in Fig. 17	Name in Table 1
SPIKE-P1-37-1-3	SPIKE-BC1-7-3
SPIKE-P1-37-7-7	SPIKE-BC1-7-7
SPIKE-P1-37-6-2	SPIKE-BC1-6-2
SPIKE-P1-37-9-6	SPIKE-BC1-9-6
SPIKE-P1-37-7-3	SPIKE-BC1-7-3
RBDNP-P9-83-IGG-LC78	RBDNP-BC9-78
RBDNP-P11-LC-9	RBDNP-BC11-9
RBDNP-P11-LC-23	RBDNP-BC11-23
RBDNP-P11-LC-31	RBDNP-BC11-31
RBDNP-P11-LC-34	RBDNP-BC11-34
RBDNP-P11-LC-47	RBDNP-BC11-47
RBDNP-P11-LC-60	RBDNP-BC11-60
RBDNP-P11-LC-78	RBDNP-BC11-78

VHH7-3-LC-37	VHH7-BC3-37
VHH7-3-LC-66	VHH7-BC3-6
VHH7-5-LC-29	VHH7-BC5-29
VHH7-5-LC-75	VHH7-BC5-75
VHH7-5-LC-82	VHH7-BC5-82
VHH7-7-LC-53	VHH7-BC7-53
VHH7-7-LC-76	VHH7-BC7-76
VHH7-7-LC-79	VHH7-BC7-79
VHH7-7-LC-85	VHH7-BC7-85
VHH7-8-LC-5	VHH7-BC8-5
VHH7-8-LC-7	VHH7-BC8-7
VHH7-8-LC-59	VHH7-BC8-59
VHH7-8-LC-62	VHH7-BC8-62
VHH7-10-LC-19	VHH7-BC10-19

[0037] Fig. 18 depicts neutralization activity of SP1-77, VHH7-5-82 and VHH7-7-53 antibodies against SARS-CoV-2 variants of concern. Data is shown the mean of two experimental replicates.

[0038] Figs. 19A-19E depict a humanized mouse model with diverse BCR repertoire derived from single human V_H and V_K recombination. (Fig. 19A) Schematic representation of modified *Igh* and *Igk* loci of V_{H1-2}/V_{K1-33} -rearranging mice. The $V_{H1-2}^{IGCR1\Delta}$ allele was made previously (33). 2 MB region upstream of V_{H1-2} that contained all mouse V_{HS} Wwase deleted to generate $V_{H1-2}^{mVHA/IGCR1\Delta}$ allele. We replaced mouse V_{K3-2} with human V_{K1-33} and deleted Cer/sis from the V_K to J_K interval to generate V_{K1-33}^{CSA} allele. (B) HTGTS-rep-seq analysis of V_K usage in 129/Sv wild-type (left panel) and V_{K1-33} -rearranging mouse splenic B cells in presence (middle panel) or absence (right panel) of Cer/sis. The x axis listed all functional V_K s from distal to the J_K -proximal ends. The histogram displayed percent usage of each V_K among all productive V_KJ_K rearrangements. (Fig. 19E-19F) Length distribution of V_{K1-33} LC CDR3 (Fig. 19E) in splenic B cells. Data were mean $\pm$ SD of three libraries from different mice. Venn diagram showed the V_{K1-33} LC CDR3 (Fig. 19F) complexity. Unique reads derived from the same libraries were in the left panel. Little overlap of V_{H1-2} HC CDR3 sequences among three independent mice indicated tremendous CDR3 complexity.

[0039] Figs. 20A-20C depict immunizing the V_{H1-2}/V_{K1-33} -rearranging mouse model with SARS-CoV-2 spike or RBD elicited multiple V_{H1-2}/V_{K1-33} antibodies. (Fig. 20A) Immunization scheme. Prime plus boost immunizations were performed at an interval of four weeks. (Fig. 20B) Binding curves showing reactivity of sera to SARS-CoV-2 spike, RBD, NTD and SARS-CoV-1 spike protein. The upper panel showed the sera from the SARS-CoV-2 Spike immunized mice at week 0, 2 and 6. The bottom panel showed the sera from VHH7-RBD immunized mice. Data

were mean $\pm$ SD of three mice. (Fig. 20C) Table showed the V_H1-2/V_K1-33 antibodies isolated from SARS-CoV-2 spike-specific or RBD-specific IgG⁺ B cells. The antibody sequences and sequence features were shown in Table 3.

[0040] Figs. 21A-21C demonstrate that SP1-77 potently neutralized SARS-CoV-2 VOCs, including Omicron sub-variants. (Fig. 21A) Table showed the neutralization activities (IC₅₀: top; IC₈₀: bottom) of three monoclonal antibodies against all variants of concern (VOCs) and some variants of interest (VOIs) in pseudovirus neutralization assays. Experiments were done in 293 T/ACE2 cells. The neutralization curves were shown in Figs. 29A-29D. The mutations on the spike proteins of different variants are provided in Example 4 below. Data were representative of 2 biologically independent experiments for most VOCs and VOIs, but one experiment for BA.3. Each independent experiment contained 2 technical replicates. IC₅₀ and IC₈₀ values were color-coded based on the key shown at the right. (Fig. 21B) Table showed the neutralization activities of three antibodies against VOCs in PRNT live virus neutralization assays. The neutralization curves were shown in Fig. 29E. Data were representative of one independent experiment with 2 technical replicates. IC₅₀ and IC₉₀ values were color-coded based on the key shown at the right. (Fig. 21C) Final 3D reconstruction of Fab-S complexes shown in top view and side view with the S in gray and the Fabs darker.

[0041] Figs. 22A-22E depict cryo-EM structures of SP1-77 Fab in complex of a full-length SARS-CoV-2 S trimer. (Fig. 22A) Cryo-EM structures of SP1-77 Fabs in complex with full-length S trimer in the one-RBD-up (2.9 Å) and three-RBD-down (2.7 Å) conformations. EM density was in gray and structures were in ribbon diagrams. Three SP1-77 Fabs bound one S trimer in both conformations. (Figs. 22B-22C) Close-up view of interactions between SP1-77 Fab and the RBD and NTD of the S trimer in RBD-down conformation. (Fig. 22B) HC CDR3 of SP1-77 made main contact with RBD away from RBM, while HC CDR2 touched the N-linked glycan from the NTD. (Fig. 22C), zoom-in views of binding interface, showing 17-residue HC CDR3 wedging into a groove formed by two segments of residues 339-346 and residues 440-445. The Asn343 glycan of RBD also interacted with Tyr99 from HC CDR3. Two glycans at Asn122 and Asn165 in the NTD were in proximity to CDR2 (Asn53, Ser54, Gly56, Thr57 and Asn58) and HC FW3 (Thr73, Ser74 and Ile75). (Figs. 22D-22E) Comparison of binding mode and footprint of SP1-77 with ACE2 and other neutralizing antibodies, including S309 and LY-CoV1404. (Fig. 22D), RBD was in surface representation in gray. ACE2 and the antibodies were in ribbon diagram. (Fig. 22E), footprints on RBD of various antibodies. The interface residues of SP1-77 were indicated with major contacting residues distinguished.

[0042] Figs. 23A-23D depict modeled SP1-77 binding site on various SARS-CoV-2 variants. (Figs. 23A-23B) The potential footprint of SP1-77 on the modeled RBDs from different SARS-CoV-2 variants in a top view. The RBD was shown in surface representation with the SP1-77 footprint highlighted and the mutations in each variant depicted. The Fv region of SP1-77 was

shown in ribbon diagram. Most mutations on spike variants were not located at the SP1-77 footprint. (Fig. 23C) Side view of a selected panel from Figs. 23A-23B. (Fig. 23D) Structural comparison of the SP1-77 binding interface among the RBDs of wildtype G614, Mu and Omicron variants. The conservative mutation Arg346Lys in Mu preserved the salt bridge between the residue 346 in the RBD and the SP1-77 Asp95. The mutations in Omicron variant reconfigured the local conformation near the N343-glycan, which is on the edge of SP1-77 footprint. The RBD, the SP1-77 heavy chain and the light chain are noted. Mutations in the Mu and Omicron variants were shown in stick and ball model.

[0043] Figs. 24A-24J demonstrate that LLSM single virus tracking revealed SP1-77 inhibited S-fragment shedding and membrane fusion. (Figs. 24A-24I) Trajectories of VSV-SARS-CoV-2-Atto 565 virus imaged every 4 seconds with volumetric LLSM beginning 5 minutes after inoculation of Vero TMPRSS2 cells with virus at MOI ~ 2 without (Figs. 24A-24D) or with 500 ng/mL SP1-77 treatment (Figs. 24E-24H). Trajectories note when virus was localized to cell surface, internalized, and from a point spread function (PSF) to a larger distribution indicating viral envelope fusion with endosomal membrane. Single virus trajectories (Figs. 24B, 24F) with insets showing x-axis projection through 4 planes (top), 3D integrated intensity profiles (Figs. 24C, 24G, bottom) and corresponding heat maps of fluorescence intensities from 4 plane z-projections (Figs. 24C, 24G, top). Intensity line-profiles through center of virions (Figs. 24D, 24H) appeared as a single point spread function at the cell surface and in endosomes in the absence (Fig. 24D, t1-t4) or presence (Fig. 24H, t1-t6) of SP1-77. Atto 565 spreaded upon fusion in the absence of SP1-77 (Fig. 24D, t5-t6). (Fig. 24I) Summary of all single virus trajectories over the course of single experiments, in the absence and presence of SP1-77, each from 3 cells imaged consecutively for 10 min. Dots indicated virus trajectories in which a TMPRSS2-dependent Atto 565 signal decrease was observed. (Fig. 24J) Quantification of 5 experiments for each condition for the number of viruses in the total cell volume of every cell imaged (left), the number of Atto 565 decreases observed in all the trajectories of virus when co-localizing to the cell surface (middle), and the number of instances of Atto 565 dye spreading within endosomes (right). Data were mean $\pm$ SD of 5 independent experiments. *p* values were accessed by unpaired two-tail t-test.

[0044] Figs. 25A-25C demonstrate that SP1-77 inhibited ACE2-mediated S1 dissociation on trypsin-treated VSV-SARS-CoV-2 Atto 565 viruses. (Fig. 25A) Histogram of the number of Atto 565 molecules on VSV-SARS-CoV-2-Atto 565 determined by single molecule counting. (Fig. 25B) Histogram of averages of peak distribution determined by a Gaussian fit of 3 independent experiments. *p* values were accessed by unpaired two-tail t-test. Treatments prior to adsorption included none (control), incubation with 1 μ g/mL trypsin for 30 min at 37°C, treatment without or with trypsin followed by an incubation with 0.5 μ M of recombinant ACE2 for 10 min at 37°C, or treatment with trypsin then incubated with 100 ng/mL SP1-77 IgG or SP1-77 Fab for 1 hour at

37°C followed by incubation with ACE2. (Fig. 25C) Schematic representation of proposed mechanism of SP1-77 inhibition of SARS-CoV-2 infection. Left panel: Without antibody treatments, the spike protein on the viral surface bound to the ACE2 receptor on the infected cell surface. Membrane fusion was activated either by TMPRSS2 protease on the cell membrane or by cathepsin L protease following endocytosis. Cleavage at the S2' site by these proteases led to dissociation of the S1 subunit, which exposed the fusion peptide on the S2, facilitating viral-host membrane fusion and viral entry into the infected cells. Middle panel: Pre-treatment of the virus with SP1-77, a non-ACE2-blocking antibody, did not appreciably impact binding of viruses to the cell surface and their endocytosis. However, SP1-77 greatly inhibited the dissociation of S1 subunit, thereby, blocking activation of the fusion peptide and membrane fusion. Right panel: Pre-treatment of the virus with VHH7-5-82, an ACE2-blocking antibody, prevented binding of the virus to the cell surface.

[0045] Figs. 26A-26C demonstrate the generation of the VH1-2/Vκ1-33-rearranging mouse model. (Figs. 26A-26B) The diagram illustrates the deletion of 2MB upstream region of human VH1-2 that contain all mouse VHs. The deletion was mediated by two gRNAs. The deleted allele was screened by PCR. The sequencing result was shown in the bottom. (Fig 26C) Similarities of amino acid sequences between mouse Js and human Js. The alignments between human Js and mouse Js were mainly based on the sequences underlined at 5' end that contribute to the CDR3 sequence. The different amino acids between mouse Js and human Js were filled in grey.

[0046] Figs. 27A-27C depict the characterization of the VH1-2/Vκ1-33-rearranging mouse. (Fig. 27A) HTGTS-rep-seq analysis of VH usage in VH1-2^{mVHA/IGCR1Δ}-rearranging mouse splenic B cells. The x axis listed all functional VHs from the distal to the D-proximal end. The histogram displayed the percent usage of each VH among all productive VHDJH rearrangements. Data were mean ± SD of 3 different mice. (Figs. 27B-27C) FACS analyses of splenic B cell and T cell populations from wild-type 129/Sv and VH1-2/Vκ1-33-rearranging mice. They were representative of three biological replicates.

[0047] Figs. 28A-28C depict immunizing the mouse model with SARS-CoV-2 spike or RBD elicited VH1-2/Vκ1-33 antibodies. (Figs. 28A-28B) Gating strategy for single cell sorting of SARS-CoV2 spike-specific or RBD-specific IgG⁺ B cells after immunization. Fig 28A - the mice immunized with SARS-CoV2 spike plus poly I:C adjuvant. Fig. 28B - the mice immunized with poly I:C (control) or VHH7-RBD plus poly I:C adjuvant. (Fig. 28C) Table showed the binding affinities of nine mAbs to the SARS-CoV-2 spike protein, RBD, NTD. Data were representative of two independent experiments. EC50 values were color-coded based on the key shown at the right.

[0048] Figs. 29A-29G* depict neutralization data of three mAbs against SARS-CoV-2 variants. (Figs. 29A-29D) Neutralization Curves of SARS-CoV-2 pseudoviruses displaying S proteins by three V_H1-2/Vκ1-33 mAbs. Data were done in 293T/ACE2 cells and also shown in Fig. 21. Data were representative of one independent experiments with two technical replicates. (Fig. 29E) Table showed

the neutralization activities of three mAbs against VOCs and VOIs in pseudovirus neutralization assays. These assays were done independently in 293T/ACE2/TMPRSS2 cells. Compared to the neutralization data done in 293T/ACE2 cells, IC₅₀ values were at the similar levels for all VOCs and VOIs except Delta which was neutralized less potently. Data were representative of one independent experiment with two technical replicates. IC₅₀ values were color-coded based on the key shown at the right. (Fig. 29F) Neutralization Curves of SARS-CoV-2 virus by three V_H1-2/V_κ1-33 mAbs in PRNT experiment. Data were done in 293T/ACE2 cells and also shown in Fig. 3B. Data were representative of one independent experiments with two technical replicates. (Fig. 29G) Table showed the neutralization activities of humanized SP1-77 and the other 4 antibodies in SP1 clonal lineage against VOCs in pseudovirus neutralization assays. These assays were done in 293T/ACE2 cells. Data were representative of two independent experiments. Each independent experiment contained 2 technical replicates. IC₅₀ values were color-coded based on the key shown at the right.

[0049] Figs. 30A-30D depict negative stain electron microscopy analysis of S complexes with three V_H1-2/V_κ1-33 neutralizing antibodies. (Fig. 30A) SPR data measuring the binding affinity between monoclonal Fabs and SARS-CoV-2 spike proteins. Each coronavirus spike (S) protein was captured on Streptavidin sensor chip and Fabs were screened at 50nM. A blank streptavidin surface along with blank buffer binding were used for double reference subtraction to account for non-specific protein binding and signal drift. Data was representative of two independent experiments. (Fig. 30B) A 3D classification of the spike-VHH7-5-82 complex data, with 158,101 particles sorted into 9 classes, shown in top and side views. (Fig. 30C) A 3D classification of the spike- VHH7-7-53 complex data, with 187,262 particles sorted into 12 classes, shown in top and side views. (Fig. 30D) A 3D classification of the spike-SP1-77 complex data, with 230,136 particles sorted into 12 classes, shown in top and side views. In the top view of Figs. 30B-30D, arrows indicate Fabs, and arrowheads indicate weak or partial Fab density; RBDs were visually assessed and assigned as up (U), down (D) or intermediate (I); and the circles indicated the selected (or combined) classes used for the final 3D reconstructions shown in Fig. 21C.

[0050] Figs. 31A-31F depict three V_H1-2/V_κ1-33 neutralizing antibodies bound to distinct regions of RBD. (Figs. 31A-31C) Binding footprint of Fabs of three V_H1-2/V_κ1-33 neutralizing antibodies, VHH7-5-82 (Fig. 31A), VHH7-7-53 (Fig. 31B), and SP1-77 (Fig. 31C) shading on the RBD in surface representation. The RBD surface was represented in 5 views (top, back, inner, front and outer). Black outline indicated the ACE2 RBM. RBD was shown as gray. (Figs. 31D-31F) SP1-77 can not block ACE2 binding to the spike protein and RBD. Fig. 31D shows the diagram of SPR assay. Fig. 31E shows the binding of ACE2, SP1-77 antibody and negative control (with ACE2 being distinguished from the other lines) to preformed SP1-77 - spike complex. Fig. 31F shows the binding of ACE2, SP1-77 antibody and negative control (with ACE2 being distinguished from the other lines) to preformed SP1-77 - RBD complex.

[0051] Figs. 32A-32C depict epitope analysis and comparison of the previously characterized VH1-2-based monoclonal antibodies. (Fig. 32A) Table showed a list of VH1-2-based mAbs for which high-resolution structures have been published with various HC CDR3s and LCs. The first three are the three antibodies identified in the instant mouse model. (Fig. 32B) Comparisons of footprints on RBD between three of the instant antibodies and nine previously characterized VH1-2-based monoclonal antibodies. Those nine previously characterized VH1-2-based antibodies show a similar RBD binding epitope that is distinct from where the present three antibodies bound.

[0052] Figs. 33A-33C depict the purification of the full-length G614 S trimer/SP1-77 Fab complex and cryo-EM analysis. (Fig. 33A) Left, the complex of the full-length G614 S trimer and SP1-77 Fab was resolved by gel-filtration chromatography on a Superose 6 column. First peak, the S trimer-Fab complex used for the cryo-EM study; second peak, the dissociated postfusion S2 trimer mixed with the monomeric S1 in complex with Fab; and third peak, free Fab. Inset, peak fractions were analyzed by Coomassie stained SDS-PAGE. Right, representative motion-corrected micrograph of the vitrified complex purified by gel-filtration chromatography. (Fig. 33B) 2D class averages (box dimension: 396Å) of the cryo-EM images of the G614 S trimer/SP1-77 Fab complex from cryoSPARC. (Fig. 33C) Data processing workflow for structure determination.

[0053] Figs. 34A-34E depict the cryo-EM structure validation of the G614 S trimer/SP1-77 Fab complex. FSC curves and the viewing direction distribution plot for the S trimer-Fab complex. From Fig. 34A-34E: the complex of the G614 S trimer in the three-RBD-down conformation and three SP1-77 Fabs; the complex of the G614 S trimer in the one-RBD-up conformation and three SP1-77 Fabs; masked local refinement of the Fab in complex with the RBD from the G614 S trimer in the three-RBD-down conformation; masked local refinement of the Fab in complex with the RBD in the up conformation from the G614 S trimer in the one-RBD-up state; masked local refinement of the Fab in complex with the RBD in the down conformation from the G614 S trimer in the one-RBD-up state.

[0054] Figs. 35A-35C depict analysis of the the G614 S trimer/SP1-77 Fab complex structure. (Fig. 35A) 3D reconstructions of SP1-77 Fab in complex with the G614 S trimer in the three-RBD-down and one-RBD-up conformations, respectively, were colored according to local resolution estimated by cryoSPARC. (Fig. 35B) 3D reconstructions from masked local refinement of the Fab in complex with a single RBD from the G614 S trimer in the three-RBD-down conformation (left), or the RBD in the up conformation from the G614 S trimer in the one-RBD-up state (middle) or the RBD in the down conformation from the G614 S trimer in the one-RBD-up state (right) were colored according to local resolution estimated by cryoSPARC. (Fig. 35C) Representative density in gray surface from EM maps with a resolution better than 3.3Å, primarily focusing on the SP1-77 Fab and its binding interface with the RBD of the G614 S trimer.

[0055] Figs. 36A-36D depict the NTD sequence alignment of SARS-CoV-2 variants. Amino acid sequence alignment of the NTD from SARS-CoV-2 variants. The sequences were aligned using ClustalW (98). Numbering was based on the sequence of the Wuhan-Hu-1 strain, as indicated on top

of the alignment. Secondary structures for the NTD were also indicated at the top. The conserved residues were colored in dark grey; the variations were highlighted in light grey. The two N-linked glycosylation sites (Asn122 and Asn165) in the NTD were marked in with an up arrow below the alignment.

[0056] Figs. 37A-37C depict the single molecule calibration of VSV-SARS-CoV-2 Atto 565 virus. (Fig. 37A) Example image of Atto 565 fluorescence of virus adsorbed for 10 minutes on a glass coverslip coated with poly-D-lysine coated slides and imaged with spinning disc confocal microscopy. (Fig. 37B) Photobleaching used to determine the intensity of single Atto 565 dye intensities allowed to establish that ~20-40 dyes were attached to each virus. (Fig. 37C) Infection assays showing neutralization of VSV-SARS-CoV-2 Atto 565 infection with SP1-77 IgG or SP1-77 Fab. A soluble eGFP reporter genetically encoded into this VSV chimera allowed for infection to be determined by fluorescence imaging on a spinning disc confocal microscope as shown in the representative examples obtained 7 hour post infection. Cell outlines were obtained with a WGA-Alexa 647 membrane stain applied to cells immediately prior to fixation. Each condition was measured with 3 independent experiments.

DETAILED DESCRIPTION

[0057] Described herein are antibodies and antibody reagents that bind specifically to SARS-CoV2 polypeptides, e.g., the spike or RBD polypeptides and which thereby inhibit the binding of SARS-CoV2 to human cells, and/or inhibit SARS-CoV2 infection processes. These compositions accordingly have therapeutic and prophylactic uses related to coronavirus infections.

[0058] As used herein, the term “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. The term also refers to antibodies comprised of two immunoglobulin heavy chains and two immunoglobulin light chains as well as a variety of forms including full length antibodies and antigen-binding portions thereof; including, for example, an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, a bispecific antibody, a functionally active epitope-binding portion thereof, and/or bifunctional hybrid antibodies.

[0059] Each heavy chain is composed of a variable region of said heavy chain (abbreviated here as HCVR or VH) and a constant region of said heavy chain. The heavy chain constant region consists of three domains CH1, CH2 and CH3. Each light chain is composed of a variable region of said light chain (abbreviated here as LCVR or VL) and a constant region of said light chain. The light chain constant region consists of a CL domain. The VH and VL regions may be further divided into hypervariable regions referred to as complementarity-determining regions (CDRs) and interspersed

with conserved regions referred to as framework regions (FR). Each VH and VL region thus consists of three CDRs and four FRs which are arranged from the N terminus to the C terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. This structure is well known to those skilled in the art.

[0060] As used herein, the term “CDR” refers to the complementarity determining regions within antibody variable sequences. The exact boundaries of these CDRs have been defined differently according to different systems. CDRs may be defined according to the Kabat system (see Kabat, E. A. *et al.*, 1991, “Sequences of Proteins of Immunological Interest”, 5th edit., NIH Publication no. 91-3242, U.S. Department of Health and Human Services). Other systems may be used to define CDRs, which as the system devised by Chothia *et al* (see Chothia, C. & Lesk, A. M., 1987, “Canonical structures for the hypervariable regions of immunoglobulins”, J. Mol. Biol., 196, 901-917) and the IMGT system (see Lefranc, M. P., 1997, “Unique database numbering system for immunogenetic analysis”, Immunol. Today, 18, 50). An antibody typically contains 3 heavy chain CDRs and 3 light chain CDRs. The term CDR or CDRs is used here to indicate one or several of these regions. A person skilled in the art is able to readily compare the different systems of nomenclature and determine whether a particular sequence may be defined as a CDR. The methods and compositions used herein may utilize CDRs defined according to any of these systems. The CDRs disclosed herein were identified via the Kabat system.

[0061] The term “antigen-binding portion” of an antibody refers to one or more portions of an antibody as described herein, said one or more portions still having the binding affinities as defined above herein. Portions of a complete antibody have been shown to be able to carry out the antigen-binding function of an antibody. In accordance with the term “antigen-binding portion” of an antibody, examples of binding portions include (i) an Fab portion, *i.e.*, a monovalent portion composed of the VL, VH, CL and CH1 domains; (ii) an F(ab')₂ portion, *i.e.*, a bivalent portion comprising two Fab portions linked to one another in the hinge region via a disulfide bridge; (iii) an Fd portion composed of the VH and CH1 domains; (iv) an Fv portion composed of the FL and VH domains of a single arm of an antibody; and (v) a dAb portion consisting of a VH domain or of VH, CH1, CH2, DH3, or VH, CH2, CH3 (dAbs, or single domain antibodies, comprising only V_L domains have also been shown to specifically bind to target epitopes).

[0062] Although the two domains of the Fv portion, namely VL and VH, are encoded by separate genes, they may further be linked to one another using a synthetic linker, and recombinant methods, making it possible to prepare them as a single protein chain in which the VL and VH regions combine in order to form monovalent molecules (known as single chain Fv (ScFv)).

[0063] The term “antigen-binding portion” of an antibody is also intended to comprise such single chain antibodies. Other forms of single chain antibodies such as “diabodies” can also be included. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker which is too short for the two domains being able to combine on

the same chain, thereby forcing said domains to pair with complementary domains of a different chain and to form two antigen-binding sites. An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art.

[0064] As used herein, the term “antibody reagent” refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. An antibody reagent can comprise an antibody or a polypeptide comprising an antigen-binding domain of an antibody. In some embodiments, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term “antibody reagent” encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, and domain antibodies (dAb) fragments as well as complete antibodies.

[0065] An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like.

[0066] Furthermore, an antibody, antigen-binding portion thereof, as described herein may be part of a larger immunoadhesion molecule formed by covalent or noncovalent association of said antibody or antibody portion with one or more further proteins or peptides. Relevant to such immunoadhesion molecules are the use of the streptavidin core region in order to prepare a tetrameric scFv molecule and the use of a cysteine residue, a marker peptide and a C-terminal polyhistidiny, ('hexahistidiny tag') in order to produce bivalent and biotinylated scFv molecules.

[0067] In some embodiments, the antibody or antigen-binding portion thereof is a fully human antibody. In some embodiments, the antibody, antigen-binding portion thereof, is a humanized antibody or antibody reagent. In some embodiments, the antibody, antigen-binding portion thereof, is a fully humanized antibody or antibody reagent. In some embodiments, the antibody or antigen-binding portion thereof, is a chimeric antibody or antibody reagent. In some embodiments, the antibody, antigen-binding portion thereof, is a recombinant polypeptide.

[0068] The term “human antibody” refers to antibodies whose variable and constant regions correspond to or are derived from immunoglobulin sequences of the human germ line, as described, for example, by Kabat *et al.* (see Kabat, *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). However, the human antibodies can contain amino acid residues not encoded by human germ line immunoglobulin sequences (for example mutations which have been introduced by random or

site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, and in particular in CDR3. Recombinant human antibodies as described herein have variable regions and may also contain constant regions derived from immunoglobulin sequences of the human germ line (see Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[0069] According to particular embodiments, however, such recombinant human antibodies are subjected to in-vitro mutagenesis (or to a somatic in-vivo mutagenesis, if an animal is used which is transgenic due to human Ig sequences) so that the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences which although related to or derived from VH and VL sequences of the human germ line, do not naturally exist *in vivo* within the human antibody germ line repertoire. According to particular embodiments, recombinant antibodies of this kind are the result of selective mutagenesis or back mutation or of both. Preferably, mutagenesis leads to an affinity to the target which is greater, and/or an affinity to non-target structures which is smaller than that of the parent antibody.

[0070] Generating a humanized antibody from the sequences and information provided herein can be practiced by those of ordinary skill in the art without undue experimentation. In one approach, there are four general steps employed to humanize a monoclonal antibody, see, *e.g.*, U.S. Pat. No. 5,585,089; No. 6,835,823; No. 6,824,989. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains; (2) designing the humanized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing process; (3) the actual humanizing methodologies/techniques; and (4) the transfection and expression of the humanized antibody.

[0071] Usually the CDR regions in humanized antibodies and human antibody variants are substantially identical, and more usually, identical to the corresponding CDR regions in the mouse or human antibody from which they were derived. In some embodiments, it is possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin or human antibody variant. In some embodiments, substitutions of CDR regions can enhance binding affinity.

[0072] The term “chimeric antibody” refers to antibodies which contain sequences for the variable region of the heavy and light chains from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions. Humanized antibodies have variable region framework residues substantially from a human antibody (termed an acceptor antibody) and complementarity determining regions substantially from a non-human antibody.

[0073] The constant region(s), if present, are also substantially or entirely from a human immunoglobulin. The human variable domains are usually chosen from human antibodies whose framework sequences exhibit a high degree of sequence identity with the (murine) variable region

domains from which the CDRs were derived. The heavy and light chain variable region framework residues can be substantially similar to a region of the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies.

[0074] In addition, techniques developed for the production of “chimeric antibodies” by splicing genes from a mouse, or other species, antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. The variable segments of chimeric antibodies are typically linked to at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, such as immortalized B-cells. The antibody can contain both light chain and heavy chain constant regions. The heavy chain constant region can include CH1, hinge, CH2, CH3, and, sometimes, CH4 regions. For therapeutic purposes, the CH2 domain can be deleted or omitted.

[0075] Additionally, and as described herein, a recombinant humanized antibody can be further optimized to decrease potential immunogenicity, while maintaining functional activity, for therapy in humans. In this regard, functional activity means a polypeptide capable of displaying one or more known functional activities associated with a recombinant antibody, antigen-binding portion thereof, as described herein. Such functional activities include binding to a SARS-CoV2 virus and/or blocking the cellular entry of a SARS-CoV2 virus.

[0076] Additionally, a polypeptide having functional activity means the polypeptide exhibits activity similar, but not necessarily identical to, an activity of a reference antibody, antigen-binding portion thereof, as described herein, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the reference antibody, antigen-binding portion thereof, , but rather substantially similar to the dose-dependence in a given activity as compared to the reference antibody, antigen-binding portion thereof, as described herein (*i.e.*, the candidate polypeptide will exhibit greater activity, or not more than about 25-fold less, about 10-fold less, or about 3-fold less activity relative to the antibodies, and/or antigen-binding portions, described herein).

[0077] In some embodiments, the antibody reagents (*e.g.*, antibodies) described herein are not naturally-occurring biomolecules. For example, a murine antibody raised against an antigen of human origin would not occur in nature absent human intervention and manipulation, *e.g.*, manufacturing steps carried out by a human. Chimeric antibodies are also not naturally-occurring biomolecules, *e.g.*, in that they comprise sequences obtained from multiple species and assembled into a recombinant molecule. In certain particular embodiments, the human antibody reagents described herein are not naturally-occurring biomolecules, *e.g.*, fully human antibodies directed against a human antigen would be subject to negative selection in nature and are not naturally found in the human body.

[0078] In some embodiments, the antibody, antibody reagent, and/or antigen-binding portion thereof, is an isolated polypeptide. In some embodiments, the antibody, antibody reagent, and/or antigen-binding portion thereof, is a purified polypeptide. In some embodiments, the antibody, antibody reagent, and/or antigen-binding portion thereof, is an engineered polypeptide.

[0079] In some embodiments of any of the aspects, the antibody reagent or antigen-binding fragment thereof is fully human or fully humanized. In some embodiments of any of the aspects, the antibody reagent or antigen-binding fragment thereof is fully humanized except for the CDR sequences. In some embodiments of any of the aspects, the antibody reagent or antigen-binding fragment is selected from the group consisting of: an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody.

[0080] In some embodiments, the antibody, antibody reagent, antigen-binding portion thereof, can comprise one or more CDRs (*e.g.*, one CDR, two CDRs, three CDRs, four CDRs, five CDRs, or six CDRs) having the sequence of a CDR described herein, *e.g.*, selected from Table 1. In some embodiments, the antibody, antibody reagent, antigen-binding portion thereof, or CAR can comprise CDRs having the sequence of the CDRs of an antibody described herein, *e.g.*, of Table 1. For example, an antibody, antibody reagent, antigen-binding fragment thereof, or chimeric antigen receptor (CAR) described herein can comprise:

- at least one heavy or light chain complementarity determining region (CDR), or
- three heavy chain CDRs, or
- three light chain CDRs, or
- three heavy chain CDRs and three light chain CDRs,

where the CDR have sequences selected from:

- a. the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
- b. the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
- c. the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
- d. the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
- e. the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
- f. the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;

- g. the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- h. the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- i. the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- j. the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- k. the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- l. the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- m. the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- n. the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- o. the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- p. the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- q. the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;
- r. the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- s. the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- t. the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- u. the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- v. the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- w. the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- x. the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;

- y. the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- z. the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- aa. the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- bb. the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- cc. the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- dd. the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ee. the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- ff. the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- gg. the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
- hh. the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
- ii. the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
- jj. the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
- kk. the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
- ll. the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
- mm. the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
- nn. the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or

a conservative substitution variant thereof.

[0081] In some embodiments of any of the aspects, an antibody, antibody reagent, or CAR or antigen-binding fragment thereof comprises:

- a VH sequence; or
- a VL sequence; or

a VH and a VL amino acid sequence selected from:

- a. SEQ ID NOs: 1 and 2;
- b. SEQ ID NOs: 9 and 10;
- c. SEQ ID NOs: 17 and 18;
- d. SEQ ID NOs: 25 and 26;
- e. SEQ ID NOs: 33 and 34;
- f. SEQ ID NOs: 41 and 42;
- g. SEQ ID NOs: 49 and 50;
- h. SEQ ID NOs: 57 and 58;
- i. SEQ ID NOs: 65 and 66;
- j. SEQ ID NOs: 73 and 74;
- k. SEQ ID NOs: 81 and 82;
- l. SEQ ID NOs: 89 and 90;
- m. SEQ ID NOs: 97 and 98;
- n. SEQ ID NOs: 105 and 106;
- o. SEQ ID NOs: 113 and 114;
- p. SEQ ID NOs: 121 and 122;
- q. SEQ ID NOs: 129 and 130;
- r. SEQ ID NOs: 137 and 138;
- s. SEQ ID NOs: 145 and 146;
- t. SEQ ID NOs: 153 and 154;
- u. SEQ ID NOs: 161 and 162;
- v. SEQ ID NOs: 169 and 170;
- w. SEQ ID NOs: 177 and 178;
- x. SEQ ID NOs: 185 and 186;
- y. SEQ ID NOs: 193 and 194;
- z. SEQ ID NOs: 201 and 2025;
- aa. SEQ ID NOs: 209 and 210;
- bb. SEQ ID NOs: 217 and 218;
- cc. SEQ ID NOs: 225 and 226;
- dd. SEQ ID NOs: 233 and 234;
- ee. SEQ ID NOs: 241 and 242;
- ff. SEQ ID NOs: 249 and 250;
- gg. SEQ ID NOs: 257 and 258;
- hh. SEQ ID NOs: 265 and 266;
- ii. SEQ ID NOs: 273 and 274;
- jj. SEQ ID NOs: 281 and 282;

- kk. SEQ ID NOs: 289 and 290;
- ll. SEQ ID NOs: 297 and 298;
- mm. SEQ ID NOs: 305 and 306;
- nn. SEQ ID NOs: 313 and 314; or

a conservative substitution variant thereof.

[0082] In one aspect of any of the embodiments, described herein is an antibody, antibody reagent, CAR, or antigen-binding portion thereof, that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with an antibody described herein, *e.g.*, selected from Table 1, or having the CDRs of an antibody selected from Table 1.

[0083] In some embodiments, the antibody, antibody reagent, CAR, and/or antigen-binding portion thereof, as described herein can be a variant of a sequence described herein, *e.g.*, a conservative substitution variant of an antibody polypeptide. In some embodiments, the variant is a conservatively modified variant. Conservative substitution variants can be obtained by mutations of native nucleotide sequences, for example.

[0084] A “variant,” as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions or substitutions. Variant polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encode a variant protein or portion thereof that retains activity, *e.g.*, antigen-specific binding activity for the relevant target polypeptide, *e.g.*, SARS-COV-2. A wide variety of PCR-based site-specific mutagenesis approaches are also known in the art and can be applied by the ordinarily skilled artisan.

[0085] One of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid and retain the ability to specifically bind the target antigen (*e.g.*, SARS-COV-2). Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles consistent with the disclosure.

[0086] Examples of substitution variants include conservative substitution of amino acids, *e.g.*, in a V_H or V_L domain, that do not alter the sequence of a CDR. A conservative substitution in a sequence not comprised by a CDR can be a substitution relative to a wild-type or naturally-occurring sequence, *e.g.*, human or murine framework and/or constant regions of an antibody sequence. In some embodiments, a conservatively modified variant of an antibody reagent can comprise alterations other than in the CDRs, *e.g.*, a conservatively modified variant of an antibody, antibody reagent, CAR, or antigen-binding portion thereof, can comprise CDRs described herein, *e.g.*, having the sequence of one

or more selected from Table 1. In some embodiments, a conservatively modified variant of an antibody, antibody reagent, CAR, or antigen-binding portion thereof, can comprise CDRs described herein, e.g., having the sequences of an antibody of Table 1.

[0087] A given amino acid can be replaced by a residue having similar physiochemical characteristics, e.g., substituting one aliphatic residue for another (such as Ile, Val, Leu, or Ala for one another), or substitution of one polar residue for another (such as between Lys and Arg; Glu and Asp; or Gln and Asn). Other such conservative substitutions, e.g., substitutions of entire regions having similar hydrophobicity characteristics, are well known. Polypeptides comprising conservative amino acid substitutions can be tested in any one of the assays described herein to confirm that a desired activity, e.g., antigen-binding activity and specificity of a native or reference polypeptide is retained.

[0088] Amino acids can be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)): (1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M); (2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q); (3) acidic: Asp (D), Glu (E); (4) basic: Lys (K), Arg (R), His (H). Alternatively, naturally occurring residues can be divided into groups based on common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; (6) aromatic: Trp, Tyr, Phe. Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Particular conservative substitutions include, for example; Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into H is; Asp into Glu; Cys into Ser; Gln into Asn; Glu into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

[0089] A variant amino acid or DNA sequence preferably is 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%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and a mutant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

[0090] Alterations of the native amino acid sequence can be accomplished by any of a number of techniques known to one of skill in the art. Mutations can be introduced, for example, at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered

nucleotide sequence having particular codons altered according to the substitution, deletion, or insertion required.

[0091] Any cysteine residue not involved in maintaining the proper conformation of the polypeptide also can be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) can be added to the polypeptide to improve its stability or facilitate oligomerization.

[0092] In particular embodiments wherein an antibody, CAR or antigen-binding portion thereof, as described herein comprises at least one CDR which is not identical to a sequence described herein, e.g., a sequence from Table 1, the amino acid sequence of that at least one CDR can be selected by methods well known to one of skill in the art. For example, Fujii, 2004, “Antibody affinity maturation by random mutagenesis” in *Methods in Molecular Biology: Antibody Engineering* 248: 345-349 (incorporated by reference herein in its entirety), particularly at Figure 2 and Section 3.3, describes methods of generating a library for any CDR of interest. This allows one of ordinary skill in the art to identify alternative CDRs, including conservative substitution variants of the specific CDR sequences described herein, which, when present in an antibody or antigen-binding portion thereof as described herein, will result in an antigen or antigen-binding portion thereof. The method described in Fujii *et al.* also permits one of ordinary skill in the art to screen for a light chain sequence which will give the desired binding behavior when combined with a known heavy chain fragment and vice versa.

[0093] In some embodiments of any of the aspects, a conservative substitution variant has at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to the applicable reference sequence (e.g., to one of the variable region sequences provided herein). In some embodiments of any of the aspects, a conservative substitution variant has at least 90% sequence identity to the applicable reference sequence (e.g., to one of the variable region sequences provided herein). In some embodiments of any of the aspects, a conservative substitution variant has at least 95% sequence identity to the applicable reference sequence (e.g., to one of the variable region sequences provided herein). In some embodiments of any of the aspects, a conservative substitution variant has at least 95% sequence identity to the applicable reference sequence (e.g., to one of the variable region sequences provided herein) and retains the wild-type activity of the reference sequence (e.g., the ability to bind specifically to SARS-COV-2 and/or to inhibit SARS-COV-2).

[0094] Variants of the sequences of Table 1 are contemplated herein, including humanized variants. For example, the following variants of SPIKE-BC1-7-7 are contemplated herein:

Variants of the heavy chain CDR3 and corresponding variants of the heavy chain variable region:

Variant heavy chain CDR3	Heavy chain variable sequence
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ARDRVLY GSSFGWY FDV (SEQ ID NO: 321)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNS GGTNFAQIFQGRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYF DVWGAGTTVTVSS (SEQ ID NO: 322)
ARDRVLY GSSFGWY FDV (SEQ ID NO: 323)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNS GGTNFAQIFQGRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYF DVWGAGTTVTVSS (SEQ ID NO: 323)
ARDRVLY GRSFGWY FDL (SEQ ID NO: 325)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNS GGTNFAQIFQGRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYF DLWGAGTTVTVSS (SEQ ID NO: 326)
ARDRVLY GSSFGWY FDL (SEQ ID NO: 327)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNS GGTNFAQIFQGRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYF DLWGAGTTVTVSS (SEQ ID NO: 328)
ARDRVLY GSSFGWY FDL (SEQ ID NO: 329)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNS GGTNFAQIFQGRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYF DLWGAGTTVTVSS (SEQ ID NO: 330)

Variants in the heavy chain framework sequences in the variable domain:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDVWGRGTTVTVSS (SEQ ID NO: 331)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDVWGAGTLVTVSS (SEQ ID NO: 332)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDVWGRGTLVTVSS (SEQ ID NO: 333)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGRGTTVTVSS (SEQ ID NO: 334)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGAGTLVTVSS (SEQ ID NO: 335)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGRGTLTVTVSS (SEQ ID NO: 336)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGRGTTTVTVSS (SEQ ID NO: 337)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGAGTLTVTVSS (SEQ ID NO: 338)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDVWGRGTLTVTVSS (SEQ ID NO: 339)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDLWGRGTTTVTVSS (SEQ ID NO: 340)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDLWGAGTLTVTVSS (SEQ ID NO: 341)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGRSFGWYFDLWGRGTLTVTVSS (SEQ ID NO: 342)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGRGTTTVTVSS (SEQ ID NO: 343)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGAGTLTVTVSS (SEQ ID NO: 344)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGRGTLTVTVSS (SEQ ID NO: 345)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGRGTTTVTVSS (SEQ ID NO: 346)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGAGTLTVTVSS (SEQ ID NO: 347)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTGTYIHWVRQAPGQGLEWMGWINPNSGGTNFAQIFQ GRVTLTRDTSISTAYMDLNRLKSDDTAVYYCARDRVLYGSSFGWYFDLWGRGTLTVTVSS (SEQ ID NO: 348)

Variants in the light chain framework sequences in the variable domain:

DIQMTQSPSSLSASVGDRTITCQASQDISDYLNWYQQQPGKAPKLLIYDASNLETGVPSRFSGSGSG
TDFTFTISSLPEDIGTYCQQYDNLPTFGQGTKLEIK (SEQ ID NO: 349)

[0095] In some embodiments of any of the aspects, an antibody, antibody reagent, CAR, or antigen-binding portion thereof comprises:

the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 321, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;

the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 323, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;

the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 325, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;

the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 327, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8; or

the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8.

[0096] In some embodiments of any of the aspects, an antibody, antibody reagent, CAR, or antigen-binding portion thereof comprises:

the VH sequences of SEQ ID NO: 1 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 322 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 324 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 326 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 328 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 330 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 331 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 332 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 333 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 334 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 335 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 336 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 337 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 338 and the VL sequence of SEQ ID NO: 2;

the VH sequences of SEQ ID NO: 339 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 340 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 341 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 342 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 343 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 344 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 345 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 346 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 347 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 348 and the VL sequence of SEQ ID NO: 2;
the VH sequences of SEQ ID NO: 1 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 322 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 324 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 326 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 328 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 330 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 331 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 332 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 333 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 334 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 335 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 336 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 337 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 338 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 339 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 340 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 341 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 342 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 343 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 344 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 345 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 346 and the VL sequence of SEQ ID NO: 349;
the VH sequences of SEQ ID NO: 347 and the VL sequence of SEQ ID NO: 349; or
the VH sequences of SEQ ID NO: 348 and the VL sequence of SEQ ID NO: 349.

[0097] Other variants of the sequences of Table 1 are contemplated herein, including humanized variants.

[0098] In some embodiments of any of the aspects, any antibody or antibody reagent described herein can comprise a heavy chain CDR3 in which the sequence “YGRS” of a heavy chain CDR3 of Table 1 is mutated to comprise “YGSS” or “YGGS.”

[0099] In some embodiments of any of the aspects, any antibody or antibody reagent described herein can comprise a heavy chain variable region in which the sequence “FDVW” of a heavy chain variable region of Table 1 is mutated to comprise “FDLW.” In some embodiments of any of the aspects, any antibody or antibody reagent described herein can comprise a heavy chain variable region in which the sequence “WGAG” of a heavy chain variable region of Table 1 is mutated to comprise “WGRG.” In some embodiments of any of the aspects, any antibody or antibody reagent described herein can comprise a heavy chain variable region in which the sequence “GTTV” of a heavy chain variable region of Table 1 is mutated to comprise “GTLV.”

[00100] In some embodiments of any of the aspects, any antibody or antibody reagent described herein can comprise a light chain variable region in which the sequence “FGGG” of a light chain variable region of Table 1 is mutated to comprise “FGQG.”

[00101] Any two or more of the foregoing mutations may be combined in a single variant antibody or antibody reagent.

[00102] In some embodiments, a CAR comprises an extracellular domain comprising an anti-SARS-CoV2 antibody or antigen-binding portion thereof as described herein, that binds one or more epitopes of a SARS-CoV2 polypeptide; a transmembrane domain, one or more intracellular co-stimulatory signaling domains, and a primary signaling domain. Exemplary anti-SARS-CoV2 antibodies and antigen-binding portions thereof, as well as exemplary epitopes, are described elsewhere herein.

[00103] As used herein, “chimeric antigen receptor” or “CAR” refers to an artificially constructed hybrid polypeptide comprising an antigen-binding domain (*e.g.*, an antigen-binding portion of an antibody (*e.g.*, a scFv)), a transmembrane domain, and a T-cell signaling and/or T-cell activation domain. CARs have the ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T-cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of immune escape.

[00104] Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T-cell receptor (TCR) alpha and beta chains. Most commonly, the CAR’s extracellular binding domain is composed of a single chain variable fragment (scFv) derived from fusing the variable heavy and light regions of a murine or humanized monoclonal antibody. Alternatively, scFvs may be used that are derived from Fab’s (instead of from an antibody, *e.g.*, obtained from Fab libraries), in various embodiments, this scFv is fused to a transmembrane domain and then to an intracellular signaling domain.

[00105] “First-generation” CARs include those that solely provide CD3zeta (CD3ζ) signals upon antigen binding, “Second-generation” CARs include those that provide both costimulation (*e.g.*, CD28

or CD 137) and activation (CD3 ζ). “Third-generation” CARs include those that provide multiple costimulatory (*e.g.*, CD28 and CD 137) domains and activation domains (*e.g.*, CD3 ζ). In various embodiments, the CAR is selected to have high affinity or avidity for the antigen. Further discussion of CARs can be found, *e.g.*, in Maus *et al.* Blood 2014 123:2624-35; Reardon *et al.* Neuro-Oncology 2014 16:1441-1458; Hoyos *et al.* Haematologica 2012 97:1622; Byrd *et al.* J Clin Oncol 2014 32:3039-47; Maher *et al.* Cancer Res 2009 69:4559-4562; and Tamada *et al.* Clin Cancer Res 2012 18:6436-6445; each of which is incorporated by reference herein in its entirety.

[00106] In some embodiments of any of the aspects, a CAR comprises an extracellular binding domain that comprises a humanized SARS-CoV2-specific binding domain; a transmembrane domain; one or more intracellular co-stimulatory signaling domains; and a primary signaling domain. As used herein, the terms, “binding domain,” “extracellular domain,” “extracellular binding domain,” “antigen-specific binding domain,” and “extracellular antigen specific binding domain,” are used interchangeably and provide a CAR with the ability to specifically bind to the target antigen of interest, *e.g.*, SARS-CoV2. The binding domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

[00107] In some embodiments, the CARs contemplated herein may comprise linker residues between the various domains, *e.g.*, added for appropriate spacing and conformation of the molecule. In particular embodiments the linker is a variable region linking sequence. A “variable region linking sequence,” is an amino acid sequence that connects the VH and VL domains and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. CARs contemplated herein, can comprise one, two, three, four, or five or more linkers. In particular embodiments, the length of a linker is about 1 to about 25 amino acids, about 5 to about 20 amino acids, or about 10 to about 20 amino acids, or any intervening length of amino acids. In some embodiments, the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids long.

[00108] In particular embodiments, the binding domain of the CAR is followed by one or more “spacer domains,” which refers to the region that moves the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. In certain embodiments, a spacer domain is a portion of an immunoglobulin, including, but not limited to, one or more heavy chain constant regions, *e.g.*, CH2 and CH3. The spacer domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region.

[00109] The binding domain of the CAR is generally followed by one or more “hinge domains,” which plays a role in positioning the antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. A CAR generally comprises one or more

hinge domains between the binding domain and the transmembrane domain (TM). The hinge domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The hinge domain can include the amino acid sequence of a naturally occurring immunoglobulin hinge region or an altered immunoglobulin hinge region. Illustrative hinge domains suitable for use in the CARs described herein include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD8 α , CD4, CD28 and CD7, which may be wild-type hinge regions from these molecules or may be altered. In another embodiment, the hinge domain comprises a CD8 α hinge region.

[00110] The “transmembrane domain” is the portion of the CAR that fuses the extracellular binding portion and intracellular signaling domain and anchors the CAR to the plasma membrane of the immune effector cell. The TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. The TM domain may be derived from (*i.e.*, comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD3 ϵ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD 16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD 134, CD137, CD152, CD 154, and PD1.

[00111] In some embodiments, CARs contemplated herein comprise an intracellular signaling domain. An “intracellular signaling domain,” refers to the part of a CAR that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit effector cell function, *e.g.*, activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target, or other cellular responses elicited with antigen binding to the extracellular CAR domain. In some embodiments, a CAR contemplated herein comprises an intracellular signaling domain that comprises one or more “co-stimulatory signaling domain” and a “primary signaling domain.”

[00112] Primary signaling domains regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Illustrative examples of ITAM containing primary signaling domains that are of particular use in the invention include those derived from TCR ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d.

[00113] As used herein, the term, “co-stimulatory signaling domain,” or “co-stimulatory domain”, refers to an intracellular signaling domain of a co-stimulatory molecule. Co-stimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Illustrative examples of such co-stimulatory molecules include CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70. In one embodiment, a CAR comprises one or more co-stimulatory signaling

domains selected from the group consisting of CD28, CD137, and CD134, and a CD3 ζ primary signaling domain.

[00114] In some embodiments, an antibody-drug conjugate is provided. In particular embodiments, an antibody-drug conjugate comprises an antibody, antibody reagent, or antigen-binding portion thereof as described herein. The drug can be, *e.g.*, an anti-viral drug or small molecule drug. In some embodiments, the antibody-drug conjugate comprises a drug directly conjugated and/or bound to an antibody or antigen-binding portion thereof. In some embodiments, binding can be non-covalent, *e.g.*, by hydrogen bonding, electrostatic, or van der Waals interactions; however, binding may also be covalent. By “conjugated” is meant the covalent linkage of at least two molecules. In some embodiments, the composition can be an antibody-drug conjugate.

[00115] In some embodiments, an antibody, antibody reagent, or antigen-binding portion thereof can be bound to and/or conjugated to multiple drug molecules. In some embodiments, an antibody-drug conjugate can be bound to and/or conjugated to multiple drug molecules. In some embodiments, the ratio of a given drug molecule to an antibody or antigen-binding portion thereof can be from about 1:1 to about 1,000:1, *e.g.*, a single antibody reagent molecule can be linked to, conjugated to, etc. from about 1 to about 1,000 individual drug molecules.

[00116] In some embodiments, an antibody, or antigen-binding portion thereof, and optionally the drug agent can be present in a scaffold material. Scaffold materials suitable for use in therapeutic compositions are known in the art and can include, but are not limited to, a nanoparticle; a matrix; a hydrogel; and a biomaterial, biocompatible, and/or biodegradable scaffold material. As used herein, the term “nanoparticle” refers to particles that are on the order of about 10^{-9} or one to several billionths of a meter. The term “nanoparticle” includes nanospheres; nanorods; nanoshells; and nanoprisms; these nanoparticles may be part of a nanonetwork.

[00117] The term “nanoparticles” also encompasses liposomes and lipid particles having the size of a nanoparticle. As used herein, the term “matrix” refers to a 3-dimensional structure comprising the components of a composition described herein (*e.g.*, an antibody or antigen-binding portion thereof). Non-limiting examples of matrix structures include foams; hydrogels; electrospun fibers; gels; fiber mats; sponges; 3-dimensional scaffolds; non-woven mats; woven materials; knit materials; fiber bundles; and fibers and other material formats (See, *e.g.*, Rockwood *et al.* Nature Protocols 2011 6:1612-1631 and US Patent Publications 2011/0167602; 2011/0009960; 2012/0296352; and U.S. Patent No. 8,172,901; each of which is incorporated by reference herein in its entirety). The structure of the matrix can be selected by one of skill in the art depending upon the intended application of the composition, *e.g.*, electrospun matrices can have greater surface area than foams.

[00118] In some embodiments, the scaffold is a hydrogel. As used herein, the term “hydrogel” refers to a three-dimensional polymeric structure that is insoluble in water but which is capable of absorbing and retaining large quantities of water to form a stable, often soft and pliable, structure. In some embodiments, water can penetrate in between the polymer chains of the polymer network, subsequently

causing swelling and the formation of a hydrogel. In general, hydrogels are superabsorbent. Hydrogels have many desirable properties for biomedical applications. For example, they can be made nontoxic and compatible with tissue, and they are highly permeable to water, ions, and small molecules. Hydrogels are super-absorbent (they can contain over 99% water) and can be comprised of natural (*e.g.*, silk) or synthetic polymers, *e.g.*, PEG.

[00119] As used herein, “biomaterial” refers to a material that is biocompatible and biodegradable. As used herein, the term “biocompatible” refers to substances that are not toxic to cells. In some embodiments, a substance is considered to be “biocompatible” if its addition to cells *in vitro* results in less than or equal to approximately 20% cell death. In some embodiments, a substance is considered to be “biocompatible” if its addition to cells *in vivo* does not induce inflammation and/or other adverse effects *in vivo*. As used herein, the term “biodegradable” refers to substances that are degraded under physiological conditions. In some embodiments, a biodegradable substance is a substance that is broken down by cellular machinery. In some embodiments, a biodegradable substance is a substance that is broken down by chemical processes.

[00120] In some embodiments, the technology described herein relates to a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein. In some embodiments, the nucleic acid is a cDNA.

[00121] As used herein, the term “nucleic acid” or “nucleic acid sequence” refers to a polymeric molecule incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one strand nucleic acid of a denatured double-stranded DNA. In some embodiments, the nucleic acid can be a cDNA, *e.g.*, a nucleic acid lacking introns.

[00122] Nucleic acid molecules encoding amino acid sequence variants of antibodies are prepared by a variety of methods known in the art. These methods include, but are not limited to preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody. A nucleic acid sequence encoding at least one antibody, portion or polypeptide as described herein can be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations can be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, antibody reagent, or antigen binding region thereof.

[00123] A nucleic acid molecule, such as DNA, is said to be “capable of expressing” a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are “operably linked” to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as peptides or antibody portions

in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art.

[00124] In some embodiments, a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein is comprised by a vector. In some of the aspects described herein, a nucleic acid sequence encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein, or any module thereof, is operably linked to a vector. The term “vector”, as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

[00125] As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term “expression” refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. “Expression products” include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term “gene” means the nucleic acid sequence which is transcribed (DNA) to RNA *in vitro* or *in vivo* when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, *e.g.*, 5' untranslated (5'UTR) or “leader” sequences and 3' UTR or “trailer” sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[00126] As used herein, the term “viral vector” refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain the nucleic acid encoding an antibody, or antigen-binding portion thereof, as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring any nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

[00127] By “recombinant vector” is meant a vector that includes a heterologous nucleic acid sequence, or “transgene” that is capable of expression *in vivo*. It should be understood that the vectors described herein can, in some embodiments, be combined with other suitable compositions and therapies. In some embodiments, the vector is episomal. The use of a suitable episomal vector provides a means of

maintaining the nucleotide of interest in the subject in high copy number extra chromosomal DNA thereby eliminating potential effects of chromosomal integration.

[00128] In one aspect of any of the embodiments, described herein is a cell comprising an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein, or a nucleic acid encoding such an antibody, antibody reagent, CAR, or antigen-binding portion thereof.

[00129] The expression of an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein can occur in either prokaryotic or eukaryotic cells. Suitable hosts include bacterial or eukaryotic hosts, including yeast, insects, fungi, bird and mammalian cells either *in vivo*, or *in situ*, or host cells of mammalian, insect, bird or yeast origin. The mammalian cell or tissue can be of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used. Further, by use of, for example, the yeast ubiquitin hydrolase system, *in vivo* synthesis of ubiquitin-transmembrane polypeptide fusion proteins can be accomplished. The fusion proteins so produced can be processed *in vivo* or purified and processed *in vitro*, allowing synthesis of an antibody or portion thereof as described herein with a specified amino terminus sequence.

[00130] Moreover, problems associated with retention of initiation codon-derived methionine residues in direct yeast (or bacterial) expression maybe avoided. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast is grown in mediums rich in glucose can be utilized to obtain recombinant antibodies or antigen-binding portions thereof as described herein. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

[00131] Production of antibodies or antigen-binding portions thereof as described herein in insects can be achieved. For example, by infecting the insect host with a baculovirus engineered to express a transmembrane polypeptide by methods known to those of ordinary skill in the art.

[00132] In some embodiments, the introduced nucleotide sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose and are known and available to those of ordinary skill in the art. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to “shuttle” the vector between host cells of different species.

[00133] Exemplary prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli*, for example. Other gene expression elements useful for the expression of cDNA encoding antibodies, or antigen-binding portions thereof, include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter, Rous sarcoma virus LTR, and Moloney murine leukemia virus; (b) splice regions and polyadenylation sites such as those derived from the SV40 late region, and (c) polyadenylation sites such as in SV40.

Immunoglobulin cDNA genes can be expressed, e.g., using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit S-globin intervening sequence, immunoglobulin and rabbit S-globin polyadenylation sites, and SV40 polyadenylation elements.

[00134] For immunoglobulin genes comprised of part cDNA, part genomic DNA, the transcriptional promoter can be human cytomegalovirus, the promoter enhancers can be cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions can be the native chromosomal immunoglobulin sequences.

[00135] In some embodiments, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

[00136] A gene is assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the chimeric immunoglobulin chain gene product are then transfected singly with an antibody, antigen-binding portion thereof, or chimeric H or chimeric L chain-encoding gene, or are co-transfected with a chimeric H and a chimeric L chain gene. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

[00137] In some embodiments, the genes encoding the antibody, antibody reagent, CAR, or antigen-binding portion thereof, or chimeric H and L chains, or portions thereof are assembled in separate expression vectors that are then used to co-transfect a recipient-cell. Each vector can contain two selectable genes, a first selectable gene designed for selection in a bacterial system and a second selectable gene designed for selection in a eukaryotic system, wherein each vector has a different pair of genes. This strategy results in vectors which first direct the production, and permit amplification, of the genes in a bacterial system. The genes so produced and amplified in a bacterial host are subsequently used to co-transfect a eukaryotic cell, and allow selection of a co-transfected cell carrying the desired transfected genes.

[00138] Non-limiting examples of selectable genes for use in a bacterial system are the gene that confers resistance to ampicillin and the gene that confers resistance to chloramphenicol. Selectable genes for use in eukaryotic transfectants include the xanthine guanine phosphoribosyl transferase gene (designated gpt) and the phosphotransferase gene from Tn5 (designated neo). Alternatively, the genes can be assembled on the same expression vector.

[00139] For transfection of the expression vectors and production of the antibodies, antibody reagents, or antigen-binding portions thereof, described herein, the recipient-cell line can be a myeloma cell.

Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin.

[00140] An expression vector carrying a chimeric, humanized, or composite human antibody construct, antibody, and/or antigen-binding portion thereof, as described herein can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment, as known to one of ordinary skill in the art.

[00141] Traditionally, monoclonal antibodies have been produced as native molecules in murine hybridoma lines. In addition to that technology, the methods and compositions described herein provide for recombinant DNA expression of monoclonal antibodies. This allows the production of humanized antibodies as well as a spectrum of antibody derivatives and fusion proteins in a host species of choice. The production of antibodies in bacteria, yeast, transgenic animals and chicken eggs are also alternatives for hybridoma-based production systems. The main advantages of transgenic animals are potential high yields from renewable sources.

[00142] In one aspect, a cell comprising an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein is provided. In some embodiments, the antibody antibody reagent, CAR, or antigen-binding portion thereof, as described herein is expressed on the cell surface. In some embodiments, the cell comprises a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein.

[00143] In some embodiments, the cell is an immune cell. As used herein, “immune cell” refers to a cell that plays a role in the immune response. Immune cells are of hematopoietic origin, and include lymphocytes, such as B-cells and T-cells; natural killer cells; myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes. In some embodiments, the cell is a T-cell; a NK cell; a NKT-cell; lymphocytes, such as B-cells and T-cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes.

[00144] In particular embodiments, a cell (*e.g.*, an immune cell) is transduced with a retroviral vector, *e.g.*, a lentiviral vector, encoding a CAR. For example, an immune effector cell is transduced with a vector encoding a CAR that comprises an anti-SARS-CoV2 antibody or antigen binding portion thereof. Thus, these transduced cells can elicit a CAR-mediated response.

[00145] Retroviruses are a common tool for gene delivery. As used herein, the term “retrovirus” refers to an RNA virus that reverse transcribes its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Once the virus is integrated into the host genome, it is referred to as a “provirus.” The provirus serves as a template for RNA polymerase II and directs the expression of RNA molecules which encode the structural proteins and enzymes needed to produce new viral particles.

[00146] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV)) and lentivirus.

[00147] As used herein, the term “lentivirus” refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In one embodiment, HIV based vector backbones (*i.e.*, HIV cis-acting sequence elements) are preferred.

[00148] Retroviral vectors and more particularly lentiviral vectors may be used in practicing particular embodiments of the present invention. Accordingly, the term “retrovirus” or “retroviral vector”, as used herein is meant to include “lentivirus” and “lentiviral vectors” respectively.

[00149] In one aspect of any of the embodiments, described herein is a composition comprising an antibody, antibody reagent, CAR or antigen-binding portion thereof, as described herein or a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein or a cell as described herein. In some embodiments, the composition is a pharmaceutical composition. As used herein, the term “pharmaceutical composition” refers to the active agent in combination with a pharmaceutically acceptable carrier accepted for use in the pharmaceutical industry. The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00150] In one aspect of any of the embodiments, described herein is a composition comprising at least one antibody, antibody reagent, CAR or antigen-binding portion thereof, as described herein (e.g, an antibody or antibody reagent comprising the CDRs of an antibody of Table 1). In some embodiments, the composition is a pharmaceutical composition. In some embodiments of any of the aspects, the composition comprises 2, 3, 4, 5, or more different antibodies or antibody reagents, each antibody or antibody reagent comprising the CDRs of an antibody of Table 1. In some embodiments of any of the aspects, the composition is an antibody cocktail. In some embodiments of any of the aspects, the composition further comprises further anti-Covid19 antibodies, e.g, casrivcimab, imdevimab, bebtelovimab (LY-CoV1404) and/or sotrovimab (s309nB).

[00151] The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art and need not be limited based on formulation. Typically, such compositions are prepared as injectable either as liquid solutions or suspensions, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation

can also be emulsified or presented as a liposome composition. The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance or maintain the effectiveness of the active ingredient.

[00152] The therapeutic composition as described herein can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

[00153] Physiologically tolerable carriers are well known in the art. Exemplary liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes. Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions. The amount of an active agent used in the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques.

[00154] In some embodiments, the composition comprising an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein or a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein can be a lyophilisate.

[00155] In some embodiments, the technology described herein relates to a syringe or catheter, including an organ-specific catheter (*e.g.*, renal catheter, biliary catheter, cardiac catheter, etc.), comprising a therapeutically effective amount of a composition described herein.

[00156] As used herein, the phrase “therapeutically effective amount”, “effective amount” or “effective dose” refers to an amount that provides a therapeutic or aesthetic benefit in the treatment, prevention, or management of a coronavirus infection, *e.g.*, an amount that provides a statistically significant decrease in at least one symptom, sign, or marker of the coronavirus infection. Determination of a therapeutically effective amount is well within the capability of those skilled in the art. Generally, a therapeutically effective amount can vary with the subject’s history, age, condition, sex, as well as the

severity and type of the medical condition in the subject, and administration of other pharmaceutically active agents.

[00157] In one aspect, the technology described herein relates to a method comprising administering an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein or a nucleic acid encoding an antibody, antibody reagent, CAR, or antigen-binding portion thereof, as described herein to a subject. In some embodiments of any of the aspects, the subject is in need of treatment for a coronavirus infection. In some embodiments of any of the aspects, the subject is in need of treatment for a coronavirus exposure. In some embodiments of any of the aspects, the subject is symptomatic. In some embodiments of any of the aspects, the subject is asymptomatic. In some embodiments of any of the aspects, the method is a method of treating a subject. In some embodiments of any of the aspects, the method is a method of treating or preventing a coronavirus infection in a subject.

[00158] In an alternative embodiment, the nucleic acid can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of a nucleic acid molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of a nucleic acid by the cell. Cationic lipids, dendrimers, or polymers can either be bound to a nucleic acid, or induced to form a vesicle or micelle (see *e.g.*, Kim SH., et al. (2008) *Journal of Controlled Release* 129(2):107-116) that encases a nucleic acid. The formation of vesicles or micelles further prevents degradation of the nucleic acid when administered systemically.

[00159] Methods for making and administering cationic- inhibitory nucleic acid complexes are well within the abilities of one skilled in the art. Some non-limiting examples of drug delivery systems useful for systemic delivery of nucleic acids include DOTAP Oligofectamine, “solid nucleic acid lipid particles”, cardiolipin, polyethyleneimine, Arg-Gly-Asp (RGD) peptides, and polyamidoamines. In some embodiments, a nucleic acid forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of nucleic acids and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety. Targeted delivery of nucleic acids is described, for example in Ikeda and Taira *Pharmaceutical Res* 2006 23:1631-1640; Soutschek *et al.*, *Nature* 2004 432:173-8 and Lorenze *et al.* *Bioorg. Med. Chem. Lett.* 14, 4975–4977 (2004); each of which is incorporated by reference herein in its entirety.

[00160] In some embodiments, the methods described herein relate to CAR-T-cell therapy. CAR-T-cell and related therapies relate to adoptive cell transfer of immune cells (*e.g.*, T-cells) expressing a CAR that binds specifically to a target (*e.g.*, SARS-CoV2) to treat a subject. In some embodiments, the cells administered as part of the therapy can be autologous to the subject. In some embodiments, the cells administered as part of the therapy are not autologous to the subject. In some embodiments, the cells are engineered and/or genetically modified to express the CAR. Further discussion of CAR-T therapies can be found, *e.g.*, in Maus *et al.* *Blood* 2014 123:2624-35; Reardon *et al.* *Neuro-Oncology* 2014 16:1441-1458; Hoyos *et al.* *Haematologica* 2012 97:1622; Byrd *et al.* *J Clin Oncol* 2014 32:3039-47;

Maher *et al.* Cancer Res 2009 69:4559-4562; and Tamada *et al.* Clin Cancer Res 2012 18:6436-6445; each of which is incorporated by reference herein in its entirety.

[00161] Provided herein are methods and compositions for the treatment and/or prevention of a coronavirus infection and/or exposure in a subject in need thereof. Such methods relate to administration of one or more of the antibodies, antibody reagent, antigen-binding portions thereof, or CARs described herein to the subject. In some embodiments of any of the aspects, described herein is a method of treating a coronavirus infection in a subject in need thereof, the method comprising administering an antibody, antibody reagent, or antigen-binding fragment thereof as described herein, and/or a cell comprising an antibody, antibody reagent, or antigen-binding fragment thereof as described herein to the subject.

[00162] “Coronaviruses” consist of a large and diverse family of enveloped, positive-sense, single-stranded RNA viruses. Every coronavirus contains four structural proteins, for example spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Coronavirus diversity is reflected in the variable S proteins, which have evolved into forms differing in their receptor interactions and their response to various environmental triggers of virus- cell membrane fusion. In particular, the RBD of the S protein is the most variable genomic part in the betacoronavirus group.

[00163] Four serologically distinct groups of coronaviruses have been described, i.e., alpha, beta (previously referred to as group 2), delta, and gamma. Within each group, viruses are characterized by their host range and genome sequence. The alphacoronaviruses and betacoronaviruses infect only mammals, while the gammacoronaviruses and deltacoronaviruses primarily infect birds, although some of them can also infect mammals. Novel mammalian coronaviruses are now regularly identified. (see e.g., Su *et al.*, Trends Microbiol. 2016; 24: 490-502). Betacoronaviruses (Beta-CoV) of known clinical importance to humans includes viruses of the A, B and C lineage and more particularly, the A lineage: OC43 (which can cause the common cold) and HKU1; the B lineage: SARS-CoV and SARS-CoV-2 (which causes the disease COVID-19); and the C lineage: MERS-CoV.

[00164] In one embodiment, the coronavirus infection described herein is a betacoronavirus infection and more particularly, an A-lineage, B-lineage, or C lineage coronavirus infection. In another particular embodiment, the coronavirus infection described herein is an infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. SARS-CoV-2 (also referred to as 2019-nCoV) was identified in January 2020 as the causative agent of Severe Acute Respiratory Syndrome 2, also referred to as Covid-19. In some embodiments of any of the aspects, the coronavirus infection described herein is an Alpha, Beta, Gamma, Delta, Epsilon, Eta, Iota, Kappa, Mu, Zeta, or Omicron SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.1.7, Q lineage, B.1.351, P.1, B.1.617.2, AY lineage, B.1.427, B.1.429, B.1.525, B.1.526, B.1.617.1, 1.617.3, B.1.621, B.1.621.1, P.2, B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.4/5 BA.2.12.1, or BA.5 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a G614 SARS-CoV-

2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.17 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.351 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a P.1 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.429 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.526 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.526 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a B.1.617.2 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.1 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.2 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.3 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.4 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.4/5 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.2.12.1 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA.5 SARS-CoV-2 infection. In some embodiments of any of the aspects, the coronavirus infection described herein is a BA/2/12/1 SARS-CoV-2 infection.

[00165] Clinical signs associated with SARS-CoV-2 include pneumonia, fever, dry cough, headache, and dyspnea, which may progress to respiratory failure and death. The incubation period for SARS-CoV-2 of 2 to 14 days can be longer than for SARS-CoV and MERS-CoV, which have a mean incubation time of 5 to 7 days.

[00166] SARS-CoV-2 was sequenced and isolated by January 2020 (see e.g., e.g., Zhou N. *N Engl J Med.*, 382 (2020), pp. 727-733). Several sequences of SARS-CoV-2 have since been released (see e.g., complete genome, SARS-CoV-2 Jan. 2020/NC_045512.2 Assembly (wuhCor1)).

[00167] In some embodiments of any of the aspects, the antibody, antibody reagent, or antigen-binding fragment thereof as described binds specifically to a coronavirus spike protein. In some embodiments of any of the aspects, the antibody, antibody reagent, or antigen-binding fragment thereof as described binds specifically to a coronavirus receptor binding domain (RBD) protein. In some embodiments of any of the aspects, the coronavirus spike protein is a MERS-CoV spike or RBD protein. In some embodiments of any of the aspects, the coronavirus spike protein is a SARS-CoV-1 spike or RBD protein. In some embodiments of any of the aspects, the coronavirus spike protein is a SARS-CoV-2 spike or RBD protein. In some embodiments, coronavirus protein (e.g., spike or RBD protein) is a protein of a variant of SARS-CoV-2, for example, that of the Alpha, Beta, Gamma, Epsilon, Delta, Kappa, Eta, or Omicron variants.

[00168] The compositions described herein can be administered to a subject to treat or prevent a coronavirus infection. In some embodiments, the methods described herein comprise administering an effective amount of an antibody, antibody reagent, CAR, or antigen-binding portion thereof, or cells described herein, to a subject in order to alleviate a symptom of a coronavirus infection.

[00169] As used herein, “alleviating a symptom” is ameliorating any condition or symptom associated with the disease. As compared with an equivalent untreated control, such reduction is by at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, 99% or more as measured by any standard technique. A variety of means for administering the compositions described herein to subjects are known to those of skill in the art. Such methods can include, but are not limited to oral, parenteral, intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), pulmonary, cutaneous, topical, or injection administration. Administration can be local or systemic.

[00170] As used herein, a “subject” means a human or animal. Usually the animal is 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, horses, pigs, deer, bison, buffalo, 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. Patients or subjects include any subset of the foregoing, *e.g.*, all of the above, but excluding one or more groups or species such as humans, primates or rodents. In certain embodiments, the subject is a mammal, *e.g.*, a primate, *e.g.*, a human. The terms, “patient”, “individual” and “subject” are used interchangeably herein.

[00171] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but are not limited to these examples. Mammals other than humans can be advantageously used, for example, as subjects that represent animal models of, for example, various autoimmune and/or autoinflammatory disorders. In addition, the methods described herein can be used to treat domesticated animals and/or pets. A subject can be male or female.

[00172] A subject can be one who has been previously diagnosed with or identified as suffering from or having a condition in need of treatment (*e.g.*, a coronavirus infection) or one or more complications related to such a condition, and optionally, but need not have already undergone treatment for a condition or the one or more complications related to the condition. Alternatively, a subject can also be one who has not been previously diagnosed as having a condition in need of treatment or one or more complications related to such a condition. For example, a subject can be one who exhibits one or more risk factors for a condition or one or more complications related to a condition or a subject who does not exhibit risk factors. A “subject in need” of treatment for a particular condition can be a subject having that condition, diagnosed as having that condition, or at risk of developing that condition.

[00173] As used herein, the terms “treat,” “treatment,” “treating,” or “amelioration” when used in reference to a disease, disorder or medical condition, refer to therapeutic treatments for a condition, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or

severity of a symptom or condition. The term “treating” includes reducing or alleviating at least one adverse effect or symptom of a condition. Treatment is generally “effective” if one or more symptoms or clinical markers are reduced. Alternatively, treatment is “effective” if the progression of a condition is reduced or halted. That is, “treatment” includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in the absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of the deficit, stabilized (*i.e.*, not worsening) state of an infection, delay or slowing of disease progression, and an increased lifespan as compared to that expected in the absence of treatment.

[00174] In some embodiments of any of the aspects, described herein is a prophylactic method of treatment. As used herein “prophylactic” refers to the timing and intent of a treatment relative to a disease or symptom, that is, the treatment is administered prior to clinical detection or diagnosis of that particular disease or symptom in order to protect the patient from the disease or symptom.

Prophylactic treatment can encompass a reduction in the severity or speed of onset of the disease or symptom, or contribute to faster recovery from the disease or symptom. Accordingly, the methods described herein can be prophylactic relative to symptoms of a coronavirus infection, a positive test for a coronavirus infection (e.g., after an exposure event), or worsening of symptoms that would require hospitalization or ventilation. In some embodiments of any of the aspects, prophylactic treatment is not prevention of all symptoms or signs of a disease.

[00175] As used herein, the term “administering,” refers to the placement of an agent, including but not limited to, an antibody, antibody reagent, or antigen-binding portion thereof, as described herein or a nucleic acid encoding an antibody, antibody reagent, or antigen-binding portion thereof, or a cell comprising such an agent, as described herein into a subject by a method or route which results in at least partial localization of the agents at a desired site. The pharmaceutical composition comprising an antibody, antibody reagent, or antigen-binding portion thereof, as described herein or a nucleic acid encoding an antibody, antibody reagent, antigen-binding portion thereof, or a cell comprising such an agent as described herein disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[00176] The administration of the compositions contemplated herein may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. In a preferred embodiment, compositions are administered parenterally. The phrases “parenteral administration” and “administered parenterally” as used herein refers to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravascular, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. In one

embodiment, the compositions contemplated herein are administered to a subject by direct injection into an affected joint or organ, lymph node, or site of infection.

[00177] It can generally be stated that a pharmaceutical composition comprising the cells, described herein may be administered at a dosage of 10^2 to 10^{10} cells/kg body weight, preferably 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein. For uses provided herein, the cells are generally in a volume of a liter or less, can be 500 mLs or less, even 250 mLs or 100 mLs or less. Hence the density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} cells.

[00178] In some aspects of the present invention, particularly since all the infused cells will be redirected to a particular target antigen, lower numbers of cells, in the range of 10^6 /kilogram (10^6 - 10^{11} per patient) may be administered. In some embodiments, the dosage can be from about 1×10^5 cells to about 1×10^8 cells per kg of body weight. In some embodiments, the dosage can be from about 1×10^6 cells to about 1×10^7 cells per kg of body weight. In some embodiments, the dosage can be about 1×10^6 cells per kg of body weight. In some embodiments, one dose of cells can be administered. In some embodiments, the dose of cells can be repeated, *e.g.*, once, twice, or more. In some embodiments, the dose of cells can be administered on, *e.g.*, a daily, weekly, or monthly basis.

[00179] The dosage ranges for the agent depend upon the potency, and encompass amounts large enough to produce the desired effect *e.g.*, slowing of disease progression or a reduction in disease activity. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the age, condition, and sex of the patient and can be determined by one of skill in the art. The dosage can also be adjusted by the individual physician in the event of any complication. In some embodiments, the dosage ranges from 0.001 mg/kg body weight to 0.5 mg/kg body weight. In some embodiments, the dose range is from 5 μ g/kg body weight to 100 μ g/kg body weight. Alternatively, the dose range can be titrated to maintain serum levels between 1 μ g/mL and 1000 μ g/mL. For systemic administration, subjects can be administered a therapeutic amount, such as, *e.g.*, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, 50 mg/kg, or more.

[00180] Administration of the doses recited above can be repeated. In some embodiments, the doses are given once a day, or multiple times a day, for example but not limited to three times a day. In some embodiments, the doses recited above are administered daily for several weeks or months. The duration of treatment depends upon the subject's clinical progress and responsiveness to therapy.

[00181] In some embodiments, the dose can be from about 2 mg/kg to about 15 mg/kg. In some embodiments, the dose can be about 2 mg/kg. In some embodiments, the dose can be about 4 mg/kg. In some embodiments, the dose can be about 5 mg/kg. In some embodiments, the dose can be about 6

mg/kg. In some embodiments, the dose can be about 8 mg/kg. In some embodiments, the dose can be about 10 mg/kg. In some embodiments, the dose can be about 15 mg/kg. In some embodiments, the dose can be from about 100 mg/m² to about 700 mg/m². In some embodiments, the dose can be about 250 mg/m². In some embodiments, the dose can be about 375 mg/m². In some embodiments, the dose can be about 400 mg/m². In some embodiments, the dose can be about 500 mg/m².

[00182] In some embodiments, the dose can be administered intravenously. In some embodiments, the intravenous administration can be an infusion occurring over a period of from about 10 minute to about 3 hours. In some embodiments, the intravenous administration can be an infusion occurring over a period of from about 30 minutes to about 90 minutes.

[00183] In some embodiments, the doses are given twice a week, once a week, biweekly, or monthly. In some embodiments, the dose can be administered weekly for from about 12 weeks to about 18 weeks. In some embodiments the dose can be administered about every 2 weeks. In some embodiments the dose can be administered about every 3 weeks. In some embodiments, the dose can be from about 2 mg/kg to about 15 mg/kg administered about every 2 weeks. In some embodiments, the dose can be from about 2 mg/kg to about 15 mg/kg administered about every 3 weeks. In some embodiments, the dose can be from about 2 mg/kg to about 15 mg/kg administered intravenously about every 2 weeks. In some embodiments, the dose can be from about 2 mg/kg to about 15 mg/kg administered intravenously about every 3 weeks. In some embodiments, the dose can be from about 200 mg/m² to about 400 mg/m² administered intravenously about every week. In some embodiments, the dose can be from about 200 mg/m² to about 400 mg/m² administered intravenously about every 2 weeks. In some embodiments, the dose can be from about 200 mg/m² to about 400 mg/m² administered intravenously about every 3 weeks. In some embodiments, a total of from about 2 to about 10 doses are administered. In some embodiments, a total of 4 doses are administered. In some embodiments, a total of 5 doses are administered. In some embodiments, a total of 6 doses are administered. In some embodiments, a total of 7 doses are administered. In some embodiments, a total of 8 doses are administered. In some embodiments, the administration occurs for a total of from about 4 weeks to about 12 weeks. In some embodiments, the administration occurs for a total of about 6 weeks. In some embodiments, the administration occurs for a total of about 8 weeks. In some embodiments, the administration occurs for a total of about 12 weeks. In some embodiments, the initial dose can be from about 1.5 to about 2.5 fold greater than subsequent doses.

[00184] In some embodiments, the dose can be from about 1 mg to about 2000 mg. In some embodiments, the dose can be about 3 mg. In some embodiments, the dose can be about 10 mg. In some embodiments, the dose can be about 30 mg. In some embodiments, the dose can be about 1000 mg. In some embodiments, the dose can be about 2000 mg. In some embodiments, the dose can be about 3 mg given by intravenous infusion daily. In some embodiments, the dose can be about 10 mg given by intravenous infusion daily. In some embodiments, the dose can be about 30 mg given by intravenous infusion three times per week.

[00185] A therapeutically effective amount is an amount of an agent that is sufficient to produce a statistically significant, measurable change in disease activity, disease progression, etc. (efficacy measurements are described below herein). Such effective amounts can be gauged in clinical trials as well as animal studies.

[00186] An agent can be administered intravenously by injection or by gradual infusion over time. Given an appropriate formulation for a given route, for example, agents useful in the methods and compositions described herein can be administered intravenously, intranasally, by inhalation, intraperitoneally, intramuscularly, subcutaneously, intracavity, and can be delivered by peristaltic means, if desired, or by other means known by those skilled in the art. It is preferred that the compounds used herein are administered orally, intravenously or intramuscularly to a patient having an autoimmune or autoinflammatory response, condition, or disorder. Local administration directly to affected sites is also specifically contemplated.

[00187] Therapeutic compositions containing at least one agent can be conventionally administered in a unit dose, for example. The term “unit dose” when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required physiologically acceptable diluent, *i.e.*, carrier, or vehicle.

[00188] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered and timing depends on the subject to be treated, capacity of the subject’s system to utilize the active ingredient, and degree of therapeutic effect desired.

[00189] Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are particular to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for *in vivo* therapies are contemplated.

[00190] In one aspect, described herein is a method of detecting SARS-COV-2, the method comprising contacting a biological sample with an antibody, antibody reagent, or antigen-binding portion thereof as described herein, wherein reaction of the antibody or antigen-binding portion thereof with SARS-COV-2 indicates the presence of SARS-COV-2. In some embodiments, a detectable signal is generated by the antibody or antigen-binding portion thereof when a SARS-COV-2 molecule is present. In some embodiments, the antibody or antigen-binding portion thereof is detectably labeled or capable of generating a detectable signal. In some embodiments, the level of the SARS-COV-2 is determined using a method selected from the group consisting of: Western blot; immunoprecipitation; enzyme-linked immunosorbent assay (ELISA); radioimmunological assay (RIA); sandwich assay; fluorescence in situ

hybridization (FISH); immunohistological staining; radioimmunometric assay; immunofluorescence assay; mass spectroscopy; FACS; and immunoelectrophoresis assay. In some embodiments, the antibody or antigen-binding portion thereof is detectably labeled or generates a detectable signal. In some embodiments, the expression level of SARS-COV-2 is normalized relative to the expression level of one or more reference genes or reference proteins. In some embodiments, the reference level of SARS-COV-2 is the expression level of SARS-COV-2 in a prior sample obtained from the subject.

[00191] In some embodiments, the level of SARS-COV-2 can be the level of SARS-COV-2 polypeptide. Detection of SARS-COV-2 polypeptides can be according to any method known in the art. Immunological methods to detect SARS-COV-2 polypeptides in accordance with the present technology include, but are not limited to antibody techniques such as immunohistochemistry, immunocytochemistry, flow cytometry, fluorescence-activated cell sorting (FACS), immunoblotting, radioimmunoassays, western blotting, immunoprecipitation, enzyme-linked immunosorbant assays (ELISA), and derivative techniques that make use of antibody reagents as described herein.

[00192] Immunochemical methods require the use of an antibody reagent specific for the target molecule (*e.g.*, the antigen or in the embodiments described herein, a SARS-COV-2 polypeptide. In some embodiments, the assays, methods, and/or systems described herein can comprise: an anti- SARS-COV-2 antibody reagent. In some embodiments, the antibody reagent can be detectably labeled. In some embodiments, the antibody reagent can be attached to a solid support (*e.g.*, bound to a solid support). In some embodiments, the solid support can comprise a particle (including, but not limited to an agarose or latex bead or particle or a magnetic particle), a bead, a nanoparticle, a polymer, a substrate, a slide, a coverslip, a plate, a dish, a well, a membrane, and/or a grating. The solid support can include many different materials including, but not limited to, polymers, plastics, resins, polysaccharides, silicon or silica based materials, carbon, metals, inorganic glasses, and membranes.

[00193] In one embodiment, an assay, method, and/or system as described herein can comprise an ELISA. In an exemplary embodiment, a first antibody reagent can be immobilized on a solid support (usually a polystyrene micro titer plate). The solid support can be contacted with a sample obtained from a subject, and the antibody reagent will bind (“capture”) antigens for which it is specific (*e.g.*, SARS-COV-2). The solid support can then be contacted with a second labeled antibody reagent (*e.g.*, a detection antibody reagent). The detection antibody reagent can, *e.g.*, comprise a detectable signal, be covalently linked to an enzyme, or can itself be detected by a secondary antibody which is linked to an enzyme through bio-conjugation. The presence of a signal indicates that both the first antibody reagent immobilized on the support and the second “detection” antibody reagent have bound to an antigen, *i.e.*, the presence of a signal indicated the presence of a SARS-COV-2 molecule. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of SARS-COV-2 polypeptides in the sample. Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates with

much higher sensitivity. There are other different forms of ELISA, which are well known to those skilled in the art.

[00194] In one embodiment, the assays, systems, and methods described herein can comprise a lateral flow immunoassay test (LFIA), also known as the immunochromatographic assay, or strip test to measure or determine the level of SARS-COV-2 polypeptide in a sample. LFIAs are a simple device intended to detect the presence (or absence) of SARS-COV-2 in a sample. There are currently many LFIA tests used for medical diagnostics either for home testing, point of care testing, or laboratory use. LFIA tests are a form of immunoassay in which the test sample flows along a solid substrate via capillary action. After the sample is applied to the test strip it encounters a colored antibody reagent which mixes with the sample, and if bound to a portion of the sample, transits the substrate encountering lines or zones which have been pretreated with a second antibody reagent. Depending upon the level of SARS-COV-2 present in the sample the colored antibody reagent can become bound at the test line or zone. LFIAs are essentially immunoassays adapted to operate along a single axis to suit the test strip format or a dipstick format. Strip tests are extremely versatile and can be easily modified by one skilled in the art for detecting an enormous range of antigens from fluid samples such as urine, blood, water samples etc. Strip tests are also known as dip stick test, the name bearing from the literal action of “dipping” the test strip into a fluid sample to be tested. LFIA strip test are easy to use, require minimum training and can easily be included as components of point-of-care test (POCT) diagnostics to be used on site in the field. LFIA tests can be operated as either competitive or sandwich assays. Sandwich LFIAs are similar to sandwich ELISA. The sample first encounters colored particles which are labeled with antibody reagents specific for a target (*e.g.*, a SARS-COV-2-specific antibody reagent). The test line will also contain antibody reagents (*e.g.*, a SARS-COV-2-specific antibody reagent). The test line will show as a colored band in positive samples. In some embodiments, the lateral flow immunoassay can be a double antibody sandwich assay, a competitive assay, a quantitative assay or variations thereof. There are a number of variations on lateral flow technology. It is also possible to apply multiple capture zones to create a multiplex test.

[00195] A typical test strip consists of the following components: (1) sample application area comprising an absorbent pad (*i. e.* the matrix or material) onto which the test sample is applied; (2) conjugate or reagent pad- this contains antibody reagent(s) specific to the target which can be conjugated to colored particles (usually colloidal gold particles, or latex microspheres); (3) test results area comprising a reaction membrane - typically a hydrophobic nitrocellulose or cellulose acetate membrane onto which antibody reagents are immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing antibodies specific for the antibody reagents conjugated to the particles or microspheres); and (4) optional wick or waste reservoir - a further absorbent pad designed to draw the sample across the reaction membrane by capillary action and collect it. The components of the strip are usually fixed to an inert backing material and may be presented in a simple dipstick format or within a plastic casing with a sample port and reaction window showing the

capture and control zones. While not strictly necessary, most tests will incorporate a second line which contains an antibody that picks up free latex/gold in order to confirm the test has operated correctly.

[00196] The use of “dip sticks” or LFIA test strips and other solid supports has been described in the art in the context of an immunoassay for a number of antigen biomarkers. U.S. Pat. Nos. 4,943,522; 6,485,982; 6,187,598; 5,770,460; 5,622,871; 6,565,808, U. S. patent applications Ser. No. 10/278,676; U.S. Ser. No. 09/579,673 and U.S. Ser. No. 10/717,082, which are incorporated herein by reference in their entirety, are non-limiting examples of such lateral flow test devices. Three U.S. patents (U.S. Pat. No. 4,444,880, issued to H. Tom; U.S. Pat. No. 4,305,924, issued to R. N. Piasio; and U.S. Pat. No. 4,135,884, issued to J. T. Shen) describe the use of “dip stick” technology to detect soluble antigens via immunochemical assays. The apparatuses and methods of these three patents broadly describe a first component fixed to a solid surface on a “dip stick” which is exposed to a solution containing a soluble antigen that binds to the component fixed upon the “dip stick,” prior to detection of the component-antigen complex upon the stick. It is within the skill of one in the art to modify the teaching of these “dip stick” technologies as necessary for the detection of SARS-COV-2 polypeptides. In some embodiments, the dip stick (or LFIA) can be suitable for use with urine samples. In some embodiments, a dip stick can be suitable for use with blood samples.

[00197] Immunochemistry is a family of techniques based on the use of a specific antibody, wherein antibodies are used to specifically target molecules inside or on the surface of cells. In some embodiments, immunohistochemistry (“IHC”) and immunocytochemistry (“ICC”) techniques can be used to detect or measure the levels of SARS-COV-2 polypeptide. IHC is the application of immunochemistry to tissue sections, whereas ICC is the application of immunochemistry to cells or tissue imprints after they have undergone specific cytological preparations such as, for example, liquid-based preparations. In some instances, signal amplification may be integrated into the particular protocol, wherein a secondary antibody, that includes a label, follows the application of an antibody reagent specific for platelets or leukocytes. Typically, for immunohistochemistry, tissue obtained from a subject and fixed by a suitable fixing agent such as alcohol, acetone, and paraformaldehyde, is sectioned and reacted with an antibody. Conventional methods for immunohistochemistry are described in Buchwalow and Bocker (Eds) “Immunohistochemistry: Basics and Methods” Springer (2010); Lin and Prichard “Handbook of Practical Immunohistochemistry” Springer (2011); which are incorporated by reference herein in their entireties. In some embodiments, immunocytochemistry may be utilized where, in general, tissue or cells obtained from a subject are fixed by a suitable fixing agent such as alcohol, acetone, and paraformaldehyde, to which is reacted an antibody. Methods of immunocytological staining of human samples is known to those of skill in the art and described, for example, in Burry “Immunocytochemistry: A Practical Guide for Biomedical Research” Springer (2009); which is incorporated by reference herein in its entirety.

[00198] In some embodiments, one or more of the antibody reagents described herein can comprise a detectable label and/or comprise the ability to generate a detectable signal (*e.g.*, by catalyzing a reaction

converting a compound to a detectable product). Detectable labels can comprise, for example, a light-absorbing dye, a fluorescent dye, or a radioactive label. Detectable labels, methods of detecting them, and methods of incorporating them into an antibody reagent are well known in the art.

[00199] In some embodiments, detectable labels can include labels that can be detected by spectroscopic, photochemical, biochemical, immunochemical, electromagnetic, radiochemical, or chemical means, such as fluorescence, chemifluorescence, or chemiluminescence, or any other appropriate means. The detectable labels used in the methods described herein can be primary labels (where the label comprises a moiety that is directly detectable or that produces a directly detectable moiety) or secondary labels (where the detectable label binds to another moiety to produce a detectable signal, *e.g.*, as is common in immunological labeling using secondary and tertiary antibodies). The detectable label can be linked by covalent or non-covalent means to the antibody reagent. Alternatively, a detectable label can be linked such as by directly labeling a molecule that achieves binding to the antibody reagent via a ligand-receptor binding pair arrangement or other such specific recognition molecules. Detectable labels can include, but are not limited to radioisotopes, bioluminescent compounds, chromophores, antibodies, chemiluminescent compounds, fluorescent compounds, metal chelates, and enzymes.

[00200] In other embodiments, the detection antibody is labeled with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can then be detected due to fluorescence. In some embodiments, a detectable label can be a fluorescent dye molecule, or fluorophore including, but not limited to fluorescein, phycoerythrin, phycocyanin, o-phthaldehyde, fluorescamine, Cy3TM, Cy5TM, allophycocyanine, Texas Red, peridenin chlorophyll, cyanine, tandem conjugates such as phycoerythrin-Cy5TM, green fluorescent protein, rhodamine, fluorescein isothiocyanate (FITC) and Oregon GreenTM, rhodamine and derivatives (*e.g.*, Texas red and tetra-rhodamine isothiocyanate (TRITC)), biotin, phycoerythrin, AMCA, CyDyesTM, 6-carboxyfluorescein (commonly known by the abbreviations FAM and F), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE or J), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA or T), 6-carboxy-X-rhodamine (ROX or R), 5-carboxyrhodamine-6G (R6G5 or G5), 6-carboxyrhodamine-6G (R6G6 or G6), and rhodamine 110; cyanine dyes, *e.g.*, Cy3, Cy5 and Cy7 dyes; coumarins, *e.g.* umbelliferone; benzimide dyes, *e.g.*, Hoechst 33258; phenanthridine dyes, *e.g.*, Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, *e.g.*, cyanine dyes such as Cy3, Cy5, etc; BODIPY dyes and quinoline dyes.

[00201] In some embodiments, a detectable label can be a radiolabel including, but not limited to ³H, ¹²⁵I, ³⁵S, ¹⁴C, ³²P, and ³³P.

[00202] In some embodiments, a detectable label can be an enzyme including, but not limited to horseradish peroxidase and alkaline phosphatase. An enzymatic label can produce, for example, a chemiluminescent signal, a color signal, or a fluorescent signal. Enzymes contemplated for use to

detectably label an antibody reagent include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

[00203] In some embodiments, a detectable label is a chemiluminescent label, including, but not limited to lucigenin, luminol, luciferin, isoluminol, thermotropic acridinium ester, imidazole, acridinium salt and oxalate ester. In some embodiments, a detectable label can be a spectral colorimetric label including, but not limited to colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, and latex) beads.

[00204] In some embodiments, antibodies can also be labeled with a detectable tag, such as c-Myc, HA, VSV-G, HSV, FLAG, V5, HIS, or biotin. Other detection systems can also be used, for example, a biotin-streptavidin system. In this system, the antibodies immunoreactive (*i.e.* specific for) with the biomarker of interest is biotinylated. Quantity of biotinylated antibody bound to the biomarker is determined using a streptavidin-peroxidase conjugate and a chromagenic substrate. Such streptavidin peroxidase detection kits are commercially available, *e.g.* from DAKO; Carpinteria, CA.

[00205] An antibody reagent can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody reagent using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[00206] The term “sample” or “test sample” as used herein denotes a sample taken or isolated from an organism, *e.g.*, a urine sample from a subject. Exemplary biological samples include, but are not limited to, a biofluid sample; serum; plasma; urine; saliva; and/or joint fluid sample, etc. The term also includes a mixture of the above-mentioned samples. The term “test sample” also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, a test sample can comprise cells from a subject. As used herein, the term “biofluid” refers to any fluid obtained from a biological source and includes, but is not limited to, blood, urine, and bodily secretions.

[00207] The test sample can be obtained by removing a sample from a subject, but can also be accomplished by using a previously isolated sample (*e.g.*, isolated at a prior timepoint and isolated by the same or another person). In addition, the test sample can be freshly collected or a previously collected sample.

[00208] In some embodiments, the test sample can be an untreated test sample. As used herein, the phrase “untreated test sample” refers to a test sample that has not had any prior sample pre-treatment except for dilution and/or suspension in a solution. Exemplary methods for treating a test sample include, but are not limited to, centrifugation, filtration, sonication, homogenization, heating, freezing and thawing, and combinations thereof. In some embodiments, the test sample can be a frozen test sample, *e.g.*, a frozen tissue. The frozen sample can be thawed before employing methods, assays and

systems described herein. After thawing, a frozen sample can be centrifuged before being subjected to methods, assays and systems described herein. In some embodiments, the test sample is a clarified test sample, for example, prepared by centrifugation and collection of a supernatant comprising the clarified test sample. In some embodiments, a test sample can be a pre-processed test sample, for example, supernatant or filtrate resulting from a treatment selected from the group consisting of centrifugation, filtration, thawing, purification, and any combinations thereof. In some embodiments, the test sample can be treated with a chemical and/or biological reagent. Chemical and/or biological reagents can be employed to protect and/or maintain the stability of the sample, including biomolecules (*e.g.*, nucleic acid and protein) therein, during processing. One exemplary reagent is a protease inhibitor, which is generally used to protect or maintain the stability of protein during processing. The skilled artisan is well aware of methods and processes appropriate for pre-processing of biological samples required for determination of the level of SARS-COV-2 as described herein.

[00209] In some embodiments, the methods, assays, and systems described herein can further comprise a step of obtaining a test sample from a subject. In some embodiments, the subject can be a human subject.

[00210] In some embodiments, the assay or method can further comprise the step of administering an anti- SARS-COV-2 therapy. In some embodiments, the anti- SARS-COV-2 therapy comprises an isolated antibody, antibody reagent, or antigen-binding portion thereof;; nucleic acid; cell; or composition as described herein.

[00211] In one aspect of any of any of the embodiments, described herein is an antibody, antibody reagent, or antigen-binding portion thereof as described herein conjugated to or coupled to a detectable label.

[00212] In one aspect of any of any of the embodiments, described herein is a solid support comprising an antibody, antibody reagent, antigen-binding fragment thereof as described herein. In some embodiments of any of the aspects, the antibody, antibody reagent or antigen-binding fragment thereof is detectably labeled. In some embodiments of any of the aspects, the solid support comprises a particle, a bead, a polymer, or a substrate.

[00213] In one aspect of any of the embodiments, described herein is a molecular complex comprising at least one antibody, antibody reagent, or antigen-binding fragment thereof, of as described herein bound to an SARS-COV-2 polypeptide.

[00214] In one aspect, described herein is a kit comprising a composition as described herein, *e.g.*, a composition comprising an antibody, antibody reagent, or antigen-binding portion thereof, as described herein. A kit is any manufacture (*e.g.*, a package or container) comprising at least one reagent, *e.g.*, an antibody, the manufacture being promoted, distributed, or sold as a unit for performing the methods described herein. In some embodiments of any of the aspects, the antibody, antibody reagent, antigen-binding fragment thereof as described herein is immobilized on a solid support. In some embodiments of any of the aspects, the solid support comprises a particle, a bead, a polymer, or a substrate. In some

embodiments of any of the aspects, the antibody, antibody reagent or antigen-binding fragment thereof is detectably labeled.

[00215] The kits described herein can optionally comprise additional components useful for performing the methods described herein. By way of example, the kit can comprise fluids (*e.g.*, buffers) suitable for composition comprising an antibody, or antigen-binding portion thereof, as described herein, an instructional material which describes performance of a method as described herein, and the like. A kit can further comprise devices and/or reagents for delivery of the composition as described herein. Additionally, the kit may comprise an instruction leaflet and/or may provide information as to the relevance of the obtained results.

[00216] For convenience, the meaning of some terms and phrases used in the specification, examples, and appended claims, are provided below. Unless stated otherwise, or implicit from context, the following terms and phrases include the meanings provided below. The definitions are provided to aid in describing particular embodiments, and are not intended to limit the claimed invention, because the scope of the invention is limited only by the claims. 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 belongs. If there is an apparent discrepancy between the usage of a term in the art and its definition provided herein, the definition provided within the specification shall prevail.

[00217] The terms “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease by a statistically significant amount. In some embodiments, “reduce,” “reduction” or “decrease” or “inhibit” typically means a decrease by at least 10% as compared to a reference level (*e.g.* the absence of a given treatment or agent) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99% , or more. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as compared to a reference level. A decrease can be preferably down to a level accepted as within the range of normal for an individual without a given disorder.

[00218] The terms “increased”, “increase”, “enhance”, or “activate” are all used herein to mean an increase by a statically significant amount. In some embodiments, the terms “increased”, “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, or any increase between 2-fold and 10-fold or greater as compared to

a reference level. In the context of a marker or symptom, a “increase” is a statistically significant increase in such level.

[00219] As used herein, the terms “protein” and “polypeptide” are used interchangeably herein to designate a series of amino acid residues, connected to each other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues. The terms “protein”, and “polypeptide” refer to a polymer of amino acids, including modified amino acids (e.g., phosphorylated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. “Protein” and “polypeptide” are often used in reference to relatively large polypeptides, whereas the term “peptide” is often used in reference to small polypeptides, but usage of these terms in the art overlaps. The terms “protein” and “polypeptide” are used interchangeably herein when referring to a gene product and fragments thereof. Thus, exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, orthologs, paralogs, fragments and other equivalents, variants, fragments, and analogs of the foregoing.

[00220] In the various embodiments described herein, it is further contemplated that variants (naturally occurring or otherwise), alleles, homologs, conservatively modified variants, and/or conservative substitution variants of any of the particular polypeptides described are encompassed. As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid and retains the desired activity of the polypeptide. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles consistent with the disclosure.

[00221] In some embodiments, the polypeptide described herein (or a nucleic acid encoding such a polypeptide) can be a functional fragment of one of the amino acid sequences described herein. As used herein, a “functional fragment” is a fragment or segment of a peptide which retains at least 50% of the wildtype reference polypeptide’s activity according to the assays described below herein. A functional fragment can comprise conservative substitutions of the sequences disclosed herein.

[00222] In some embodiments, the polypeptide described herein can be a variant of a sequence described herein. In some embodiments, the variant is a conservatively modified variant. Conservative substitution variants can be obtained by mutations of native nucleotide sequences, for example. A “variant,” as referred to herein, is a polypeptide substantially homologous to a native or reference polypeptide, but which has an amino acid sequence different from that of the native or reference polypeptide because of one or a plurality of deletions, insertions or substitutions. Variant polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that encode a variant protein or fragment thereof that retains activity. A wide variety of PCR-based site-specific mutagenesis approaches are known in the art and can be applied by the ordinarily skilled artisan.

[00223] As used herein, the term “nucleic acid” or “nucleic acid sequence” refers to any molecule, preferably a polymeric molecule, incorporating units of ribonucleic acid, deoxyribonucleic acid or an analog thereof. The nucleic acid can be either single-stranded or double-stranded. A single-stranded nucleic acid can be one nucleic acid strand of a denatured double-stranded DNA. Alternatively, it can be a single-stranded nucleic acid not derived from any double-stranded DNA. In one aspect, the nucleic acid can be DNA. In another aspect, the nucleic acid can be RNA. Suitable DNA can include, e.g., genomic DNA or cDNA. Suitable RNA can include, e.g., mRNA.

[00224] In some embodiments of any of the aspects, a polypeptide, nucleic acid, or cell as described herein can be engineered. As used herein, “engineered” refers to the aspect of having been manipulated by the hand of man. For example, a polypeptide is considered to be “engineered” when at least one aspect of the polypeptide, e.g., its sequence, has been manipulated by the hand of man to differ from the aspect as it exists in nature. As is common practice and is understood by those in the art, progeny of an engineered cell is typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[00225] In some embodiments, a nucleic acid encoding a polypeptide as described herein (e.g. an antibody or antibody reagent) is comprised by a vector. In some of the aspects described herein, a nucleic acid sequence encoding a given polypeptide as described herein, or any module thereof, is operably linked to a vector. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, chromosome, virus, virion, etc.

[00226] As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term “expression” refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. “Expression products” include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term “gene” means the nucleic acid sequence which is transcribed (DNA) to RNA in vitro or in vivo when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, e.g. 5' untranslated (5'UTR) or “leader” sequences and 3' UTR or “trailer” sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[00227] The term “isolated” or “partially purified” as used herein refers, in the case of a nucleic acid or polypeptide, to a nucleic acid or polypeptide separated from at least one other component (e.g., nucleic acid or polypeptide) that is present with the nucleic acid or polypeptide as found in its natural source and/or that would be present with the nucleic acid or polypeptide when expressed by a cell, or secreted

in the case of secreted polypeptides. A chemically synthesized nucleic acid or polypeptide or one synthesized using *in vitro* transcription/translation is considered “isolated.” The terms “purified” or “substantially purified” refer to an isolated nucleic acid or polypeptide that is at least 95% by weight the subject nucleic acid or polypeptide, including, for example, at least 96%, at least 97%, at least 98%, at least 99% or more. In some embodiments, the antibody, or antigen-binding portion thereof, described herein is isolated. In some embodiments, the antibody, antibody reagent, or antigen-binding portion thereof, described herein is purified.

[00228] As used herein, “engineered” refers to the aspect of having been manipulated by the hand of man. For example, an antibody, antibody reagent, or antigen-binding portion thereof, is considered to be “engineered” when the sequence of the antibody, antibody reagent, or antigen-binding portion thereof, is manipulated by the hand of man to differ from the sequence of an antibody as it exists in nature. As is common practice and is understood by those in the art, progeny and copies of an engineered polynucleotide and/or polypeptide are typically still referred to as “engineered” even though the actual manipulation was performed on a prior entity.

[00229] As used herein, an “epitope” can be formed on a polypeptide both from contiguous amino acids, or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation. An “epitope” includes the unit of structure conventionally bound by an immunoglobulin VH/VL pair. Epitopes define the minimum binding site for an antibody, and thus represent the target of specificity of an antibody. In the case of a single domain antibody, an epitope represents the unit of structure bound by a variable domain in isolation. The terms “antigenic determinant” and “epitope” can also be used interchangeably herein. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics.

[00230] “Avidity” is the measure of the strength of binding between an antigen-binding molecule (such as an antibody or antigen-binding portion thereof described herein) and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule, and the number of pertinent binding sites present on the antigen-binding molecule. Typically, antigen-binding proteins (such as an antibody or portion of an antibody as described herein) will bind to their cognate or specific antigen with a dissociation constant (K_D of 10^{-5} to 10^{-12} moles/liter or less, such as 10^{-7} to 10^{-12} moles/liter or less, or 10^{-8} to 10^{-12} moles/liter (*i.e.*, with an association constant (K_A) of 10^5 to 10^{12} liter/moles or more, such as 10^7 to 10^{12} liter/moles or 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^{-4} mol/liter (or any K_A value lower than 10^4 M^{-1}) is generally considered to indicate non-specific binding. The K_D for biological interactions which are

considered meaningful (*e.g.*, specific) are typically in the range of 10^{-10} M (0.1 nM) to 10^{-5} M (10000 nM). The stronger an interaction, the lower is its K_D . For example, a binding site on an antibody or portion thereof described herein will bind to the desired antigen with an affinity less than 500 nM, such as less than 200 nM, or less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as other techniques as mentioned herein.

[00231] Accordingly, as used herein, “selectively binds” or “specifically binds” refers to the ability of a peptide (*e.g.*, an antibody or portion thereof) described herein to bind to a target, such as an antigen present on the cell-surface, with a K_D 10^{-5} M (10000 nM) or less, *e.g.*, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. Specific binding can be influenced by, for example, the affinity and avidity of the polypeptide agent and the concentration of polypeptide agent. The person of ordinary skill in the art can determine appropriate conditions under which the polypeptide agents described herein selectively bind the targets using any suitable methods, such as titration of a polypeptide agent in a suitable cell binding assay. A polypeptide specifically bound to a target is not displaced by a non-similar competitor. In certain embodiments, an antibody, or antigen-binding portion thereof, is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

[00232] In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of 10^{-5} M (10000 nM) or less, *e.g.*, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or less. In some embodiments, an antibody, antigen-binding portion thereof, and/or CAR as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-5} M to 10^{-6} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-6} M to 10^{-7} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-7} M to 10^{-8} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-8} M to 10^{-9} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-9} M to 10^{-10} M. In some embodiments, an antibody, antigen-binding portion thereof, and/or CAR as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-10} M to 10^{-11} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of from about 10^{-11} M to 10^{-12} M. In some embodiments, an antibody, and/or antigen-binding portion thereof, as described herein binds to SARS-COV-2 with a dissociation constant (K_D) of less than 10^{-12} M.

[00233] As used herein, the term “administering,” refers to the placement of a compound as disclosed herein into a subject by a method or route which results in at least partial delivery of the agent at a desired site. Pharmaceutical compositions comprising the compounds disclosed herein can be administered by any appropriate route which results in an effective treatment in the subject.

[00234] The term “statistically significant” or “significantly” refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[00235] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean $\pm 1\%$.

[00236] As used herein, the term “comprising” means that other elements can also be present in addition to the defined elements presented. The use of “comprising” indicates inclusion rather than limitation.

[00237] The term “consisting of” refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[00238] As used herein the term “consisting essentially of” refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[00239] The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, “e.g.” is derived from the Latin *exempli gratia*, and is used herein to indicate a non-limiting example. Thus, the abbreviation “e.g.” is synonymous with the term “for example.”

[00240] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[00241] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of

Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter *et al.* (eds.), The Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN 047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entirety.

[00242] In some embodiments of any of the aspects, the disclosure described herein does not concern a process for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

[00243] Other terms are defined herein within the description of the various aspects of the invention.

[00244] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00245] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible

within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[00246] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[00247] The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting.

[00248] Some embodiments of the technology described herein can be defined according to any of the following numbered paragraphs:

1. The technology described herein provides an antibody, antibody reagent, or antigen-binding fragment thereof, that specifically binds a SARS-COV-2 polypeptide, said antibody reagent, or antigen-binding fragment thereof, comprising at least one heavy or light chain complementarity determining region (CDR) selected from Table 1.
2. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment thereof, comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
3. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
4. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment comprises heavy chain CDRs having the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
5. In one embodiment of any aspect, a first antibody, antibody reagent, or antigen-binding fragment that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with a second antibody comprising a heavy chain CDRs having

the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.

6. In one embodiment of any aspect, the first antibody, antibody reagent, or antigen-binding fragment comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
7. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
8. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
9. In one embodiment of any aspect, the first antibody reagent, or antigen-binding fragment comprises heavy chain CDRs having the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
10. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment, comprises the heavy chain variable region sequence selected from Table 1.
11. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment, comprises the light chain variable region sequence selected from Table 1.
12. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment, comprises heavy chain variable region sequence selected from Table 1 and the light chain variable region sequence selected from Table 1.
13. In one embodiment of any aspect, the antibody, antibody reagent, antigen-binding fragment from Table 1 thereof, further comprises a conservative substitution in a sequence not comprised by a CDR.
14. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment from Table 1 is fully human or fully humanized.
15. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment from Table 1 is fully humanized except for the CDR sequences.
16. In one embodiment of any aspect, the antibody, antibody reagent, or antigen-binding fragment from Table 1 is selected from the group consisting of an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody

17. In one embodiment of any aspect, a nucleic acid sequence encodes the antibody, antibody reagent, or antigen-binding fragment from Table 1.
18. In one embodiment of any aspect, a cell comprises the antibody, or antigen-binding fragment from Table 1 or the related nucleic acid sequence.
19. In one embodiment of any aspect, a pharmaceutical composition comprising the antibody, antibody reagent, or antigen-binding fragment from Table 1 and the accompanying cell, and a pharmaceutically acceptable carrier.
20. In one embodiment of any aspect, a solid support comprises the antibody, antibody reagent, antigen-binding fragment thereof, or CAR from Table 1.
21. In one embodiment of any aspect, the solid support is detectably labeled.
22. In one embodiment of any aspect, the solid support comprises a particle, a bead, a polymer, or a substrate.
23. In one embodiment of any aspect, a kit for the detection of a SARS-COV-2 polypeptide in a sample, comprises at least a first antibody, antibody reagent, antigen-binding fragment thereof, or CAR from Table 1 immobilized on a solid support and comprises a detectable label.
24. In one embodiment of any aspect, a molecular complex comprises at least one antibody, antibody reagent, or antigen-binding fragment bound to a SARS-COV-2 polypeptide.
25. In one embodiment of any aspect, a method of treating a coronavirus infection in a subject in need thereof, the method comprises administering an antibody from Table 1 and a cell.

[00249] In some embodiments, the present technology may be defined in any of the following numbered paragraphs:

1. An antibody, antibody reagent, antigen-binding fragment thereof, or chimeric antigen receptor (CAR), that specifically binds a SARS-COV-2 polypeptide, said antibody reagent, or antigen-binding fragment thereof comprising at least one heavy or light chain complementarity determining region (CDR) selected from Table 1.
2. The antibody, antibody reagent, or antigen-binding fragment thereof, of paragraph 1, which comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
3. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-2, which comprises light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
4. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-3, which comprises:

heavy chain CDRs having the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.

5. A first antibody, antibody reagent, or antigen-binding fragment thereof, that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with a second antibody comprising:

heavy chain CDRs having the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.

6. The first antibody, antibody reagent, or antigen-binding fragment thereof, of paragraph 5, comprising at least one heavy or light chain complementarity determining region (CDR) selected from Table 1.
7. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 5-6, which comprises heavy chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
8. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 5-7, which comprises light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.
9. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 5-8, which comprises:

heavy chain CDRs having the amino acid sequences selected from Table 1 and light chain CDRs having the amino acid sequences selected from Table 1 or a conservative substitution variant of such amino acid sequence.

10. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-9, comprising the heavy chain variable region sequence selected from Table 1.
11. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-10, comprising the light chain variable region sequence selected from Table 1.
12. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-11, comprising:

the heavy chain variable region sequence selected from Table 1 and the light chain variable region sequence selected from Table 1.

13. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-12, further comprising a conservative substitution in a sequence not comprised by a CDR.
14. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-13, wherein the antibody reagent or antigen-binding fragment thereof is fully human or fully humanized.

15. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-14, wherein the antibody reagent or antigen-binding fragment thereof is fully humanized except for the CDR sequences.
16. The antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-15, wherein the antibody reagent or antigen-binding fragment is selected from the group consisting of:
 - an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody.
17. A nucleic acid sequence encoding the antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16.
18. A cell comprising the antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16 or the nucleic acid sequence of paragraph 17.
19. A pharmaceutical composition comprising the antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16; or the cell of paragraph 18, and a pharmaceutically acceptable carrier.
20. A solid support comprising the antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16.
21. The solid support of paragraph 20, wherein the antibody, antibody reagent or antigen-binding fragment thereof is detectably labeled.
22. The solid support of any one of paragraphs 20-21, wherein the solid support comprises a particle, a bead, a polymer, or a substrate.
23. A kit for the detection of a SARS-COV-2 polypeptide in a sample, the kit comprising at least a first antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16 immobilized on a solid support and comprising a detectable label.
24. A molecular complex comprising at least one antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16 bound to a SARS-COV-2 polypeptide.
25. A method of treating a coronavirus infection in a subject in need thereof, the method comprising administering
 - (i) the antibody, antibody reagent, or antigen-binding fragment thereof, of any one of paragraphs 1-16
 - (ii) the cell of paragraph 18, to the subject.

[00250] In some embodiments, the present technology may be defined in any of the following numbered paragraphs:

1. An antibody, antibody reagent, chimeric antigen receptor (CAR), or antigen-binding fragment thereof, that specifically binds a SARS-COV-2 polypeptide, said antibody, antibody reagent,

CAR, or antigen-binding fragment thereof comprising at least one heavy or light chain complementarity determining region (CDR) selected from:

- a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
- b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
- c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
- d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
- e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
- f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
- g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
- h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- m) the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n) the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- o) the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- p) the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;

- q) the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- r) the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;
- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t) the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u) the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;

- ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
 - jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
 - kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
 - ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
 - mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
 - nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
 - oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
- a conservative substitution variant thereof.
2. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof, of paragraph 1, which comprises three heavy chain CDRs or three light chain CDRs having amino acid sequences selected from:
- a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
 - b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
 - c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
 - d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
 - e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
 - f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
 - g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
 - h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;

- i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- m) the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n) the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- o) the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- p) the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- q) the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- r) the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;
- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t) the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u) the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;

- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
 - bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
 - cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
 - dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
 - ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
 - ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
 - gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
 - hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
 - ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
 - jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
 - kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
 - ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
 - mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
 - nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
 - oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
a conservative substitution variant thereof.
3. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of paragraph 1, which comprises three heavy chain CDRs and three light chain CDRs having amino acid sequences selected from:
- a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;

- b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
- c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
- d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
- e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
- f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
- g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
- h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- m) the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n) the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- o) the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- p) the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- q) the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- r) the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;

- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t) the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u) the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
- ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
- jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;

- kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
 - ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
 - mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
 - nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
 - oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
 - a conservative substitution variant thereof.
4. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of paragraph 1, which comprises VH and/or VL amino acid sequences selected from:
- a) SEQ ID NOs: 1 and 2;
 - b) the VH sequences of one of SEQ ID NOs: 1, 322, 324, 326, 328, and 330-348 and the VL sequences of SEQ ID NO: 2 and 349;
 - c) SEQ ID NOs: 9 and 10;
 - d) SEQ ID NOs: 17 and 18;
 - e) SEQ ID NOs: 25 and 26;
 - f) SEQ ID NOs: 33 and 34;
 - g) SEQ ID NOs: 41 and 42;
 - h) SEQ ID NOs: 49 and 50;
 - i) SEQ ID NOs: 57 and 58;
 - j) SEQ ID NOs: 65 and 66;
 - k) SEQ ID NOs: 73 and 74;
 - l) SEQ ID NOs: 81 and 82;
 - m) SEQ ID NOs: 89 and 90;
 - n) SEQ ID NOs: 97 and 98;
 - o) SEQ ID NOs: 105 and 106;
 - p) SEQ ID NOs: 113 and 114;
 - q) SEQ ID NOs: 121 and 122;
 - r) SEQ ID NOs: 129 and 130;
 - s) SEQ ID NOs: 137 and 138;
 - t) SEQ ID NOs: 145 and 146;
 - u) SEQ ID NOs: 153 and 154;
 - v) SEQ ID NOs: 161 and 162;
 - w) SEQ ID NOs: 169 and 170;

- x) SEQ ID NOs: 177 and 178;
 - y) SEQ ID NOs: 185 and 186;
 - z) SEQ ID NOs: 193 and 194;
 - aa) SEQ ID NOs: 201 and 2025;
 - bb) SEQ ID NOs: 209 and 210;
 - cc) SEQ ID NOs: 217 and 218;
 - dd) SEQ ID NOs: 225 and 226;
 - ee) SEQ ID NOs: 233 and 234;
 - ff) SEQ ID NOs: 241 and 242;
 - gg) SEQ ID NOs: 249 and 250;
 - hh) SEQ ID NOs: 257 and 258;
 - ii) SEQ ID NOs: 265 and 266;
 - jj) SEQ ID NOs: 273 and 274;
 - kk) SEQ ID NOs: 281 and 282;
 - ll) SEQ ID NOs: 289 and 290;
 - mm) SEQ ID NOs: 297 and 298;
 - nn) SEQ ID NOs: 305 and 306;
 - oo) SEQ ID NOs: 313 and 314; or
a conservative substitution variant thereof.
5. A first antibody, antibody reagent, CAR, or antigen-binding fragment thereof that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with a second antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-4.
 6. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-5, further comprising a conservative substitution in a sequence not comprised by a CDR.
 7. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-6, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is fully human or fully humanized.
 8. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-7 wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is fully humanized except for the CDR sequences.
 9. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-8 wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof comprises one or more of:
 - a) a heavy chain CDR3 in which the sequence "YGRS" of the heavy chain CDR3 is mutated to comprise "YGSS" or "YGGS";

- b) a VH in which the sequence "FDVW" of the VH is mutated to comprise "FDLW";
 - c) a VH in which the sequence "WGAG" of the VH is mutated to comprise "WGRG";
 - d) a VH in which the sequence "GTTV" of the VH is mutated to comprise "GTLV"; and
 - e) a VL in which the sequence "FGGG" of the VL is mutated to comprise "FGQG."
10. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-9, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is selected from the group consisting of:
 - an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody.
 11. A nucleic acid sequence encoding the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10.
 12. A cell comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10 or the nucleic acid sequence of paragraph 11.
 13. A pharmaceutical composition comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10; or the cell of paragraph 12, and a pharmaceutically acceptable carrier.
 14. The pharmaceutical composition of paragraph 13, comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof and at least one of casirivimab, imdevimab, or sotrovimab.
 15. A solid support comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10.
 16. The solid support of paragraph 15, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is detectably labeled.
 17. The solid support of any one of paragraphs 15-16, wherein the solid support comprises a particle, a bead, a polymer, or a substrate.
 18. A kit for the detection of a SARS-COV-2 polypeptide in a sample, the kit comprising at least a first antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10 immobilized on a solid support and comprising a detectable label.
 19. A molecular complex comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10 bound to a SARS-COV-2 polypeptide.
 20. A method of treating a coronavirus infection in a subject in need thereof, the method comprising administering
 - (i) the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10;
 - (ii) the composition of paragraph 13 or 14; and/or

(iii) the cell of paragraph 12, to the subject.

21. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10; the composition of paragraph 13 or 14; and/or the cell of paragraph 12 for use in a method of treating a coronavirus infection in a subject in need thereof, the method comprising administering antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of paragraphs 1-10; the composition of paragraph 13 or 14; and/or the cell of paragraph 12 to the subject.
22. The method or antibody, antibody reagent, CAR, or antigen-binding fragment thereof of paragraph 20 or 21, wherein the coronavirus infection is a SARS-CoV-2 infection.
23. The method or antibody, antibody reagent, CAR, or antigen-binding fragment thereof of paragraph 20 or 21, wherein the coronavirus infection is a B.1.1.7, Q lineage, B.1.351, P.1, B.1.617.2, AY lineage, B.1.427, B.1.429, B.1.525, B.1.526, B.1.617.1, 1.617.3, B.1.621, B.1.621.1, P.2, B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.4/5, BA.2.12.1, or BA.5 SARS-CoV-2 infection.

[00251] The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting.

EXAMPLES

Example 1: Novel Mouse Models for HIV-vaccine and SARS-CoV-2-neutralizing Antibody Discovery.

[00252] HIV-1 vaccine strategies seek to elicit HIV-1 broadly neutralizing antibodies (bnAbs), such as the potent VRC01-class, in humans. Antibody heavy chain (HC) and light chain (LC) variable region exons are assembled by V(D)J recombination. V(D)J junctional regions encode complementarity-determining-region 3 (CDR3), an antigen-contact region immensely diversified in human HC and LC repertoires by terminal deoxynucleotidyl transferase (TdT). Precursor human VRC01-lineage B cells uniformly express receptors (BCRs) with a HC V_H1-2 and a LC V associated with an exceptionally short CDR3, which severely reduces precursor frequency. Prior VRC01-based HIV-1 vaccine mouse models had non-physiological precursor levels and/or limited precursor diversity. Here, we describe "complete" VRC01-lineage mice in which precursors physiologically rearrange human V_H1-2 and VRC01-lineage LC Vs with diverse CDR3s, including, human-like frequencies of short LC CDR3s mediated by enforced, human-like TdT expression. Priming immunization of these models with eOD-GT8 to engage VRC01 precursors induced strong and, for some, TdT-enhanced VRC01-class germinal center B cell responses. Thus, complete VRC01 models will facilitate testing diverse prime and boost immunization strategies designed to elicit VRC01-class bnAbs. With this model, we also tested the postulate that mice with BCR repertoires from single human HC and LC Vs associated with immensely diverse CDR3s could generate potential therapeutic antibodies. Indeed, SARS-CoV-2 Spike protein

immunization of a complete VRC01 model elicited novel humanized spike protein-targeting antibodies, including lineages with potent neutralizing activity against known SARS-CoV-2 variants.

[00253] Diverse antibody variable region exons are assembled in developing B cells from V, D, and J gene segments for HCs and from Vs and Js for Igk or Iyλ LCs. In humans, there are 55 germline HC Vs (V_{HS}) and 70 Igk and Igλ LC Vs. Vs encode most of the HC and LC variable region, including CDR1 and CDR2 sequences that vary among different HC and LC Vs. BCR diversity is achieved partly assorting HC and LC Vs. However, it is immensely augmented by TdT-based non-templated nucleotide additions (N regions) to V(D)J junctions, which generate CDR3 length and sequence diversity suppress recurrent CDR3s resulting from microhomology (MH)-mediated joining. VRC01-class bnAb HCs employ human V_H1-2, which encodes residues that contact the HIV-1 envelope protein (Env) CD4 binding site. VRC01-class LC variable regions can be encoded by a handful of Vs; but all are associated with an exceptionally short 5 amino acids (5aa) CDR3, which avoids steric clash with Env and contributes to Env interaction. As both requirements can be achieved by V(D)J recombination, they are predicted attributes of primary VRC01-lineage precursor BCRs. However, deduced primary VRC01 BCRs lack detectable affinity for Env. In this regard, following BCR antigen-activation, primary B cells are driven into GC reactions where they undergo rounds of variable region exon somatic hyper-mutation (SHM) followed by selection of SHMs that increase BCR antigen-binding affinity. This process ultimately leads to high-affinity antibody production. Correspondingly, a third VRC01-class bnAb attribute is abundant variable region SHMs with a subset contributing to Env-binding and potent VRC01 bnAb activity, suggesting VRC01-class bnAbs evolve with HIV-1 over long infection times and many SHM/selection cycles.

[00254] To mature VRC01 precursors into bnAbs, sequential VRC01 vaccine immunization approaches propose a priming immunogen to drive precursors into GCs followed by boost immunogens designed to lead them through rounds of SHM/affinity maturation. Based on e0D-GT8 priming immunogen-binding, potential human VRC01-like precursor B cell frequency was estimated at 1 in 300,000 or fewer. As sequential vaccine testing in humans is not practical, mouse models were developed for this purpose. Early models expressed knock-in V_H1-2 HCs and, in some, VRC01-class LC Vs, both with deduced CDR3s. These models were non-physiological as their BCR repertoire is dominated by a single human HC/LC combination or a single human HC with diverse mouse LCs. Mice with fully human HC and LC gene segment loci assembled by V(D)J recombination were also tested; but precursor frequencies were far too low, likely due to inability to express immense human-like CDR3 repertoires in mice with orders of magnitude fewer B cells. A V_H1-2-rearranging mouse model generated diverse V_H1-2 HC CDR3, but employed a deduced VRC01 precursor LC with a 5 AA CDR3. While useful for HC maturation studies during sequential immunization, this model was limited by over-abundance of VRC01 lineage LC precursors. Recently, eOD-GT8-binding precursor B cells from transgenic VRC01 HC/LC knock-in mice were adoptively transferred into WT mice at human-like frequencies.

Immunization with eOD-GT8 drove such precursors into GC reactions. However, this elegant approach remained limited to OD-GT8 priming and testing only a tiny subset of potential VRC01 precursors. To address issues of prior models, we developed complete VRC01 mouse models in which individual B cells express one of a multitude of different VRC01 precursors at human-like levels, based on enforced rearrangement of both V_{H1-2} and VRC01 class V_{κ} s along with enforced human TdT expression (Fig.1A).

[00255] Generation of mice with VRC01-rearranging human HC and LC Vs.

[00256] All of our complete VRC01 models employ our previously described V_{H1-2} -rearranging HC allele in which the most D proximal mouse V_{H5-2} (" V_{H81X} ") was replaced with human V_{H1-2} . We also deleted the CTCF-binding site (CBE)-based IGCR1 element in the V_H to D interval which leads to nearly exclusively proximal V_H rearrangement in an otherwise intact V_H locus by unleashing linear V(D)J recombination chromatin-scanning to most proximal V_H . As high-level V_{H5-2} utilization via scanning in the absence of IGCR1 depends on recombinational targeting by its associated CBE, we maintained this CBE in association with V_{H1-2} on the replacement allele. As predicted, V_{H1-2} rearrangements, on homozygous replacement alleles in our new models represent nearly 80% of primary V(D)J rearrangements and are associated with immense CDR diversity (Fig. 5A). Due to counter selection of lower frequency upstream V_H rearrangements, V_{H1-2} contribution to primary B cell BCRs is reduced to 40%, but still with immense CDR3 diversity (Fig. 5B-5D), which is critical as V_{H1-2} -encoded HC CDR3 diversity was implicated Env by precursor VRC01 BCRs and in the maturation VRC01-class bnAbs. In this regard, while 10% or more BCRs with V_{H1-2} HCs paired with VRC01-class germline LCs can be engaged by priming immunogens, their binding affinities to priming immunogen vary over a wide range.

[00257] Human $V_{\kappa 1-33}$ and $V_{\kappa 3-20}$ are frequently represented in VRC01-class bnAb LC variable regions. To generate human $V_{\kappa 1-33}$ -rearranging mice, we tested a strategy similar to that used for V_{H1-2} (Fig.1A). As the Cer/Sis CBE-based element in the V_{κ} to J_{κ} interval was implicated in promoting distal versus proximal V_{κ} rearrangements, we deleted Cer/Sis in ES cells and the mouse germline (Figs. 6A, 6B). While all V_{H} s are oriented for deletional rearrangement via linear scanning, V_{κ} s occur, mostly in clusters, in both deletional and inversional orientations relative to J_{κ} s. HTGTS-Rep-Seq analyses of splenic B cells revealed that homozygous Cer/Sis deletion increased rearrangement of 7 of the 11 the most J_{κ} -proximal V_{κ} s up to 8-fold; notably, all those increased were in deletional orientation, consistent with recombinational access via linear scanning from J_{κ} s (Fig. 1B and 6C). In Cer/Sis-deleted mice, these 7 V_{κ} s generated the vast majority of BCRs, as upstream deletional and inversional oriented V_{κ} rearrangements were abrogated. We generated mice homozygous for Ig κ alleles on which the penultimate mouse J_{κ} -proximal $V_{\kappa 3-2}$ was replaced with human $V_{\kappa 1-33}$ (VRC01 ^{$V_{\kappa 1-33}$} mice) and also VRC01 ^{$V_{\kappa 1-33}$} mice homozygous for the Cer/Sis deletion (VRC01 ^{$V_{\kappa 1-33}$ -CS} mice). (Figs. 1A, 7A, 7B). HTGTS-rep-seq revealed that, similar to $V_{\kappa 3-2}$, human $V_{\kappa 1-33}$ accounted 2% of primary V_{κ}

rearrangements in VRC01^{Vκ1-33} mice and 18% when in VRC01^{Vκ1-33-CS} mice (Fig. 7C). Vκ1-33 also contributed at a similar frequency, 2.2% and 14.5% of splenic BCR repertoires in these mice, respectively (Fig. 1C). We also generated mice homozygous for Igκ alleles in which mouse proximal Vκ3-7 was replaced with human Vκ3-20 (VRC01^{Vκ3-20} mice), in which Vκ3-20 contributed about 6% of Vκ rearrangements and similar frequencies of primary B BCRs (Figs. 1A, 1C, 7D-7F). Unlike proximal V_{HS}, proximal V_{KS}, lack closely associated CBEs. To determine whether such CBEs influence rearrangement frequency, we added CBEs to both inserted human V_{KS} and found, compared to mouse V_{KS} they replaced, associated CBEs had, at most, a modest effect on Vκ1-33 or Vκ3-20 rearrangement. Analyzing these model by fluorescence-activated cell sorting (FACS) of several cell surface markers, splenic B and T cell populations were comparable to wild-type 129/Sv mice (Fig. 7G).

[00258] Enforced TdT Expression diversifies LC repertoires

[00259] VRC01-class bnAb LCs commonly have a 5-aa LC CDR3 with a relatively conserved sequence (QQYEF). However, our VRC01 Igκ-rearranging models had a very low frequency of 5-aa LC CDR3s (0.02%) in their mouse V_{KS}, and Vκ1-33 or Vκ3-20 LC BCR repertoires (Fig. 8A), compared to a 25- to 50-fold higher frequency 5-aa LC CDR3s in human BCR repertoires. In this regard, approximately 80% of human 5-aa LC CDR3s are encoded by sequences with TdT-generated N regions (Fig. 8B), which suppress recurrent MH-mediated V(D)J joins. Such MH-mediated joins are relatively abundant in primary mouse Igκ LC repertoires, as mouse precursor B cells rearranging V_{KS} do not express TdT. Thus, we tested effects of enforced human TdT expression from the mouse Rosa locus (Figs. 2A, 8C-8E) on LC CDR3 diversity. Indeed, splenic B cells of TdT-knock in mice had markedly increased frequencies of N regions in mouse Vκ to Jκ junctions (Fig. 2B), and, correspondingly, more diverse CDRs (Fig. 2C). Notably, while enforced N region addition increased the profragment of longer LC CDR3s (> 9-aa), it also increased, up to 5-fold, the profragment of short LC CDR3s (< 7-aa), including 5-aa CDR3s (Fig. 2D). Similar to human LC CDR3s, most short and long LC CDR3s in TdT-expressing mice had N regions (Fig. 8F). Endogenous TdT expression is robust in mouse B cell progenitors at the time of IgH V(D)J recombination; correspondingly, human TdT-expressing mice had a very modest effect on HC CDR3 length and diversity (Fig. 8G). We further introduced the TdT allele in the VRC01^{Vκ1-33-CS} and VRC01^{Vκ3-20} mice to generate VRC01^{Vκ1-33-CS/TdT} and VRC01^{Vκ3-20/TdT} models. Analyses of splenic B cells from these models revealed little effect of enforced TdT expression overall utilization of the Vκ1-33 and Vκ3-20 segments (Extended Data Fig. 5a-d). However, in VRC01^{Vκ1-33-CS/TdT} and VRC01^{Vκ3-20/TdT} mice, the diversity of Vκ1-33 and Vκ3-20 LC CDR3s was increased along with significantly increased, frequencies of 5-aa Vκ1-33 (10-fold) and Vκ3-20 (5-fold) LC CDR3s at least in part by suppressing recurrent MH-mediated joins that diminish their chance of occurring (Figs. 2F-2I).

[00260] TdT enhanced VRC01-class GC responses induced by eOD-GT8

[00261] VRC01^{Vκ1-33-CS}, VRC01^{Vκ1-33-CS/TdT}, VRC01^{Vκ3-20}, and VRC01^{Vκ3-20/TdT} mice were tested in parallel for response to priming eOD-GT8 60-mer immunogen along with poly I:C adjuvant (Fig.3A). All developed CD4-binding site (CD4bs) specific germinal center (GC) responses on day 8 post-immunization (Figs. 10A-10C). Analyses of VRC01-class GC B cells revealed that the frequencies of VRC01/Vκ1-33 GC B cells were extremely high, up to 70% of CD4bs-specific GC B cells and showed no changes between VRC01^{Vκ1-33-CS} and VRC01^{Vκ1-33-CS/TdT} mice (Fig. 3B and Fig. 10D). However, VRC01/Vκ3-20 and VRC01/mL C GC B cells frequencies were substantially increased, approximately 6-fold in VRC01^{Vκ3-20/TdT} compared to VRC01^{Vκ3-20} mice (Figs.3B and 10E). In addition, the Glu (E) residue, a conserved residue in the 4th position of VRC01-class LC CDR3s, were dominantly selected by eOD-GT8 in all 5-aa LC CDR3s, except Vκ3-20 and mouse LC CDR3s in VRC01^{Vκ3-20} mice on day 8 post-immunization (Figs. 3C and 10F-10H), indicating the Vκ3-20 and mouse LCs can not form the E residue independent of N regions added by TdT, also as proved by Vκ3-20 and Jκ germline sequences. The mouse LCs data was consistent with previous studies in V_H1-2 model using mouse LCs which did not enrich the E residue in mouse 5-aa LC CDR3s at early GCs induced by eOD-GT8. Therefore, TdT expression substantially enhanced the VRC01/Vκ3-20 GC response induced by eOD-GT8, since it increased the frequency of Vκ3-20 5-aa CDR L3, and also, importantly, the frequency of E residue formation.

[00262] VRC01-class B cells develop SHM and affinity maturation in GCs.

[00263] Both our VRC01^{Vκ1-33-CS/TdT} and VRC01^{Vκ3-20/TdT} models responded to eOD-GT8 efficiently. To make it more human-like, we bred these two Vκ segments together. Vκ1-33 and Vκ3-20 LCs were expressed in 7.8% and 3.4% of splenic B cells, respectively (Fig. 11A). However, on day 8 post-immunization with eOD-GT8 60mer, the VRC01/Vκ1-33 B cells were dominant in the GCs, while the VRC01/Vκ3-20 B cells can not be engaged, indicating the real frequency of VRC01/Vκ1-33 precursors was much higher than that of VRC01/Vκ3-20 precursors (Fig. 11B). Thus, we further generated a model in with Cer/sis still present on Vκ1-33 allele (VRC01^{Vκ1-33/TdT}) which reduced B cells with Vκ1-33 LC expression to just 1% of splenic B cells, 5-fold lower than Vκ3-20 LCs (Fig. 12A). These relative levels are more comparable to those in human naïve B cells. On day 8 post-immunization with eOD-GT8 60mer, high levels of both VRC01/Vκ1-33 and VRC01/Vκ3-20 GC responses were detected, similar to those of VRC01^{Vκ1-33-CS/TdT} and VRC01^{Vκ3-20/TdT} mice (Figs. 12B-12E).

[00264] To test if these VRC01-class GC B cells can develop SHM and affinity maturation, we boosted the mice with eOD-GT8 60 mer, and evaluated the GC responses on day 36 (Fig. 3D). Sequencing analyses of these VRC01-class antibodies cloned from both day 8 and day 36 GCs revealed extensive SHM (Fig. 3E and 12F). Compare to the intrinsic mutations generated from non-productive rearrangements of GC B cells without affinity selection, the Q62R mutant which has been seen in VRC01-class bnAbs was significantly enriched in day 36 VRC01-class HCs (Fig. 3F and 12G). The

length of HC CDR3 was in a range from 9-aa to 16-aa, but the HC CDR3s paired with V κ 1-33 LCs were slightly shorter (1 aa) than that paired with V κ 3-20 LCs (Fig. 12H). The E residues in LC CDR3s were dominant in all types of day 36 VRC01-class antibodies (Fig. 12I). We expressed several VRC01-class antibodies with different LCs cloned from both GCs. Antibodies from day 8 GCs showed a range of binding affinities, approximately 100nM K_D, to eOD-GT8 (Fig. 3G). For the antibodies from day 36 GCs, about 50% showed much higher binding activities, below 1nM K_D, representing an average affinity improvement of 100-fold (Fig. 3G). We also tested our VRC01 mouse models with another prime immunogen, 426c-degly3 Ferritin. 426c-degly3 was also designed to engage germline VRC01-class bnAbs, but with lower binding affinity compared to eOD-GT8. On day 8 post-immunization with 426c-degly3 Ferritin, all VRC01^{V κ 1-33/V κ 3-20/TdT} mice developed CD4bs-specific GC B cells, but at much lower frequencies compared to that induced by eOD-GT8 and VRC01/V κ 1-33 and VRC01/V κ 3-20 BCRs were detected, at low levels, in GC B cells in 3 out of 5 mice (Fig. 13A-13D). Altogether, the data show that the VRC01-class B cells develop SHMs and affinity maturation in GCs induced by eOD-GT8 and also indicate that the complete VRC01 models will be useful for comparing efficacies of different priming immunogens.

[00265] Novel SARS-CoV2 human antibodies isolated from VRC01 mouse model

[00266] We immunized a modified version of the VRC01^{V κ 1-33-CS/TdT} with several SARS-CoV-2 spike protein-derived immunogens, to test notion mice with BCR repertoires from single human HC and LC Vs associated with immensely diverse CDR3s could be useful for generating potential therapeutic antibodies²⁰. To avoid the potential competition B cells expressing mouse HC variable region exons, we deleted the 2MB region upstream of V_H1-2 that contains all the mouse VHs to generate the VRC01^{VH1-2 ONLY/V κ 1-33-CS/TdT} mouse in which human V_H1-2 and V κ 1-33 represented contribute to BCRs in, respectively, 100% and 13% of splenic B cells (Figs. 4A and 14A-14B). Analyzing this model by fluorescence-activated cell sorting (FACS) of several cell surface markers, splenic B and T cell populations were comparable to wild-type 129/Sv mice, even though they were generated from a single human VH1-2 rearranging allele (Fig. 14C, 14D).

[00267] As an initial test, we immunized 4 VRC01^{VH1-2 ONLY/V κ 1-33-CS/TdT} mice twice, four weeks apart, with SARS-CoV-2 spike protein (D614G) with poly I:C adjuvant (Fig. 4B). All mice developed strong antibody responses to SARS-CoV-2 spike, Receptor binding domain (RBD), N-terminal domain (NTD) and also cross-reactive to SARS-CoV-1 spike, as demonstrated by the IgG titer (Fig. 14E). 384 SARS-CoV-2 spike-specific IgG⁺ B cells were fluorescence-activated single-cell sorted from spleens on week 7 post-immunization. 20 of them expressed paired V_H1-2 HC and V κ 1-33 LC antibodies that derived from 5 B cell lineages defined as common CDR3s (Fig. 4C). The CDR3s of these 5 lineages varied in length and sequence (Fig. 4C). mAbs from the same lineages also contained different extensive mutations (Tables 1 and 2).

[00268] To access the binding properties, we express these 20 mAbs. ELISAs showed that 19 bound to SARS-CoV-2 spike, 5 from SPIKE-P1 lineage bound to RBD and none of them bound to NTD (Figs. 4D and 14F-14H). We further detected their cross-reactivities to the spikes of 7 coronavirus strains. The P1 mAb targeting RBD showed strong binding to BCoV_RaTG13 and weak binding to SARS-CoV1, while P2, P3 and P4 mAbs showed wide cross-reactivity to all 7 strains, indicating they targeted conserved regions of spike (Fig. 14I-14J).

[00269] The blocking assays showed that SPIKE-P1 mAb inhibited over 90% ACE2 binding to CoV2 spike, suggesting a potential neutralizing activity, and did not competed by the DH1041, 1042 and 1047 mAbs indicating a different RBD-binding epitope of SPIKE-P1 (Fig. 4E). The SPIKE-P1 can also bind to the variants of RBDs shown by ELISA, including K417N, E484K and N501Y (Fig. 4F). To determine if the SPIKE-P1 mAb had neutralizing activity, we test it against the SARS-CoV2 pseudovirus and live virus. The pseudovirus neutralization assays showed that SPIKE-P1 mAb potently neutralized all known SARS-CoV2 variants with IC₅₀ values ranging from 0.005 to 0.057 ug/ml (Fig. 4G). The plaque reduction neutralization titer (PRNT) live virus assay also showed high potent neutralization by SPIKE-P1 against all tested variants with IC₅₀ value ranging from 0.0008 to 0.0011 ug/ml (Fig. 4H). To further define the binding characteristics of SPIKE-P1 mAb, we imaged SARS-CoV2 S-Fab complexes using negative-stain electron microscopy (NSEM). The results showed SPIKE-P1 bound to the 'down' RBD (Fig. 4I). The footprint of SPIKE-P1 overlapped with ACE2 and two known mAbs identified from convalescent donors, S309 and REGN10987, which encoded by different V, D, J segments with different CDR3s. And the angle of approach of SPIKE-P1 is more similar to REGN10987 (Fig. 4J).

[00270] To generate more SARS-CoV2 neutralizing antibodies, we further immunized our mice with SARS-CoV2 RBD immunogens since the RBD is the major target for neutralizing antibodies. We used 3 RBD immunogens, including RBD monomer, RBD-Ferritin (24 mer) and nanobody-based RBD (VHH7-RBD) (Fig. 15A). All induced RBD-specific antibodies in mice, but the RBD-Ferritin and VHH7-RBD worked better than RBD monomer, as demonstrated by the higher IgG titers detected in RBD-Ferritin and VHH7-RBD immunized mice (Fig. 15B). 37 V_H1-2/V_κ1-33 antibodies were cloned from 576 RBD-specific IgG⁺ B cells on week 7 RBD-Ferritin or VHH7-RBD post-immunization (Figs. 15C, 15D), and 23 of them from 6 B cell lineages showed high binding activities to both SARS-CoV2 RBD and Spike (Fig. 15E). These newly identified RBD antibodies are different in the CDR3s compared to SPIKE-P1 (Fig. 15D). One RBD lineage that contain 11-aa HC CDR3 and 8-aa LC CDR3 was identified in both RBD-Ferritin and VHH7-RBD immunized mice (Fig. 15D).

[00271] Thus, our results proved that the mouse model with BCR repertoires derived from single human HC and LC Vs associated with immensely diverse CDR3s can respond to other potential therapeutic targets, not only to HIV-1 vaccines, and generate novel human antibodies. The antibodies contained various CDR3s and developed high levels of SHM. As we have ES cells derived from the VRC01^{VH1-2 ONLY/Vκ1-33-CS/TdT} mice, we can rapidly generate a series of ES cells by replacing the VH1-2 and/or Vκ1-

33 with other human VH and VL sequences and while the mouse Ds employed in the current model greatly overlap with sequences of human Ds we can also add additional human Ds that have sequences not found in mouse Ds. Such ES cells can be used to rapidly generate cohorts of mice for immunization via RAG-2 deficient blastocyst complementation as we have previously described.

Example 2: Antibody Sequences

[00272] Table 1: Antibody sequences. The protein sequences of variable regions, heavy chain (HC), and light chain (LC) for the antibodies are listed below in Table 1:

Ab Name	Heavy Chain Variable Region	Light Chain Variable Region	Heavy Chain CDR1	Heavy Chain CDR2	Heavy Chain CDR3	Light Chain CDR1	Light Chain CDR2	Light Chain CDR3
>SPIK E- BC1- 7-7	QVQLVQSGAEVKKP	DIQMTQSPSSLSASVG	GYTFTGT	INPNSGGT	ARDRVLYG	QDISDY	DAS	QQYDNLPT (SEQ
	GASVKVSCKASGYT	DRVITTCQASQDISDY	(SEQ ID NO: 3)	(SEQ ID NO: 4)	RSFGWYFD	(SEQ ID NO: 6)	(SEQ ID NO: 7)	ID NO: 8)
	FTGTIHWVRQAPG	LNWYQQQPGKAPKL			V (SEQ ID NO: 5)			
	QGLEWMGWNPNSG	LIYDASNLETGVPSRF						
>SPIK E- BC1- 1-3	GTNFAIFQGRVTLT	SGSGSGTDFITISLL	GYTFTDSY	INPHSGGT	ARDRVLYG	QDISDY	DAS	QQYDNLPT (SEQ
	RDTSTAYMDLNLRL	QPEDIGTYCYCQQYDN	(SEQ ID NO: 11)	(SEQ ID NO: 12)	RSYGWYFD	(SEQ ID NO: 14)	(SEQ ID NO: 15)	ID NO: 16)
	KSDDTAVYYCARDR	LPTFGGGTKLEIK			V (SEQ ID NO: 13)			
	VLYGRSFGWYFDV	(SEQ ID NO: 2)						
	WGAGTTVTVSS							
	(SEQ ID NO: 1)							
	QVQLVQSGAEVKKP	DIQMTQSPSSLSASVG						
	GASVTVSCKASGYT	DRVITTCQASQDISD						
	FTDSLHWVRQAPG	YLNWYQQKPGKAPK						
	QGLEWMGWNPNSG	LLIYDASNLETGVPSR						
	GTNYAQKFGQGRVT	FSGSGSGTDFITISLL						
	MTRDTSISTVYMELS	QPEDIAITYCYCQQYDN						
	RLRSDDTAVYYCAR							

	DRVLYGRSYGWYFD VWGAGTTTVSS (SEQ ID NO: 9)	LPTFGGGTKLEIK (SEQ ID NO: 10)							
>SPIK E- BC1- 6-2	QVQLVQSGAEVKKP GASVKVSCCKTSGYT FSDSYIHVVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSISTAYMDLS RLKSDDDTAVYYCAR DRVLYGRSYGWYFD VWGAGTTTVSS (SEQ ID NO: 17)	DIQMTQSPSSLSASVG DRVITTCQASQDIND YLNWYQQQPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFITSL QPEDIGTYCQQYDN LPTFGGGTKLEIK (SEQ ID NO: 18)	GYTFSDSY (SEQ ID NO: 19)	INPNSGGT (SEQ ID NO: 20)	ARDRVLYG RSYGWYFD V (SEQ ID NO: 21)	QDINDY (SEQ ID NO: 22)	DAS (SEQ ID NO: 23)	QQYDNLPT (SEQ ID NO: 24)	
>SPIK E- BC1- 7-3	QVQLVQSGAEVKKP GASVKVSCCKASGYT FTGTIHHVVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDDTAVYYCAR DRVLYGRSYGWYFD VWGAGTTTVSS (SEQ ID NO: 25)	DIQMNQSPSSLSASV GDRVITTCQASQDISD YLNWYQQQHPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFITSL QPEDIGTYFCQQYDN LPTFGGWTKLEIK (SEQ ID NO: 26)	GYTFTGT (SEQ ID NO: 27)	INPNSGGT (SEQ ID NO: 28)	ARDRVLYG RSYGWYFD V (SEQ ID NO: 29)	QDISDY (SEQ ID NO: 30)	DAS (SEQ ID NO: 31)	QQYDNLPT (SEQ ID NO: 32)	
>SPIK E-	QVQLVQSGAEVKKP GASVKVSCCKASGYT FTDSYIHVVVRQAPG	DIQMTQSPSSLSASVG DRVITTCQASQDISD YLNWYQQQPGKAPK	GYTFTDSY (SEQ ID NO: 35)	INPNSGGT (SEQ ID NO: 36)	ARDRVLYG RSYGWYFD	QDISDY (SEQ ID NO: 38)	DAS (SEQ ID NO: 39)	QQYDNLPT (SEQ ID NO: 40)	

BC1-9-6	QGLEWMGWPNPNSG GTNYAQNFQGRVT MTRDTSISTAYMDLS RLKSDDTAVVYCAR DRVLYGRSYGWYFD VWGAGTTVTVSS (SEQ ID NO: 33)	LLIYDASNLETGVPSR FSGSGGTDFTFTISSL QPEDIAITYYCCQQYDN LPTFGGGTKLEIK (SEQ ID NO: 34)			V (SEQ ID NO: 37)			
>SPIK E- BC2-1-6	QVQLVQSGAEVKKP GASVKVSCKASGDF IGYYMHVWRQAPG QGLEWMGWPNPNSG GTNYVKKFQGRVT MTRDTSISKAYMEL RRLRSDDTAVVYCA RERRVIHYSGLYAL DYWGQGTALTIVSS (SEQ ID NO: 41)	DIQMNQSPSSLSASV GDRVTTTCQASQDITN YLNWYQQKPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFTISSL QPEDIAITYYCCQQYDN LHMFTFGGGTKLEIK (SEQ ID NO: 42)	GDIFIGYY (SEQ ID NO: 43)	INPNSGGT (SEQ ID NO: 44)	ARERRVIH YSGILYAL DY (SEQ ID NO: 45)	QDITNY (SEQ ID NO: 46)	DAS (SEQ ID NO: 47)	QQYDNLHMFT (SEQ ID NO: 48)
>SPIK E- BC2-12-2	QVQLVQSGAEVKKP GASVKVSCKASGDT FTGYSMHVWRQAP QGLEWMGWPNPNS GGTNYVQNFQDRVT MTRDTSISTAYMELS RLRSDDTAVVYCAR ERRVIHYGILYALD YWGQGTALTIVSS	DIQMTQSPSSLSASVG DRVTTTCQASQDITNY LNWYQQKPGKAPKL LLIYDASNLETGVPSRF SGSGGTDFTFTISSL QPEDIAITYYCCQQYDN LHMFTFGGGTKLEIK	GDITFTGYS	INPNSGGT	ARERRVIH YYGILYAL DY	QDITNY	DAS	QQYDNLHMFT

>SPIK E- BC2- 12-5	QVQLVQSGAEVKKP GASVKVSCKASGDP FTGYMHWRQAP GQGLEWMGWNPNS GGTNYVQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR ERRVIHYSGLYALD YWGQGTALTIVSS (SEQ ID NO: 49)	DIQMNQSPSSLSASV GDRVITTCQASQDITN YLNWYQQKPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFITSSL QPEDIAITYYCQQYDN LHMFTFGGWTKEIK (SEQ ID NO: 50)	GDPTGY (SEQ ID NO: 51)	INPNSGGT (SEQ ID NO: 52)	ARERRVIH YSGILYAL DY (SEQ ID NO: 53)	QDITNY (SEQ ID NO: 54)	DAS (SEQ ID NO: 55)	QQYDNLHMFT (SEQ ID NO: 56)
>SPIK E- BC2- 4-8	QVQLVQSGAEVKKP GASVKVSCKASGDT LTGYMHWRQAP GQGLEWMGWNPNS GGTNYVQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR ERRVIHYGILYAM DYWGQGTAVTVSS (SEQ ID NO: 57)	DIQMTQSPSSLSASV DRVITTCQASQDITNY LNWYQQKPGKAPKL LLIYDASNLETGVPSRF SGSGGTDFTFITSSL QPEDIAITYYCQQYDD LHMFTFGGWTKEIK (SEQ ID NO: 58)	GDILTGY (SEQ ID NO: 59)	INPNSGGT (SEQ ID NO: 60)	ARERRVIH YYGILYAM DY (SEQ ID NO: 61)	QDITNY (SEQ ID NO: 62)	DAS (SEQ ID NO: 63)	QQYDDLHMFT (SEQ ID NO: 64)
>SPIK E- BC3- 10-1	QVQLVQSGAEVKKP GASVKVSCKASGYT FTAYIHWRQVPG QGLEWMGWNPITG GTNYAQNFQGRVT MTRDTPRTAYMEL	DIQMNQSPSSLSASV GDRVITTCQARQDIN NYLNWYQQKPGKAP KVLIVDYSNLETGVP SRFSGSRGTDFTFTIS SLQPEDIGITYCQQY	GYTFTAY (SEQ ID NO: 67)	INPITGGT (SEQ ID NO: 68)	AITGRRPW FPF (SEQ ID NO: 69)	QDINNY (SEQ ID NO: 70)	DVS (SEQ ID NO: 71)	QQYDNLPLYT (SEQ ID NO: 72)

	RRLRSDDTAVFYCAI TGRRPWFPGQGT LVTVSA (SEQ ID NO: 65)	DNLPTFGGWTKEI K (SEQ ID NO: 66)							
>SPIK E- BC3- 4-2	QVQLVQSGAEVKKP GASVKVCKASGYT FTAYYIHWMRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSVRTAYMEL SRLTSDDTAVYYCAI TGRRPWFPGQGT LVTVSA (SEQ ID NO: 73)	DIQMNQSPSSLSASV GDRVITTCQASQDIN NYLNWYQQKPGKAP KVLIDYDTSNLETGVPS RFGSRSGTDFTFITIS SLHPEDIAITYYCQY DYLPTFGGWTKEI K (SEQ ID NO: 74)	GYTFTAYY (SEQ ID NO: 75)	INPNSGGT (SEQ ID NO: 76)	AITGRRPW FPY (SEQ ID NO: 77)	QDINNY (SEQ ID NO: 78)	DTS (SEQ ID NO: 79)	QQYDYLPT (SEQ ID NO: 80)	
>SPIK E- BC3- 4-6	QVQLVQSGAEVKKP GASVKVCKSSGYT FTAYYMHWRQAP GQGLKWMGWNP NGGTNYAQEFQGRV TMRDTSIKTVYME LSRLRSDDTAVYYC AITGRRPWFANWQ GTLVTVSA (SEQ ID NO: 81)	DIQMTQSPSSLSASV DRVITTCQASQDINN YLNWYQQKPGKAPK VLIYDVSNLETGVPSR FSGSRSGTDFTFITISL QPEDIAIFYCQYDY LPTFGGWTKEI (SEQ ID NO: 82)	GYTFTAYY (SEQ ID NO: 83)	INPHNGGT (SEQ ID NO: 84)	AITGRRPW FAN (SEQ ID NO: 85)	QDINNY (SEQ ID NO: 86)	DVS (SEQ ID NO: 87)	QQYDYLPT (SEQ ID NO: 88)	

>SPIK E- BC3- 9-8	QVQLVQSGAEVKKP GASVKVSCKASGYT FTAYYHWMRQAPG QGLEWMGWNPNR GDTNYAQKFQGRVT MTRDTSIRIAYMELS RLKSDDTAVYYCTIT GRRPFPPYWGQGT VTVSA (SEQ ID NO: 89)	DIQMNQSPSSLSASV GDRVTTTCQASQDIN NYLNWYQQKPGKVP KLLIYDASNLETGVPS RFGSRSGTDFSTISS LPEDIAITYYCCQYD NLPYTFGGWTKLEIK (SEQ ID NO: 90)	GYTFTAYY (SEQ ID NO: 91)	INPNRGDT (SEQ ID NO: 92)	TITGRRPFF PY (SEQ ID NO: 93)	QDINNY (SEQ ID NO: 94)	DAS (SEQ ID NO: 95)	QQYDNLPLYT (SEQ ID NO: 96)
>SPIK E- BC4- 10-2	QVQLVQSGAEVKKP GASVKVSCKASGYT FTVYNIHWIRQAPGQ GLEWMGWNPYSGG TNSAQNFQGRVTMT RDTSTTAYMELSSL RSDDTALYYCARGE RRSWFTYGGQGT TVSA (SEQ ID NO: 97)	DIQMNQSPSSLSASV GDRVTTTCQASQDINT YLNWYQQKAGKAPK VLIYDASNLETGVPSR FSGSLGSDFTICISL QPEDIAITYYCCQYDN LPYTFGGGKLEIK (SEQ ID NO: 98)	GYTFTVYN (SEQ ID NO: 99)	INPYSGGT (SEQ ID NO: 100)	ARGERRSW FTY (SEQ ID NO: 101)	QDINTY (SEQ ID NO: 102)	DAS (SEQ ID NO: 103)	QQYDNLPLYT (SEQ ID NO: 104)
>SPIK E- BC4- 11-3	QVQLVQSGAEVKKP GASVKVSCKASGYT FTVYNIHWIRQVPG QGLEWMGWNPYSG GTNSAQNFQGRVTM TRDTSITAYMELSS	DIQMNQSPSSLSASV GDRVTTTCQASQDINT YLNWYQQKAGKAPK VLIYDASNLETGVPSR FSGSRSGTDFITISL QPEDIAITYYCCQYDN	GYTFTVYN (SEQ ID NO: 107)	INPYSGGT (SEQ ID NO: 108)	ARGERRSW FTY (SEQ ID NO: 109)	QDINTY (SEQ ID NO: 110)	DAS (SEQ ID NO: 111)	QQYDNLPLYT (SEQ ID NO: 112)

	LRSDDTALYYCARG ERRSWFTYWGQGT VTVSA (SEQ ID NO: 105)	LPYTFGGWTKLEIK (SEQ ID NO: 106)							
>SPIK E- BC4- 7-3	QVQLVQSGAELEKPK GASVKVSCKASGYT FTVYNIHWIRQAPGQ GLEWMGWINPYSGG TNSAQNFQGRVTMT RDTISITAYMELSSL RSDDTALYYCARGE RRSWFTYWGQGT TVSA (SEQ ID NO: 113)	DIQMTQSPSSLSASVG DRVITTCQASQDINTY LNWYQQKAGKAPKV LIYDASNLETGVPSRF SGSRSGTDFTFITSSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 114)	GYTFTVYN (SEQ ID NO: 115)	INPYSGGT (SEQ ID NO: 116)	ARGRRSW FTY (SEQ ID NO: 117)	QDINTY (SEQ ID NO: 118)	DAS (SEQ ID NO: 119)	QQYDNLPLYT (SEQ ID NO: 120)	
>SPIK E- BC4- 1-5	QVQLVQSGAEVKKP GASMKVSCKASGYT FTTYHHWVRQAPG QGLEWMGWIIPHS DTNYAQKFQGRVT MTRDTSINTAYMEL TRLRSDDTAVYYCA RGGRRTWFAWGWQ GTLVTVSA (SEQ ID NO: 121)	DIQMTQSPSSLSASVG DRVITTCQASQDINN YLNWYQQKPGKAPK LIYDASNLETGVPSR FSGSRSGTHFTITSSL QPEDIAITYYCQQYDY LPYTFGGGKLEIK (SEQ ID NO: 122)	GYTFTTYH (SEQ ID NO: 123)	IIPHSGDT (SEQ ID NO: 124)	ARGRRRT WFAY (SEQ ID NO: 125)	QDINNY (SEQ ID NO: 126)	DAS (SEQ ID NO: 127)	QQYDYLPLYT (SEQ ID NO: 128)	

>SPIK E- BC4- 6-5	QVQLVQSGAEVKKP GASVNVSCKASGYT FTVYNNMHWVRQAP GQGLEWMGWITPYS GATNYAQKFQGRVT MTRDTSIPTVYMEL NRLRSDDTAMYCA RGARRTFAYWGQ GTLVTVSA (SEQ ID NO: 129)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGKAPKF LIYDASNLETGVPSRF SGSRSGTDFFTISSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 130)	GYTFTVYN (SEQ ID NO: 131)	ITPYSGAT (SEQ ID NO: 132)	ARGARRT WFAY (SEQ ID NO: 133)	QDISNY (SEQ ID NO: 134)	DAS (SEQ ID NO: 135)	QQYDNLPLYT (SEQ ID NO: 136)
	QVQLVQSGAEVKKP GASMKVSCKASGYT FTTYHHWVRQAPG QGLEWMGWIPNNG DTNYAQKFQGRVT MTRDTSINTAYMEL TRLRSDDTAVYYCA RGGRRTFAYWGQ GTLVTVSA (SEQ ID NO: 137)	DIQMTQSPSSLSASVG DRVITTCQASQDINN YLNWYQQKPGKAPK FLYDASNLETGVPSR FSGSRSGTDFFTISSL QPEDIAITYYCQQYDY LPYTFGGGKLEIK (SEQ ID NO: 138)	GYTFTTYH (SEQ ID NO: 139)	IIPNNGDT (SEQ ID NO: 140)	ARGGRRT WFAY (SEQ ID NO: 141)	QDINNY (SEQ ID NO: 142)	DAS (SEQ ID NO: 143)	QQYDYLPLYT (SEQ ID NO: 144)
	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYMHWVRQAP GQGLEWMGWINPNS GGTNYAQKFQDRVT MTRDTSISTAYMELS	DIQMTQSPSSLSASVG DRVITTCQASQDIYN YLNWFQQKPGKAPK LLIYDASNLETGVPSR FNGSGGTDFTFIAISS LQPEDIAITYYCQQYD	GYTFTGYY (SEQ ID NO: 147)	INPNSGGT (SEQ ID NO: 148)	ARGKGNPA WFAY (SEQ ID NO: 149)	QDIYNY (SEQ ID NO: 150)	DAS (SEQ ID NO: 151)	QQYDNLPWTT (SEQ ID NO: 152)
	>RBD NP- BC9- HC78							

	RLRSDDTAVYYCAR GKGNPWFAYWGQ GTLVTVSA (SEQ ID NO: 145)	NLPWTFGGGKLEIK (SEQ ID NO: 146)							
>RBD NP- BC11- 23	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYMHWRQAP QGQLEWMGWNPNS GGTNYAQKFQGRVT MTRDTSISTACMELS RLRSDDTAVYYCAR DRVGRFAYWGQ TLVTVSA (SEQ ID NO: 153)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGRAPKV LIYDASHLETGVPSRF SGSGSGTDFTFTISL QPEDIAYYCQQYDN LWTFGGGKLEIK (SEQ ID NO: 154)	GYTFTGYY (SEQ ID NO: 155)	INPNSGGT (SEQ ID NO: 156)	ARDRVQQR FAY (SEQ ID NO: 157)	QDISNY (SEQ ID NO: 158)	DAS (SEQ ID NO: 159)	QQYDNLWT (SEQ ID NO: 160)	
>RBD NP- BC11- 31	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYIHWRQAPG QGQLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DRVGRFPYWGQGT LVTVSA (SEQ ID NO: 161)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISL QPEDIAYYCQQYDN LWTFGGGKLEIK (SEQ ID NO: 162)	GYTFTGYY (SEQ ID NO: 163)	INPNSGGT (SEQ ID NO: 164)	ARDRVQQR FPY (SEQ ID NO: 165)	QDISNY (SEQ ID NO: 166)	DAS (SEQ ID NO: 167)	QQYDNLWT (SEQ ID NO: 168)	

>RBD NP- BC11- 34	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYIHVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DRVGRFPYWGQGT LVTVSA (SEQ ID NO: 169)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISL QPEDIATYYCQQYDN LWTFGGGKLEIK (SEQ ID NO: 170)	GYTFTGYY (SEQ ID NO: 171)	INPNSGGT (SEQ ID NO: 172)	ARDRVQQR FPY (SEQ ID NO: 173)	QDISNY (SEQ ID NO: 174)	DAS (SEQ ID NO: 175)	QQYDNLWT (SEQ ID NO: 176)
>RBD NP- BC11- 47	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYIHVVRQAPG QGLEWMGWNPNSG VTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DRVGRFPYWGQGT LVTVSA (SEQ ID NO: 177)	DIQMTQSPSSLSASVG DRVITTCQGSQDISNY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISSP QPEDIGTYTCQQYDN LWTFGGGKLEIK (SEQ ID NO: 178)	GYTFTGFY (SEQ ID NO: 179)	INPNSGVT (SEQ ID NO: 180)	ARDRVQQR FPY (SEQ ID NO: 181)	QDISNY (SEQ ID NO: 182)	DAS (SEQ ID NO: 183)	QQYDNLWT (SEQ ID NO: 184)
>RBD NP- BC11- 60	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYIHVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSISTAYMELS	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISL QPEDIATYYCQQYDN	GYTFTGYY (SEQ ID NO: 187)	INPNSGGT (SEQ ID NO: 188)	ARDRVQQR FPY (SEQ ID NO: 189)	QDISNY (SEQ ID NO: 190)	DAS (SEQ ID NO: 191)	QQYDNLWT (SEQ ID NO: 192)

	RLRSDDTAVYYCAR DRVGRFPYWGQGT LVTVSA (SEQ ID NO: 185)	LWTFGGGKLEIK (SEQ ID NO: 186)								
>RBD NP- BC11- 78	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYMHWRQAP QGQLEWMGWNPNS GGTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DRVGRFPYWGQGT LVTVSA (SEQ ID NO: 193)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISL QPEDIAITYYCQQYDN LWTFGGGKLEIK (SEQ ID NO: 194)	GYTFTGYY (SEQ ID NO: 195)	INPNSGGT (SEQ ID NO: 196)	ARDRVQQR FPY (SEQ ID NO: 197)	QDISNY (SEQ ID NO: 198)	DAS (SEQ ID NO: 199)	QQYDNLWT (SEQ ID NO: 200)		
>RBD NP- BC11- 9	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYHWRQAPG QGQLEWMGWNPSSG GTNYAQKFQGRVT MTRDTSITAYMEL NRLRSDDTAVYYCA RDRVGRFPYWGQ GTLVTVSA (SEQ ID NO: 201)	DIQMTQSPSSLSASVG DRVITTCQASQDISNC LNWYQKPGKAPKL LIYDASNLETGVPSRF SGSGSGTDFTFTISL QPEDIAITYYCQQYDN LWTFGGGTRLEIK (SEQ ID NO: 202)	GYTFTGYY (SEQ ID NO: 203)	INPSSGGT (SEQ ID NO: 204)	ARDRVQQR FPY (SEQ ID NO: 205)	QDISNC (SEQ ID NO: 206)	DAS (SEQ ID NO: 207)	QQYDNLWT (SEQ ID NO: 208)		

>VHH 7- BC3- 37	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYMHWRQAP GQGLEWMGWNPNS GGTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DLGDDYDAWFAYWG QGTLVTVSA (SEQ ID NO: 209)	DIQMTQSPSSLSASVG DRVITTCQASQDISNH LNWYQQKPGKAPKV LIYDTFNLETGVPSRF SGSGGTDFTFTRSL QPEDIAITYYCQQYDN LRWTFGGGKLEIK (SEQ ID NO: 210)	GYFTGYY (SEQ ID NO: 211)	INPNSGGT (SEQ ID NO: 212)	ARDLGDDYD AWFAY (SEQ ID NO: 213)	QDISNH (SEQ ID NO: 214)	DTF (SEQ ID NO: 215)	QQYDNLRWLT (SEQ ID NO: 216)
>VHH 7- BC3- 66	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYMHWRQAP GQGLEWMGWNPNS GGTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR DLGDDYDAWFAYWG QGTLVTVSA (SEQ ID NO: 217)	DIQMTQSPSSLSASVG DRVITTCQASQDISNH LNWYQQKPGKAPKV LIYDAFNLETGVPSRF SGSGGTDFTFTRSL QPEDIAITYYCQQYDN LRWTFGGGKLEIK (SEQ ID NO: 218)	GYFTGYY (SEQ ID NO: 219)	INPNSGGT (SEQ ID NO: 220)	ARDLGDDYD AWFAY (SEQ ID NO: 221)	QDISNH (SEQ ID NO: 222)	DAF (SEQ ID NO: 223)	QQYDNLRWLT (SEQ ID NO: 224)
>VHH 7- BC5- 29	QVQLVQSGAEVKKP GASVKVSCKASGYF TGYVHWIRQAPGQ GLEWMGWNPDSGG THFAKKFQGRVLT RDTSTTAYMEL SRL	DIQMTQSPSSLSASVG DRVITTCQGSQDISNY LNGYQQKLGKAPKL LISDASNLETGVPSRF SGRSGTTFTRTSSP QPEDIAITYYCQQYDN	GYFTGYY (SEQ ID NO: 227)	INPDSGGT (SEQ ID NO: 228)	ARDRVQGR FVY (SEQ ID NO: 229)	QDISNY (SEQ ID NO: 230)	DAS (SEQ ID NO: 231)	QQYDNLWT (SEQ ID NO: 232)

	RSDDTAVYYCARDR VGQRFVYWGQGT VTVSA (SEQ ID NO: 225)	LWTFGGGKLEIK (SEQ ID NO: 226)							
>VHH 7- BC5- 75	QVQLVQSGAEVKKP GASVKVSCKASGYT FTDFYHHWVRQAPG QGLEWMGWNPNSV VTFYAPKFQGRVTM TRDTSISTAYMELNR LRSDDSAVYYCARD RVGQRFPPYWGQGT VTVSA (SEQ ID NO: 233)	DIQMTQSPSSLSASVG DRVTITCQASQDISN CLNYYQQKPGKAPK LLYDASNLETGVPSR FSGSGGTDFFTISSL QPEDFATYYCQQYD YLVTFGGGSKLEIK (SEQ ID NO: 234)	GYTFTDFY (SEQ ID NO: 235)	INPNSVVT (SEQ ID NO: 236)	ARDRVQQR FPY (SEQ ID NO: 237)	QDISNC (SEQ ID NO: 238)	DAS (SEQ ID NO: 239)	QQYDYLWT (SEQ ID NO: 240)	
>VHH 7- BC5- 82	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYHHWVRQAPG QGLEWMGWNPNSG GSHYAQKFQGRVTL TRDTSISTAYMELTR LRSDDTAVYYCARD RVGQRFPPYWGQGT VTVSP (SEQ ID NO: 241)	DIQMTQSPSSLSASVG DRVTITCQASQDIFNC LNYYQQKPGKAPKL LIYDASNLETGVPSRF SGSGGTDFFTISSL QPEDVATYFCQQYD NLWTFGGGKLEIK (SEQ ID NO: 242)	GYTFTGYY (SEQ ID NO: 243)	INPNSGGS (SEQ ID NO: 244)	ARDRVQQR FPY (SEQ ID NO: 245)	QDIFNC (SEQ ID NO: 246)	DAS (SEQ ID NO: 247)	QQYDNLWT (SEQ ID NO: 248)	

>VHH 7- BC7- 53	QVQLVQSGAEVKKP GASVRVSCCKASGFTF TGYQHVVVRQAPGQ GLEWMGWNPCKSGG TNYAQKFQGRVTMT RDTSTAYMELSRL RSDDTAVVYCARRN CDYWGQGTTTLTVSS (SEQ ID NO: 249)	DIQMTQSPSSLSASVG DRVITTCQASQDISTRY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGGTDFITFISSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 250)	GFTFTGYQ (SEQ ID NO: 251)	INPKSGGT (SEQ ID NO: 252)	ARRNCDY (SEQ ID NO: 253)	QDISTY (SEQ ID NO: 254)	DAS (SEQ ID NO: 255)	QQYDNLPYT (SEQ ID NO: 256)
>VHH 7- BC7- 76	QVQLVQSGAEVKKP GASVKVSCCKASGFT FTGYQHVVVRQAPG QGLEWMGWNPNSG VTNYAHKFQGRVT MTRDTSISTAYMELS RLRSDDTAVVYCAR RNC DYWGQGTTTLTV SS (SEQ ID NO: 257)	DIQMTQSPSSLSASVG DRVITTCQASQDISTRY LNWYQQKPGKAPKL LIYDASNLETGVPSRF SGSGGTDFITFISSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 258)	GFTFTGYQ (SEQ ID NO: 259)	INPNSGVT (SEQ ID NO: 260)	ARRNCDY (SEQ ID NO: 261)	QDISTY (SEQ ID NO: 262)	DAS (SEQ ID NO: 263)	QQYDNLPYT (SEQ ID NO: 264)
>VHH 7- BC7- 79	QVQLVQSGAEVKKP GASVKVSCCKASGFT FTGYQHVVVRQAPG QGLEWMGWNPCKSG GTNYAQKFQGRVT MTRDTSISTAYMELS RLRSDDTAVVYCAR	DIQMTQSPSSLSASVG DRVITTCQASQDISTRY LNWYQQKPGKAPNL LIYDASNLETGVPSRF SGSGGTDFITFISSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 266)	GFTFTGYQ (SEQ ID NO: 267)	INPKSGGT (SEQ ID NO: 268)	ARRNCDY (SEQ ID NO: 269)	QDISTY (SEQ ID NO: 270)	DAS (SEQ ID NO: 271)	QQYDNLPYT (SEQ ID NO: 272)

	RNC DYWGQGTTLTV SS (SEQ ID NO: 265)	DIQMTQSPSSLSASVG DRVITTCQASQDSTY LNWYQQKPGKAPKL LIFDASNLETGVPSRF SGGSGTDFTFITSSL QPEDIAITYYCQQYDN LPYTFGGGKLEIK (SEQ ID NO: 274)	GYFTGHQ (SEQ ID NO: 275)	INPNSGGT (SEQ ID NO: 276)	ARRNCDN (SEQ ID NO: 277)	QDISTY (SEQ ID NO: 278)	DAS (SEQ ID NO: 279)	QQYDNLPYT (SEQ ID NO: 280)	
>VHH 7- BC7- 85	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGHQIHVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVT MTRDTSITAYMELS RLRSDDTAVYYCAR RNC DNWGQGTTLTV SS (SEQ ID NO: 273)	DIQMTQSPSSLSASVG DRVITTCQASQDIGN YLNWFQQRPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFITSSL QPEDIAITYYCQQYDN LYTFGGGKLEIK (SEQ ID NO: 282)	GYIFTGYN (SEQ ID NO: 283)	INPNSDFT (SEQ ID NO: 284)	ARGRGGTS WLA Y (SEQ ID NO: 285)	QDIGNY (SEQ ID NO: 286)	DAS (SEQ ID NO: 287)	QQYDNLYT (SEQ ID NO: 288)	
>VHH 7- BC8- 59	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYYIHVVRQAPG QGLEWMGWNPNSG DTNYAQKFQGRVT	DIQMTQSPSSLSASVG DRVITTCQASQDIYN YLNWYQQKPGKAPK LLIYDASNLETGVPSR FSGSGGTDFTFITSSL	GYFTGVY (SEQ ID NO: 291)	INPNSGDT (SEQ ID NO: 292)	ARGKGGTS WFAY (SEQ ID NO: 293)	QDIYNY (SEQ ID NO: 294)	DAS (SEQ ID NO: 295)	QQYDNLCY (SEQ ID NO: 296)	

	MTRDTSISTAYMELS RLRSDDTAVYYCAR GKGGTSWFAYWGQ GTLVTVSA (SEQ ID NO: 289)	QPEDIAYYCQQYDN LCTFGGGTKLEIK (SEQ ID NO: 290)							
>VHH 7- BC8- 62	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYYIHVVRRQAPG QGLEWMGWNPNSG DTNYAQKFQVRVT MTRDTSISTAYMELS RLRSDDTAVYYCAR GKGGTSWFAYWGQ GTLVTVSA (SEQ ID NO: 297)	DIQMTQSPSSLSASVG DRVITTCQASQDIYN YLNWYQQKPGKAPK LLIYDASNLETGVPSR FSGSGGTDFFTFISSL QPEDIAYYCQQYDN LYTFGGGKLEIK (SEQ ID NO: 298)	GYTFTGYY (SEQ ID NO: 299)	INPNSGDT (SEQ ID NO: 300)	ARGKGGTS WFAY (SEQ ID NO: 301)	QDIYNY (SEQ ID NO: 302)	DAS (SEQ ID NO: 303)	QQYDNLYT (SEQ ID NO: 304)	
>VHH 7- BC8-7	QVQLVQSGAEVKKP GASVKVSCKASGYT FTGYYLHVVRQAPG QGLEWMGWNPNSG DTHYAQKFQGRVT MPRDTISISTAYMELS RLRSDDTAVYYCAR GRGGTSWFAYWGQ GTLVTVSA (SEQ ID NO: 305)	DIQMTQSPSSLSASVG DRVITTCQASQDIYN YLNWYQQKPGKAPK LLIYDASNLETGVPSR FSGSGGTDFFTFISSL QPEDIAYYCQQYDN LYTFGGGKLEIK (SEQ ID NO: 306)	GYTFTGYY (SEQ ID NO: 307)	INPNSGDT (SEQ ID NO: 308)	ARGRGGTS WFAY (SEQ ID NO: 309)	QDIYNY (SEQ ID NO: 310)	DAS (SEQ ID NO: 311)	QQYDNLYT (SEQ ID NO: 312)	

>VHH 7-BC10-19	QVQLVQSGAEVKKP GASVKVSCKASEYT FTGYYIHVVVRQAPG QGLEWMGWNPNSG GTNYAQKFQGRVSM TGDTSIRTA YMELSR LRSDDTAVFYCARD PLTGIFDYWGQGTTL TVSS (SEQ ID NO: 313)	DIQMTQSPSSLSASVG DRVITTCQASQDISNY LNWYQQKPGKAPKL LIYDASNLETGVPLRF SGSGSGTDFTFTISL QPEDIAITYYCQQYDN LPYMYTFGGGKLEI K (SEQ ID NO: 314)	EYFTFGYY (SEQ ID NO: 315)	INPNSGGT (SEQ ID NO: 316)	ARDPLTGIF DY (SEQ ID NO: 317)	QDISNY (SEQ ID NO: 318)	DAS (SEQ ID NO: 319)	QQYDNLPYMYT (SEQ ID NO: 320)
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[00273] Table 2: Characteristics of antibodies of Table 1. Antibodies with italicized and bolded names were selected for further binding and neutralization experiments.

Rank	Ab Name	Lineage	Binding to spike	Binding to RBD	immunogen
1	> <i>SPIKE-BC1-7-7</i>	SPIKE-BC1	+	+	SARS-CoV2 spike
2	>SPIKE-BC1-1-3		+	+	SARS-CoV2 spike
3	>SPIKE-BC1-6-2		+	+	SARS-CoV2 spike
4	>SPIKE-BC1-7-3		+	+	SARS-CoV2 spike
5	>SPIKE-BC1-9-6		+	+	SARS-CoV2 spike
6	>SPIKE-BC2-1-6	SPIKE-BC2	+	-	SARS-CoV2 spike
7	>SPIKE-BC2-12-2		+	-	SARS-CoV2 spike
8	> <i>SPIKE-BC2-12-5</i>		+	-	SARS-CoV2 spike
9	>SPIKE-BC2-4-8		+	-	SARS-CoV2 spike
10	>SPIKE-BC3-10-1	SPIKE-BC3	+	-	SARS-CoV2 spike
11	>SPIKE-BC3-4-2		+	-	SARS-CoV2 spike
12	> <i>SPIKE-BC3-4-6</i>		+	-	SARS-CoV2 spike
13	>SPIKE-BC3-9-8		+	-	SARS-CoV2 spike
14	>SPIKE-BC4-10-2	SPIKE-BC4	+	-	SARS-CoV2 spike
15	>SPIKE-BC4-11-3		+	-	SARS-CoV2 spike
16	>SPIKE-BC4-7-3		+	-	SARS-CoV2 spike
17	>SPIKE-BC4-1-5		+	-	SARS-CoV2 spike
18	>SPIKE-BC4-6-5		+	-	SARS-CoV2 spike
19	> <i>SPIKE-BC4-8-7</i>		+	-	SARS-CoV2 spike
20	> <i>RBDNP-BC9-HC78 also referred to herein as RBDNP-BC9-78</i>	RBDNP-BC9	+	+	RBD-Ferritin nanoparticle
21	>RBDNP-BC11-23	RBDNP-BC11			RBD-Ferritin nanoparticle
22	>RBDNP-BC11-31				RBD-Ferritin nanoparticle
23	> <i>RBDNP-BC11-34</i>		+	+	RBD-Ferritin nanoparticle
24	>RBDNP-BC11-47				RBD-Ferritin nanoparticle
25	>RBDNP-BC11-60				RBD-Ferritin nanoparticle
26	>RBDNP-BC11-78				RBD-Ferritin nanoparticle
27	>RBDNP-BC11-9				RBD-Ferritin nanoparticle
28	>VHH7-BC3-37	VHH7-BC3			VHH7-RBD (nanobody fused RBD)
29	> <i>VHH7-BC3-66</i>		+	+	VHH7-RBD (nanobody fused RBD)
30	>VHH7-BC5-29	VHH7-BC5			VHH7-RBD (nanobody fused RBD)

31	>VHH7-BC5-75	VHH7-BC7			VHH7-RBD (nanobody fused RBD)
32	> VHH7-BC5-82		+	+	VHH7-RBD (nanobody fused RBD)
33	> VHH7-BC7-53		+	+	VHH7-RBD (nanobody fused RBD)
34	>VHH7-BC7-76				VHH7-RBD (nanobody fused RBD)
35	>VHH7-BC7-79				VHH7-RBD (nanobody fused RBD)
36	>VHH7-BC7-85	VHH7-BC8			VHH7-RBD (nanobody fused RBD)
37	>VHH7-BC8-5				VHH7-RBD (nanobody fused RBD)
38	> VHH7-BC8-59		+	+	VHH7-RBD (nanobody fused RBD)
39	>VHH7-BC8-62				VHH7-RBD (nanobody fused RBD)
40	>VHH7-BC8-7	VHH7-BC10			VHH7-RBD (nanobody fused RBD)
41	> VHH7-BC10-19		+	+	VHH7-RBD (nanobody fused RBD)

EXAMPLE 3

[00274] The SARS-CoV-2 Omicron variant has spread worldwide and become a public health concern. Omicron has an unprecedented number of spike gene mutations, is resistant to most existing SARs-CoV-2 neutralizing antibodies, and evades antibodies induced by past infections or vaccinations. The need for an antibody that broadly neutralizes omicron and all current variants of concern is important for managing omicron and future variants. While humanized mouse models provide many therapeutic antibodies, they have yet to produce Omicron-neutralizing antibodies. To address this need, described herein is the isolation of such an antibody from a novel mouse model that generates a primary B cell receptor repertoire from a single human V_H1-2 and V_κ1-33 in association with an immense diversity of antigen contact complementarity-determining-region 3 sequences (CDR3s) generated by V(D)J recombination. Immunizing this model with SARS-CoV-2 D614G prototype spike protein immunogens elicited multiple V_H1-2/V_κ1-33 SARS-CoV-2 neutralizing antibodies. Of these, SP1-77 (also referred to herein as SPIKE-BC1-7-7) potently neutralized all known variants of concern, including Omicron. SP1-77 binds the spike protein receptor binding domain outside of ACE2 binding motif, via a different mode compared to well-characterized V_H1-2-based SARs-CoV-2 neutralizing antibodies—all of which are sensitive to E484K mutation present in Beta and Gamma variants. The novel binding and broad neutralization characteristics of SP1-77 can guide generation of therapeutic antibodies and vaccines against newly-arising VOCs. Likewise, the humanized mouse model described herein can provide a continuing source of potent humanized antibodies against SARS-CoV-2 and other pathogens.

[00275] The SPIKE-BC1-7-7 (SP1-77), VHH7-BC5-82 (VHH7-5-82), and VHH7-BC7-53 (VHH7-7-53) antibodies were screened for their ability to neutralize wild-type coronaviruses and variants of concern. Fig. 18. The neutralization experiments were performed in two different facilities.

[00276] The SPIKE-BC1-7-7 (SP1-77) is notable for being able to strongly neutralize the Omicron Covid19 variant and having potent activity against all tested variants of concern.

[00277] The IC_{50} for pseudovirus neutralization indicates that SPIKE-BC1-7-7 (SP1-77) is significantly more potent for Omicron neutralization than sotrovimab. Neither casirivcimab or imdevimab neutralize Omicron.

EXAMPLE 4

[00278] SARS-CoV-2 Omicron sub-variants have generated a world-wide health crisis due to resistance to most approved SARS-CoV-2 neutralizing antibodies and evasion of vaccination-induced antibodies. To manage Omicron sub-variants and prepare for potential new variants, additional means of isolating broad and potent humanized SARS-CoV-2-neutralizing antibodies are desirable. Described herein is a mouse model in which the primary B cell receptor (BCR) repertoire is generated solely through V(D)J recombination of a human V_{H1-2} heavy chain (HC) and, substantially, a human $V_{\kappa 1-33}$ light chain (LC). Thus, primary humanized BCR repertoire diversity in these mice derives from immensely diverse HC and LC antigen-contact complementarity-region-3 (CDR3) sequences generated by non-templated junctional modifications during V(D)J recombination. Immunizing the human $V_{H1-2}/V_{\kappa 1-33}$ -rearranging mouse model with SARS-CoV-2 (Wuhan-Hu-1) spike protein immunogens elicited several $V_{H1-2}/V_{\kappa 1-33}$ -based neutralizing antibodies that bound RBD in a different mode from each other and from those of many prior human patient-derived V_{H1-2} -based neutralizing antibodies. Of these, SP1-77 potently and broadly neutralized all SARS-CoV-2 variants through BA.5. Cryo-EM studies revealed that SP1-77 bound RBD away from the receptor-binding-motif via a CDR3-dominated recognition mode. Lattice-light-sheet-microscopy-based studies showed that SP1-77 did not block ACE2-mediated viral attachment or endocytosis, but rather blocked viral-host membrane fusion. The broad and potent SP1-77 neutralization activity and non-traditional mechanism of action indicate the therapeutic potential of this antibody. Likewise, the SP1-77 binding epitope can further inform on vaccine strategies. Finally, the general class of humanized mouse models we have described can contribute to identifying therapeutic antibodies against future SARS-CoV-2 variants and other pathogens.

[00279] INTRODUCTION

[00280] SARS-CoV-2 Omicron and its sub-variants, including the recently emergent BA.5 sub-variant, have spread worldwide and remain the cause of a public health crisis (1–3). Very recently, yet another Omicron sub-variant, BA.2.75 emerged in India and has begun to spread (4–6). Omicron-variants have an unprecedented number of mutations in their spike proteins, are resistant to most prior SARS-CoV-2-neutralizing antibodies, and evade antibodies induced by vaccinations (1–3, 7–12). The few published human monoclonal antibodies that broadly neutralize omicron sub-variants came from B cells of vaccinated individuals and human patients previously infected by SARS-CoV-2 ancestral strains before Omicron (1, 13–15). To manage Omicron sub-variants and prepare for potential future SARS-CoV-2 variants of concern (VOCs), new human or humanized antibodies that robustly

neutralize all SARS-CoV-2 VOCs are needed. In addition, a characterization of their mechanism of action will be essential.

[00281] Binding of the SARS-CoV-2 spike protein to its obligate angiotensin-converting enzyme 2 (ACE2) receptor on the target cell surface initiates infection (16). The spike protein is made up of non-covalently linked S1 and S2 subunits (17). The receptor-binding-domain (RBD) for ACE2 is located in the S1 subunit, while the S2 subunit anchors the spike protein in the viral membrane. The S2 subunit also contains other sequences that mediate viral/host cell membrane fusion for viral entry. The RBD can adopt two distinct conformations: “down” and “up”; the “down” state is shielded from ACE2 binding, while the “up” state is ACE2-accessible (18). Engagement of ACE2 by an “up” RBD exposes the S2’ cleavage site on the S2 subunit to either the serine 2 (TMPRSS2) transmembrane protease at the infected cell surface, or to cathepsin L in the endosomal compartment following endocytosis of the ACE2/SARS-CoV-2 complex (19). Cleavage of the S2 subunit by these proteases leads to dissociation of the S1 subunit, which exposes the fusion peptide on the S2 subunit and ultimately leads to fusion pore formation, viral-host membrane fusion, and viral entry into the infected cell (20).

[00282] Therapeutic SARS-CoV-2 neutralizing antibodies that block virus entry into host cells have demonstrated substantial efficacy for treating COVID-19 infections. The majority of such antibodies target the SARS-CoV-2 RBD and inhibit viral entry by binding to the ACE2 receptor binding motif (RBM), thereby, directly impeding its binding to the ACE2 receptor (7). Other such antibodies bind outside of the RBM, but sterically inhibit ACE2 binding (21). Some SARS-CoV-2 neutralizing antibodies bind outside of the RBM and do not inhibit ACE2 binding, but can still potently neutralize SARS-CoV-2 VOCs (7, 22–25). Several antibodies in this latter class destabilize the SARS-CoV-2 Spike trimer *in vitro*, which has been speculated to be their neutralization mechanism *in vivo* (24, 25). However, the neutralization mechanism of most antibodies in this class is unknown.

[00283] A vast portion of the primary antibody repertoire diversity lies in the antigen-contact CDR3-encoding variable region sequences generated *de novo* by non-templated V(D)J junctional modifications during V(D)J recombination (26). Indeed, the number of CDR3 sequences that can be generated through junctional diversification exceeds the number of B cells in mammalian immune systems by many orders of magnitude (27). Thus, the relative size of the primary BCR repertoire in humans and mice is determined by the number of primary B cells in their immune system (28). Immunoglobulin (Ig) gene-humanized mice capable of rearranging the full complement of human *Igh* and *IgL* variable region gene segments have yielded many therapeutic monoclonal antibodies, including antibodies that neutralize several SARS-CoV-2 VOCs (21, 29). However, humanized mouse models have not previously been described that yielded monoclonal antibodies that neutralize all of the currently dominant Omicron sub-variants. There could be many possible explanations for the lack of isolation of antibodies that more broadly neutralize SARS-CoV-2 VOCs from Immunoglobulin (Ig) gene-humanized mice. One of these possibilities is that mice express only a

tiny fraction of the CDR3-diversity present in human primary BCR repertoires, as a mouse has 1000-fold fewer B cells than a human. Early experiments indicated that BCR repertoires generated from rearrangement of a single V_H and V_K could make potent responses to diverse antigens due to immense CDR3 diversification (30). Described herein is a mouse model that generates more human-like CDR3 diversity in association with rearrangement of a single human V_H and V_K that can elicit potent SARS-CoV-2 neutralizing antibodies.

[00284] RESULTS

[00285] Single human V_H - and V_K -rearranging mouse model for human antibody discovery

[00286] For these studies, the “ V_H1-2/V_K1-33 -rearranging mouse model” that generates primary BCR repertoires via exclusive rearrangement of a single human V_H1-2 and predominant rearrangement of human V_K1-33 was generated, with the primary humanized BCR repertoire diversification based on immense CDR3 diversification (Fig. 19A). In this regard, V_H1-2 is frequently represented in SARS-CoV-2 neutralizing antibodies derived from human patients, but has not contributed to antibodies that broadly neutralize the different VOCs (31). For the *Igh* locus in this model, the proximal mouse V_H5-2 (also known as V_H81X , (32)) was replaced with human V_H1-2 on an allele in which the IGCR1 regulatory element in the V_H to D intervening region was deleted (33). Deletion of IGCR1 permits direct cohesin-mediated loop extrusion-based V(D)J recombination scanning to the proximal V_H from the J_H based V(D)J recombination center (34–36). Such scanning is impeded at the inserted V_H1-2 due to its association with the CTCF-binding element (CBE) downstream of the mouse V_H5-2 (33, 34). Impeded scanning by this linked CBE increases V_H1-2 accessibility to the V(D)J recombination apparatus and leads to its dominant usage (34). All functional mouse V_H s were further deleted from this allele to avoid any competition with the dominantly rearranged V_H1-2 (Fig. 19A and 26A-26B). For the *Igk* locus in this model, the proximal mouse V_K3-2 , that is expressed in 2.7% of splenic B cells, was replaced with human V_K1-33 , which resulted in its expression in 2.2% of splenic B cells (Fig. 19A-19D; 7A, 7B). Then, the IGCR1-related Cer/Sis element in the V_K - J_K interval was deleted; which, based on prior studies (37), was predicted to increase V_K1-33 utilization. Indeed, Cer/Sis deletion increased V_K1-33 utilization to 11% (Fig. 19A-19D; 6A, 6B), which it is contemplated herein also results from increasing direct V(D)J recombinational scanning to proximal V_K s from a J_K -based recombination center. The mouse J_H s and J_K s were retained in this model as their framework sequences outside of CDR3 were largely conserved between mouse and human (Fig. 26C). The mouse Ds were also retained, as mouse Ds are highly related to a subset of human Ds and, in any case, contribution of D segments to CDR3 are often unassignable due to extensive non-templated junctional diversification (26).

[00287] In the resulting V_H1-2/V_K1-33 -rearranging mouse model, human V_H1-2 and V_K1-33 contributed to the BCRs expressed by 100% and 11%, respectively, of splenic B cells (Figs. 19B-19D, 27A). Splenic B and T cell populations in this mouse model were similar in numbers and characteristics to those of wild-type as assessed by cytofluorimetry (Figs. 27B-27C). Moreover,

analysis of CDR3s in primary BCR repertoires of these mice revealed an immensely diverse V_H1-2-based CDR3 repertoire, as well as a diverse V_κ1-33 repertoire (Fig. 14A-14B, 19E-19F). Following immunization, it was predicted that the massive CDR3 diversity would contribute to selection of B cells with human V_H1-2- and V_κ1-33-based BCRs into germinal centers, where somatic hypermutations could further diversify variable region exons and contribute to affinity maturation.

[00288] Identification of potent SARS-CoV-2 neutralizing antibodies

[00289] The human V_H1-2/V_κ1-33-rearranging model was immunized with recombinant stabilized soluble SARS-CoV-2 Wuhan-Hu-1 spike trimer, or with an RBD monomer fused to a nanobody (“VHH7-RBD”) that targets MHC II-complex antigens (38) to potentially increase immunogenicity—given the poor immunogenicity of RBD monomer (39) (Fig. 20A). Immunizations were done twice, four weeks apart, with each immunogen plus poly I:C adjuvant (Fig. 20A). All mice developed strong antibody responses to the SARS-CoV-2 spike or RBD immunogens, as demonstrated by serum IgG titers at 2 weeks and 6 weeks post immunization (Fig. 20B). At 3 weeks after the second immunization, 96 antigen-specific IgG⁺ B cells were fluorescence-activated single-cell sorted from each mouse and their IgH (HC) and IgL (LC) variable region exons were sequenced (Figs. 28A-28B). These experiments identified 9 separate spike-specific B cell lineages, each of which expressed BCRs with unique sets of V_H1-2 and V_κ1-33-associated CDR3s (Fig. 20C). In each CDR3-based lineage, individual members had unique patterns of somatic hypermutations (Table 3). The V_H1-2-based HC CDR3s of these lineages were also distinct from CDR3s of previously reported V_H1-2-based anti-SARS-CoV-2 antibodies. To assess binding properties, we expressed one monoclonal antibody from each lineage with human IgG1 or IgG4 and human Ig_κ constant regions. ELISAs showed that all bound to the SARS-CoV-2 spike protein and 6 bound to the RBD, with one of the latter, termed SP1-77, derived from spike protein immunization (Fig. 28C).

[00290] SARS-CoV-2 pseudovirus neutralization assays revealed that 3 of the RBD-binding monoclonal antibodies potently neutralized the SARS-CoV-2 G614 virus including VHH7-5-82 (IC₅₀: 38 ng/ml), VHH7-7-53 (IC₅₀: 68 ng/ml), and SP1-77 (IC₅₀: 20 ng/ml) (Fig. 21A; Figs. 29A-29D). VHH7-5-82 also potently neutralized the Alpha (IC₅₀: 20 ng/ml) and Iota (K484E) (IC₅₀: 43 ng/ml) VOCs (Fig. 21A; Figs. 29A-29D). VHH7-7-53 potently neutralized Alpha (IC₅₀: 43 ng/ml), Epsilon (IC₅₀: 62 ng/ml), Iota (K484E) (IC₅₀: 43 ng/ml) and Delta (IC₅₀: 96 ng/ml) VOCs (Fig. 21A; Figs. 29A-29D). Notably, SP1-77 potently neutralized all currently known SARS-CoV-2 VOCs through BA.5, including robust neutralization of the recently emerged Omicron sub-variants, BA.1 (IC₅₀: 6.5 ng/ml), BA.2 (IC₅₀: 33 ng/ml), BA.3 (IC₅₀: 7 ng/ml), BA.4/BA.5 (IC₅₀: 16 ng/ml) and BA.2.12.1 (IC₅₀: 8 ng/ml) (Fig. 21A; Figs. 29A-29D). The IC₈₀ neutralization values of SP1-77 against these variants were equally compelling (Fig. 21A). Similar neutralization profiles for SP1-77 were found with an independent pseudovirus neutralization assay (Fig. 29E). Robust neutralization of all assayed variants (which did not include Omicron sub-variants) by SP1-77 also was seen in a plaque reduction neutralization test (PRNT) on live SARS-CoV-2 virus, with IC₅₀ values ranging from 0.8 to 9.6 ng/ml

(Fig. 21B and Fig. 29F). An SP1-77-derived antibody was also generated in which J_H and J_κ framework sequences outside of CDR3 were fully humanized and found that it retained similar robust neutralization activities against G614, Delta, and Omicron sub-variants as SP1-77 (Fig. 29G). Finally, the other four antibodies from the SP1 clonal lineage were expressed, each of which has unique pattern of somatic hypermutations compared to SP1-77. All had similar broad and potent neutralization activities (Fig. 29G). The mutations of each strain are shown below in Table 4

[00291] Table 4

Variant	Lineage	Substitutions (relative to Wuhan Hu-1 - deletions indicated by -)
D614G	B1	D614G
Alpha	B.1.1.7	H69-, V70-, Y144-, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
Beta	B.1.351	L18F, D80A, D215G, L241-, L242-, A243-, K417N, E484K, N501Y, D614G, A701V (L241-, L242-, A243- is equivalent to L242-, A243-, L244-)
Delta	B.1.617.2	T19R, G142D, E156-, F157-, R158G, L452R, T478K, D614G, P681R, D950N (E156-, F157-, R158G is equivalent to E156G, F157-, R158-)
Epsilon	B.1.429	S13I, W152C, L452R, D614G
Gamma	P.1	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
Iota	B.1.526	L5F, T95I, D253G, E484K, D614G, A701V
Iota_K484E	B.1.526_K484E	L5F, T95I, D253G, D614G, A701V
Omicron_BA.1	BA.1	A67V, H69-, V70-, T95I, G142D, V143-, Y144-, Y145-, N211-, L212I, in214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
Omicron_BA.2	BA.2	T19I, L24S, P25-, P26-, A27-, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron_BA.3	BA.3	A67V, H69-, V70-, T95I, G142D, V143-, Y144-, Y145-, N211-, L212I, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron_BA.4/BA.5	BA.4/BA.5	T19I, L24S, P25-, P26-, A27-, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
Omicron_BA.2.12.1	BA.2.12.1	T19I, L24S, P25-, P26-, A27-, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452Q, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K
Omicron_BA.2.75	BA.2.75	T19I, L24S, P25-, P26-, A27-, G142D, K147E, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K,

	S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K
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[00292] Surface plasmon resonance (SPR) confirmed interactions of the Fabs of the 3 RBD-binding neutralizing antibodies with SARS-CoV-2 spike protein (Fig. 30A). Spike-Fab complexes were further imaged using negative-stain electron microscopy (NSEM) to elucidate binding characteristics (Fig. 21C and Fig. 30B-30D). Three-dimensional NSEM reconstructions indicated that VHH7-5-82, VHH7-7-53, and SP1-77 all bound to both up and down RBDs (Fig. 21C and Fig. 30B-30D).

However, these reconstructions further indicated that these 3 antibodies had distinct binding footprints. Views from the top, front, inner, outer and back faces of the RBD showed that: VHH7-5-82 bound the top and outer faces of RBD, overlapping with the RBM (Fig. 31A); VHH7-7-53 bound the top face of the RBD, near the front, overlapping more widely with the RBM (Fig. 31B); and SP1-77 bound the back and outer faces, completely outside of the RBM (Fig. 31C). Indeed, a surface plasmon resonance competition assay showed that SP-77 did not compete with ACE2 for binding to the RBD or spike trimer (Figs. 31D-31F). According to the Bjorkman nomenclature (22), VHH7-5-82 and SP1-77 were Class 2 and Class 3 antibodies, respectively, while VHH7-7-53 was somewhat intermediate between Class 1 or Class 2 antibodies. According to the Veesler nomenclature (40), SP1-77 bound site IV, VHH7-7-53 bound site Ib, and VHH7-5-82 bound somewhat intermediate between sites Ib and IV. Notably, 9 well-characterized V_H1-2-based SARS-CoV-2 neutralizing antibodies isolated from SARS-CoV-2 Wuhan-Hu-1 or G614 virus-infected human patients all had very similar RBD binding footprints, which were distinct from those of VHH7-5-83, VHH7-7-53, and SP1-77 (21, 22, 31, 41-45) (Figs. 32A-32C).

[00293] cryo-EM structure of G614 S trimer in complex with the SP1-77 Fab

[00294] As SP1-77 neutralized SARS-CoV-2 with remarkable potency and breadth, we determined cryo-EM structures of the full-length G614 S trimer in complex with the SP1-77 Fab (46) (Figs. 33A-33C) 3D classification revealed two distinct conformations, including a three-RBD-down conformation and a one-RBD-up conformation, that both bound to three Fabs per S trimer (Fig. 22A) -- consistent with negative stain EM results (Fig. 30D). These structures were refined to 2.7Å and 2.9Å resolution, respectively (Figs. 33A-33C, 34A-34E, 35A-35C). To improve resolution near the RBD/SP1-77 interface, local refinement was performed, leading to a 3.2Å map that covered the Fab-RBD interface from the S trimer in the three RBD-down conformation. From the S trimer in the one-RBD-up conformation, maps were obtained for the Fab-RBD interfaces in the down conformation and in the up conformation at 3.3Å and 4.8Å resolution, respectively (Figs. 33A-33C, 34A-34E, 35A-35C). These structures confirmed that the SP1-77 epitope sit on the opposite side of RBD from the ACE2 binding site (Figs. 22B-22E), consistent with the negative stain EM data and the surface plasmon resonance competition data showing that SP1-77 did not impact ACE2 binding.

[00295] SP1-77 mainly made direct contacts with the RBD, but also touched the N-terminal domain (NTD) from a neighboring protomer (Figs. 22B-22C). The interaction buried an 939 Å² surface area on the antibody and an 936 Å² area from the spike protein. The HC and LC of SP1-77 contributed to 83% and 17% of the binding surface area, respectively. In particular, part of the 17-residue HC CDR3 wedged into a short groove formed by two segments in the RBD encompassing residues 343-346 and 441-444, respectively, while the tip of the 343-346 segment (mainly Thr345 and Arg346) projected into a ring-like structure formed by the HC CDR3 (Figs. 22B-22C). The intertwined contacts between the HC CDR3 and the RBD accounted for 78% of the binding surface area contributed by the HC. There appeared to be an extensive hydrogen bond network between Asn343, Thr345, Arg346, Asn440, Leu441 from the RBD and Val97, Leu98, Tyr99, Gly100, Arg100A, Ser100B from the HC CDR3. Notably, there was also a salt bridge between Arg346 of the RBD and Asp95 of the HC CDR3. Tyr99 of the HC CDR3 also packed against the fucose residue from the fucosylated Asn-343 glycan (47), probably forming a CH- π interaction, which buried a surface area of ~150 Å² (Figs. 22B-22C). In addition, there were contacts between the 441-444 segment (Lys444 and Val445) and Tyr32 of the LC CDR1 and Asp92 of the LC CDR3 (Figs. 22B-22C). Finally, the SP1-77 HC CDR2 and FW3 made additional contacts with the Asn122 and Asn165 glycans on the NTD, probably by forming hydrogen bonds or water-mediated hydrogen bonds (Figs. 22B-22C). It was noted that these two glycosylation sites were highly conserved among different VOCs (Figs. 36A-36D). Overall, the Cryo-EM structure revealed that, compared to other SARS-CoV-2 neutralizing antibodies, SP1-77 bound a different RBD epitope outside of the ACE2 binding motif via an interaction substantially mediated by the HC CDR3.

[00296] The binding epitopes of SP1-77 were compared with those of the LY-CoV1404 (Bebtelovimab) and S309 neutralizing antibodies. The RBD epitope targeted by SP1-77 overlapped very modestly with that of the LY-CoV1404, which is currently approved for therapeutic use (14) (Figs. 22D-22E). The RBD-binding site of LY-CoV1404 included the 440-445 segment of the RBD that was also bound by SP1-77 (Figs. 22D-22E). Like SP1-77, LY-CoV1404 potently neutralizes all currently known VOCs (14). However, the bulk of the LY-CoV1404 footprint overlaps with the RBM, which mechanistically allows LY-CoV1404 to achieve neutralization by blocking ACE2 binding. S309 and its Sotrovimab derivative, previously used therapeutically, bind outside of the RBM (23). However, S309 is quite distinct from SP1-77 in that it fails to neutralize Omicron BA.2 *in vitro* (48); although it does prevent BA.2 infection *in vivo* through utilization of Fc effector function interactions (49). In this regard, the S309 footprint covered both the 339-346 and 440-451 segments that were also in the SP1-77 footprint, with the long axis of the S309 footprint aligning along the exposed ridge of the RBD in the closed S trimer (Figs. 22D-22E). Notably, however, the same axis of the SP1-77 footprint was almost perpendicular to that of S309, which made it possible for SP1-77 to reach the NTD (Figs. 22B-22C).

[00297] Comparison of SP1-77 footprint to the mutations on the RBD of variants.

[00298] When the SP1-77 footprint was projected onto modeled RBDs from different SARS-CoV-2 variants (Figs. 23A-23C), it was evident that most mutations were located outside of the SP1-77 binding site, except for the Arg346Lys in the Mu and Omicron-BA.1.1 variants, and the Gly339Asp and Asn440Lys in all current Omicron sub-variants (50). The modeled interfaces of SP1-77 bound to the Mu and Omicron RBDs, based on the SP1-77/G614 S complex, indicated that the conservative Arg346Lys mutation would preserve the interaction with HC CDR3 and would not have much impact on binding. Likewise, the Gly339Asp and Asn440Lys mutations were at the edge of the SP1-77 epitope with their side chains pointing away from the binding interface, explaining why Omicron variants were sensitive to SP1-77 neutralization (Fig. 23D). While mutations near the Asn343-glycan in Omicron reconfigure the orientation such that the carbohydrate projects away from the protein surface (51), such mutations had minimal impact on binding and neutralization activity of SP1-77 (Fig. 23D). Thus, these studies provided a structure-driven hypothesis for why SP1-77 potently and broadly neutralized all tested SARS-CoV-2 variants through Omicron BA.5. Finally, the SP1-77 footprint was compared with the new mutations on the recently emerged BA.2.75 sub-variant. The N460K mutation in BA.2.75 was far from the SP1-77 footprint (Figs. 23A-23C); while the BA.2.75 G339H and G446S mutations were close to but did not overlap with SP1-77 footprint as described for BA.1. These findings indicate that SP1-77 also will bind and robustly neutralize BA.2.75.

[00299] SP1-77 neutralized SAR2-CoV-2 by inhibiting viral-host membrane fusion.

[00300] To address the SP1-77 neutralization mechanism, a lattice light sheet microscopy (LLSM) approach was applied to monitor target cell infection by a chimeric vesicular stomatitis virus in which the coat glycoprotein was replaced by the SARS-CoV-2 (Wuhan strain) S protein (VSV-SARS-CoV-2) (Figs. 24A-24J and 37A-37C) (52). This chimeric virus was surface labeled with Atto565-NHS-ester (VSV-SARS-CoV-2-Atto 565) (Figs. 37A-37B) and encoded a soluble eGFP that was expressed by infected cells. ACE2-expressing Vero cells that ectopically expressed TMPRSS2 protease (Vero TMPRSS2) were used as host cells. LLSM imaging revealed Atto 565-labeled viruses on cell surfaces at the earliest time points (Figs. 24A-24D, 24I). Single stepwise decreases in fluorescence intensity, corresponding to 25-30% of the total Atto 565 fluorescence, were observed for cell surface virus (Figs. 24B-24D, 24I, t2 to t3). As these stepwise decreases were blocked by pharmacological inhibition of TMPRSS2 (Camostat Mesylate) (Fig. 24J, middle panel), they likely resulted from TMPRSS2 protease activity and, correspondingly, reflected S1 dissociation after S2' cleavage. In these experiments, viral-host membrane fusion was not observed on the cell surface as these experiments were done with culture medium at neutral pH, while membrane fusion requires acidic pH (52). A subset of labeled chimeric viruses were internalized via endocytosis within a recorded 10-min time series (Fig. 24A-24D, 24I), followed by fluorescence-spreading, indicative of membrane fusion within the endosome (Figs. 24B-24D, 24I, t5 to t6; the method of single virus tracking and image analysis).

[00301] VSV-SARS-CoV-2-Atto 565 chimeric virus infectivity of Vero TMRSS2 cells was potentially neutralized by its pre-incubation with SP1-77 IgG ($IC_{50} \sim 15$ ng/ml) (Fig. 37C). Pre-incubation of VSV-SARS-CoV-2 Atto 565 with 500 ng/ml of SP1-77 did not appreciably impact its binding to the Vero TMRSS2 cell surface (Figs. 24E-24I). This finding was consistent with the SP1-77 footprint lying outside of the RBM and the inability of SP1-77 to block ACE2 engagement (Figs. 22C, 31C-31F). In addition, pre-incubation with SP1-77 greatly diminished the number of Atto 565 fluorescence intensity step decreases of chimeric virus at the cell surface (Figs. 24F-24J, t_2 to t_3). This result indicated that SP1-77 may either inhibit S2' cleavage or inhibit S1 dissociation (or inhibit both). While pre-incubation of viruses with SP1-77 had modest impact on virus endocytosis (Figs. 24F-24I), it dramatically inhibited fluorescence-spreading within the endosome--indicative of a viral-endosomal membrane fusion block (Figs. 24F-24J). Without wishing to be limited by theory, together, these findings indicated that SP1-77 neutralized the SARS-CoV-2 by blocking membrane fusion (Figs. 24I-24J). For comparison, LLSM single virion visualization revealed that pre-incubation with 500 ng/ml of the VHH7-5-82 antibody fully blocked binding of the chimeric virus to the cell surface (Fig. 24J), consistent with our findings that the VHH7-5-82 footprint overlapped with the RBM (Fig. 31A).

[00302] SP1-77 inhibited S1 dissociation

[00303] To distinguish between the possible effect of SP1-77 on cleavage of S2' versus dissociation of S1, these two processes were reconstructed *in vitro*. Activation of VSV-SARS-CoV-2-Atto 565 by brief treatment with trypsin for 30 minutes followed by the addition of the trypsin inhibitor aprotinin bypassed the requirement for host cell proteases TMPRSS2 and cathepsin L for infection--implicating that trypsin treatment cleaved the S2' site (52, 53). Incubation of the trypsin-activated virus with recombinant ACE2 induced a signal decrease of ~75% of the total Atto565 fluorescence, as quantified by single particle imaging; while cleavage by trypsin alone or incubation with ACE2 alone did not induce this signal decrease (Figs. 25A-25B). Assuming that an ~75% signal decrease *in vitro* reflected complete S1 dissociation from VSV-SARS-CoV-2 virus, then the corresponding 25-30 % signal decrease *in vivo* likely reflected S1 dissociation for a subset of spike proteins when the virus was attached to the cell surface (Figs. 24B-24D, t_2 to t_3). In contrast, the decrease in signal no longer occurred when trypsin-activated VSV-SARS-CoV-2-Atto 565 was incubated with SP1-77 and then exposed to ACE2 (Figs. 25A-25B). This finding indicated that SP1-77 inhibited ACE2-induced dissociation of S1 from the pre-cleaved S1/S2 complex. Finally, the SP1-77 Fab also inhibited S1 dissociation in this assay and, correspondingly, robustly neutralized VSV-SARS-CoV-2 virus (Fig. 25B and 37C). Together, these findings indicated that SP1-77 can achieve neutralization activity by blocking S1 dissociation via a monovalent interaction (Fig. 25C).

[00304] DISCUSSION

[00305] Described herein is the engineering of a human V_H1-2/V_K1-33 -rearranging mouse line that produced immunodiverse BCR repertoires through V(D)J recombination of these single human V_H and V_K sequences in association with diverse CDR3 sequences generated by V(D)J junctional

diversification mechanisms. Immunization of this mouse line with SARS-CoV-2 (Wuhan-Hu-1) spike protein immunogens elicited several humanized V_H1-2/V_κ1-33-based SARS-CoV-2-neutralizing antibodies that bound RBD in a different mode from each other and from those of many prior human patient-derived V_H1-2-based neutralizing antibodies. Of these three antibodies, two (VHH7-5-82 and VHH7-7-53), like many other previously described SARS-CoV-2 neutralizing antibodies, bound an epitope overlapping with the RBM. In this regard, the LLSM-based mechanism of action study directly demonstrated that VHH7-5-82 neutralized SARS-CoV-2 by blocking its binding to the host cell. The third antibody, SP1-77, bound RBD on the opposite side from the RBM. SP1-77 showed much broader neutralizing activity than the other two, and potently neutralized all major SARS-CoV-2 variants through the recently emergent BA.5 variant. Cryo-EM studies revealed that SP1-77 binding epitope on the SARS-CoV-2 RBD was different from those of previously characterized neutralizing antibodies. However, future mutagenesis studies are needed to more precisely define residues that are critical for SP1-77 binding to the RBD. The LLSM assay further revealed that SP1-77 did not inhibit SARS-CoV-2 binding to the host cell or its endocytosis. Instead, SP1-77 inhibited a downstream step in the SARS-CoV-2 infection process that involved fusion of the virus membrane with that of the host cell.

[00306] The SP1-77 neutralization mechanism described herein provided further insight into how a non-ACE2 blocking antibody can potently neutralize SARS-CoV-2. Besides the S309 neutralizing antibody mentioned above, several other Omicron sub-variant neutralizing antibodies that have binding epitopes outside of the RBM have been reported (1, 54). However, the mechanisms by which these antibodies neutralize SARS-CoV-2 remain unknown. The LLSM-based approach we employed to elucidate the SP1-77 mechanism should be readily applicable for mechanistic studies of other neutralizing antibodies that do not function via an ACE2 blocking mechanism. In the current study, the broad and potent activity of SP1-77 in neutralization of SARS-CoV-2 has only been demonstrated via pseudovirus assays. It is contemplated that the broad and potent SP1-77 neutralization activity against SARS-CoV-2 variants is maintained *in vivo*, making it a therapeutic against current and newly-arising VOCs. Also, SP1-77 can be useful in a cocktail with other neutralizing antibodies, such as LY-CoV1404, that potently neutralizes all tested VOCs through an ACE2-blocking mechanism. In addition, the non-traditional neutralization mechanism of SP1-77 may inspire the design of new vaccine strategies to more broadly target BA.5 and other VOCs.

[00307] The use of a solely human V_H1-2-rearranging mouse to obtain SP1-77 posed a rigorous test for this for the ability of such a mouse model to provide potent SARS-CoV-2-neutralizing humanized antibodies. As described above, full characterized V_H1-2-based SARS-CoV-2 neutralizing antibodies isolated from Wuhan-Hu-1 or G614 SARS-CoV-2 virus-infected human patients bind a similar RBD epitope (21, 22, 31, 41-45). None of these antibodies neutralize Beta, Gamma or Omicron variants, due to their sensitivity to the E484K/A mutation in these variants (21, 22, 31, 41-45). Indeed, the striking binding and neutralization similarities of all patient-derived V_H1-2-based neutralizing

antibodies characterized in depth suggests that most V_H1-2-based SARS-CoV-2-neutralizing antibodies in infected humans are selected by a common prototype neutralization pathway mediated by predisposition of the germline-encoded V_H1-2 variable region to bind to the RBD (31). Notably, the footprints of the present 3 V_H1-2-based SARS-CoV-2 neutralizing antibodies obtained from the initial immunization of the human V_H1-2/V_κ1-33-rearranging mouse were distinct from those of V_H1-2 based SARS-CoV-2 neutralizing antibodies isolated from human patients. This finding highlights the ability of V_H1-2/V_κ1-33-rearranging type of mouse model to permit isolation of potent human V_H1-2-based SARS-CoV-2 neutralizing antibodies with different binding characteristics relative to those, thus far, derived from patients.

[00308] The V_H1-2/V_κ1-33-rearranging mouse model has served as a very successful prototype for documenting the potential utility of this humanized antibody-discovery approach. In this regard, this initial study provided the VHH7-7-53 humanized antibody that potently neutralized multiple SARS-CoV-2 variants including Delta and the humanized SP1-77 antibody that potently neutralized all SARS-CoV-2 variants tested, including all Omicron sub-variants through BA.5. In this regard, among the thousands of SARS-CoV-2 neutralizing antibodies isolated from hundreds of human patients and vaccinated individuals, only a few broadly and potently neutralize all SARS-CoV-2 variants (1, 13–15).

[00309] The present study demonstrates that the mouse model described herein can be used to identify humanized antibodies with potent activity against SARS-CoV-2 variants.

[00310] MATERIALS AND METHODS

[00311] Study Design. The main objective of this study was to develop a humanized mouse model that through appropriate immunizations could be used to generate antibodies that broadly and robustly neutralize SARS-CoV-2 VOCs. The specific hypothesis tested was that such mice could be generated by programming them to produce sufficiently diverse antibody precursor repertoires through diversification mechanisms associated with the rearrangement of just a single human antibody heavy and light chain variable region sequence out of the 100 s present in the human genome. Such mice were generated through standard gene targeted mutational *Igh* and *Igκ* locus modifications that exploited the discovery of genetic elements that could be modified to enhance the rearrangement and diversification of single human variable region sequences. High throughput antibody repertoire analyses were employed to document production in these mice of highly diverse antibody repertoires from single human antibody variable region sequences via mechanisms that somatically diversify V(D)J rearrangements. The mice were then immunized with SARS-CoV-2 spike protein constructs and their antibody responses analyzed. It was found that the mice indeed could make humanized antibodies that potently neutralized SARS-CoV-2. We focused on characterizing one of these antibodies that broadly and potently neutralized all major SARS-CoV-2 variants at least through the recently emergent BA.5 variant. Cryo-EM studies were performed that revealed that this antibody binds to an overall epitope on the SARS-CoV-2 Spike protein receptor binding domain that was

different than any found to date. A lattice-light-sheet-microscopy-based assay was used to reveal that this antibody inhibits a downstream step in the SARS-CoV-2 infection process that involves fusion of the virus membrane with that of the host cell.

[00312] V_H1-2/V_κ1-33-rearranging mouse model and embryonic stem cells. All the genetic modifications were introduced into previously generated V_H1-2^{IGCR1Δ} ES cells (129/Sv and C57BL/6 F1 hybrid background) (33), using targeting strategies described previously (33, 55). In the *Igh* locus, the deletion of all mouse V_Hs was mediated by two guide RNAs that, respectively, targeted the upstream of the most distal mouse V_H1-86P and the upstream of the human V_H1-2 that replaced V_H5-2 in this modified ES cell line. In the *Igκ* locus, the mouse V_κ3-2 segment was replaced with human V_κ1-33 segment with an attached CTCF-binding element (CBE) inserted 50 bp downstream of human V_κ1-33 segment. The replacement was mediated by homologous recombination using a PGKneolox2DTA.2 (Addgene #13449) construct and two guide RNAs that targeted the mouse V_κ3-2 segment. The Cer/sis regulatory element in this ES cell line was deleted via the use of two guide RNAs. A human TdT gene was expressed in this mouse model. The human TdT cDNA was inserted into the first intron of mouse Rosa26 gene by homologous recombination using a CTV (Addgene #15912) construct.

[00313] The V_H1-2^{mVHA/IGCR1Δ}/V_κ1-33^{CSA}-rearranging mouse was generated by blastocyst injection of the ES cells described above and several rounds of breeding to get germline transmission and homozygous mice. All mouse experiments were performed under protocol 20-08-4242R approved by the Institutional Animal Care and Use Committee of Boston Children's Hospital.

[00314] Splenic B Cell Purification and HTGTS-Repertoire-seq Analysis. Splenic B cells were purified from 5-8 week old mice by MACS Microbeads according to the manufacturer's protocol. 10 ug of genomic DNA from purified splenic B cells was used for generating HTGTS-repertoire-seq (HTGTS-rep-seq) libraries as previously described (56). Four bait primers that target mouse J_H1, J_H2, J_H3 and J_H4 were mixed to capture the *Igh* heavy chain repertoire in one library. Likewise, four 4 bait primers that target mouse J_κ1, J_κ2, J_κ4 and J_κ5 were mixed to capture the *Igκ* light chain repertoire in one library. The sequences of mouse J_H and J_κ primers were as same as the previously reported (56). These HTGTS-rep-seq libraries were sequenced by Illumina NextSeq 2 x 150-bp paired end kit and analyzed with the HTGTS-Rep-seq pipeline (57).

[00315] Immunogens and Immunizations. HexaPro Spike immunogen was produced and purified as previously described (58). HexaPro Spike was stabilized by the introduction of six prolines in the S2 region and contained an HRV 3C-cleavable C-terminal twinStrepTagII-8 × His tag to facilitate purification (PMID: 32703906). The VHH7-RBD immunogen was made as previously described (38). The VHH7-RBD immunogen is a recombinant protein that consists of a fusion between VHH7 nanobody which targets class II MHC antigens and the SARS-CoV-2 RBD (Wuhan Hu-1 strain). For each immunization, 200 ul of immunogen mix, containing 25 ug of filter-sterilized protein

immunogen and 60 ug of Poly I:C in PBS, was injected into the peritoneum of each mouse. 8 weeks old mice were immunized twice with same immunogen, 4 weeks apart.

[00316] Antigen-specific B Cell Sorting and Single Cell RT-PCR. Mouse spleen samples from immunized mice were processed for single B cell sorting as previously described (56). Antigen-specific IgG⁺ B cells were selected for the phenotype B220⁺ (PE-Cy7: BD 552772), IgD⁻ (BV711: BioLegend 103255), IgG1⁺ (BV605: BD 563285), IgG2a⁺ (BV605: BD 564024), Spike⁺ (Alex647) or RBD⁺ (Alex647). SARS-CoV-2 spike and RBD were conjugated with Alex647 fluorescence (Thermo Fisher A30009). Single cell RT-PCR was performed as described previously (33). In brief, single antigen-specific IgG⁺ B cell was sorted into 96-well plate. Single cell cDNA was generated by reverse transcription (Thermo Fisher 18080093) using a primer mixture that specifically target C_u, C_γ1, C_γ2a and C_κ. V_H1-2 and V_κ1-33 V(D)J exons were amplified by two rounds of nested PCR and identified by sanger sequencing.

[00317] Monoclonal Antibody and Fab Production. The antibody expression constructs containing the heavy-chain and the light-chain variable region exons (Table 3), with human constant region sequences (IgG4 or IgG1, Ig κ) at the C terminus were made by Genscript. Monoclonal antibodies were generated using the Expi293 expression system (Thermo Fisher Scientific) and purified by high-performance liquid chromatography (HPLC) coupled with HiTrap Protein A HP columns (Cytiva). Fabs were made as previously described (59). Full-length antibodies at ~20 mg/ml concentrate were digested by papain-agarose resin. Then, the Fab fragments were collected by rProtein A Sepharose Fast Flow (Cytiva).

[00318] ELISA. ELISA was performed as previously described (60). In brief, 96 well plates (Thermo Fisher 446612) were coated with SARS-CoV-2 spike, RBD, NTD or SARS-CoV-1 spike at 100 ng/well. Sera were added to the plates in the dilution series from 1:200. After washing, antibodies retained on the plates were detected with AP anti-mouse IgG antibody (Southern Biotech, 1030-04).

[00319] Pseudovirus Neutralization Tests. The pseudovirus neutralization assay in 293 T/ACE2 cells was performed at Duke (61). The mutations on the spike protein of SARS-CoV-2 variants were listed in table S4. Spike-pseudovirus was incubated with 8 serial 5-fold dilutions of antibody in duplicate for 1 hr. at 37°C in 96-well culture plates. 293 T/ACE2-MF cells were detached from culture flasks, suspended in growth medium (GM), and immediately added to all wells. One set of 8 wells received cells + virus (virus control) and another set of 8 wells received cells only (background control). After 71-73 hrs of incubation, medium was removed and Promega 1X lysis buffer was added to all wells. After a 10-minute room temperature incubation, Bright-Glo luciferase reagent was added to all wells. After 1-2 minutes, the cell lysate was transferred to a black/white plate. Luminescence was measured using a GloMax Navigator luminometer (Promega). Neutralization titers were the inhibitory concentration (IC) of antibody at which relative luminescence units were reduced by 50% (IC₅₀) compared to virus control wells.

[00320] Plaque Reduction Neutralization Test (PRNT). SARS-CoV-2 Plaque Reduction Neutralization Test (PRNT) were performed in the Duke Regional Biocontainment Laboratory BSL3 (Durham, NC) as previously described with virus-specific modifications (62). Briefly, two-fold dilutions of antibody (the start concentration is 10 ug/ml) were incubated with 50 PFU SARS-CoV-2 virus (WA1, Alpha, Beta, Gamma and Delta) for 1 hour. The antibody/virus mixture was used to inoculate Vero E6 cells in a standard plaque assay (63, 64). Briefly, infected cultures were incubated at 37°C, 5% CO₂ for 1 hour. At the end of the incubation, 1 mL of a viscous overlay (1:1 2X DMEM and 1.2% methylcellulose) was added to each well. Plates were incubated for 4 days. After fixation, staining and washing, plates were dried and plaques from each dilution of each sample were counted. Data were reported as the concentration at which 50% of input virus was neutralized. A known neutralizing control antibody was included in each batch run (Clone D001; SINO, CAT# 40150-D001).

[00321] Antibody Affinity and Competition Assays by Surface Plasmon Resonance (SPR). SPR measurements of monoclonal Fabs binding to SARS-CoV-2 spike (S) proteins were performed using a Biacore S200 instrument (Cytiva) and as described previously (58). Antibody binding competition and blocking were measured by SPR using methods described previously (58).

[00322] NSEM Methods. Fab-spike complexes were negatively stained with 2 g/dL uranyl formate, imaged on a Philips EM420 electron microscope; and 3D reconstructions were calculated using standard protocols with Relion 3.0.

[00323] Cryo-EM single particle analysis. Cryo-EM single particle analysis was carried out following the detailed protocols published previously (65, 66). Briefly, to prepare cryo EM grids, the full-length G614 spike trimer (3.1 mg/ml) (65) and the SP1-77 Fab (7 mg/ml) were mixed at a molar ratio of 1:9 and the complex was also purified by gel filtration chromatography. The grids were first screened for ice thickness and particle distribution. Selected grids were used to acquire images by a Titan Krios transmission electron microscope (ThermoFisher Scientific) operated at 300 keV and equipped with a BioQuantum GIF/K3 direct electron detector. Automated data collection was carried out using SerialEM version 3.8.6 (67) with a defocus range of 0.5-2.2 μ m.

[00324] Cryo-EM images were processed in cryoSPARC v.3.3.1. (46) with the drift correction performed and contrast transfer function (CTF) estimated. Motion corrected sums with dose-weighting were used for subsequent image processing. Template-based particle picking was performed for all 28,899 recorded images with 7,657,522 particles extracted in total. The particles were subjected to multiple rounds of 2D and 3D classification, giving two major classes, one representing the three-RBD-down S trimer bound with three SP1-77 Fabs and another representing the one-RBD-up S trimer in complex with three SP1-77 Fabs. The two classes class were refined to 2.9 Å resolution from 250,319 particles and 3.1 Å resolution from 209,249 particles, respectively. To further improve the density for the Fab and RBD, different local refinements were performed with a soft mask covering a single RBD and the bound Fab were performed. All resolutions were reported

from the gold-standard Fourier shell correlation (FSC) using the 0.143 criterion and local resolution determined using cryoSPARC. For model building, we used our G614 S trimer structures (PDB ID: 7KRQ and PDB ID: 7KRR (66)) as the initial templates for the spike protein, and the S309 antibody structure (PDB ID: 6WS6 (23)) for SP1-77. Several rounds of manual building were performed in Coot (68), and iterative refinement was performed in both Phenix (69) and ISOLDE (70).

[00325] Generation of VSV-SARS-CoV-2 Atto 565. The generation of recombinant VSV chimeras expressing eGFP where the glycoprotein G was replaced with spike (S) protein Wuhan-Hu-1 strain (VSV-SARS-CoV-2) was previously described (71). VSV-SARS-CoV-2 at a concentration of ~150 µg/mL viral RNA was conjugated with Atto565-NHS ester (Sigma-Aldrich, cat.72464) (52). The labeled virus was first tested for its infection efficacy in Vero-TMPRSS2 cells as previously described (71). Then, 1-hour incubation of label virus with 100 ng/ml SP1-77 IgG or SP1-77 Fab before infection was performed to test the infection inhibition efficacy (neutralization activity) of SP1-77 IgG or SP1-77 Fab.

[00326] Lattice light sheet microscopy (LLSM). The single virus tracking LLSM experiments were performed as previous described (52). In brief, cells were plated onto 5 mm coverslips in a 35 mm culture dish at 60% confluence the day prior to each experiment. Approximately 10 µl of a solution containing 2.5 µg/mL viral RNA of VSV-SARS-CoV-2 Atto 565 (MOI ~2) without antibody (control), with 500 ng/ml SP1-77 or with VHH7-5-82 antibodies were incubated at 37°C for 1 hour and then added to the coverslips. The cells were imaged in phenol red free media (FluoBright) supplemented with 5% FBS and 25 mM HEPES, pH 7.4. The samples were volumetrically imaged as a time series using a dithered multi-Bessel lattice light sheet using a sample scan with 0.5 µm spacing between each plane every 4 seconds.

[00327] Single virus tracking and image analysis. The single virus tracking and image analysis was performed as previously described (52). The 3D stacks from the LLSM experiments were deskewed and the diffraction limited spots were detected and tracked using an automated detection algorithm that uses least-squares numerical fitting with a model of the microscope PSF approximated by a 3D Gaussian function and implemented in MATLAB previously developed (72). Estimated fluorescence intensities associated with each spot were calculated from the corresponding amplitudes of the fitted 3D Gaussian and compared to single virus bound to poly-D-lysine coated glass imaged under the same condition as the experiments. Tracks of virus were then exported into a custom-made program written in LabView for visualizing trajectories, which was available for download (github.com/VolkerKirchheim/TrackBrowser_Matlab.git). Each virus trajectory was visually examined for localization to the cell surface, internalization into the cell, and spreading of the Atto565 dye fluorescence. The virus is 80-100 nm, which made it a diffraction limited object. The spot limited by the wavelength of the dye being emitted was 300 nm. The endosome was 600 nm and larger. The fusion of the viral envelope to the endosomal membrane was observed by a spreading of the fluorescent signal from a point spread limited object to 500-1000 nm over the endosomal membrane.

[00328] S1 dissociation reconstitution. The S1 dissociation reconstitution was performed as previously described (52). In brief, VSV-SARS-CoV-2 Atto 565 was treated with 5 different conditions: trypsin only, ACE2 only, trypsin followed by ACE2, trypsin followed by SP1-77 IgG followed by ACE2 and trypsin followed by SP1-77 Fab followed by ACE2. The trypsin treatment was performed by incubation of VSV-SARS-CoV-2 Atto 565 with 1 µg/mL trypsin for 30 minutes at 37°C, and then the reaction was stopped by the addition of Aprotinin to a final concentration 10 µM. The ACE2 treatment was performed by incubation of VSV-SARS-CoV-2 Atto 565 with soluble ACE2 for 10 minutes at 37°C. The antibody treatment was performed by incubation of VSV-SARS-CoV-2 Atto 565 with 100 ng/ml IgG or Fab for 1 hour at 37°C. After treatment, virus was bound to poly-D-lysine-coated glass and single molecule Atto565 dye calibration was performed as previously described (52).

[00329] Statistics. Statistical analyses in this paper were performed in Prism (v.8, GraphPad Software). *p* values were calculated by unpaired, two-tail t-test.

[00330] SUPPLEMENTAL MATERIALS AND METHODS

[00331] **V_H1-2/V_κ1-33-rearranging mouse model and embryonic stem cells.** All the genetic modifications were introduced into previously generated V_H1-2^{IGCR1Δ} ES cells (129/Sv and C57BL/6 F1 hybrid background) (73), using targeting strategies described previously (73, 74). In the *Igh* locus, the deletion of all mouse V_Hs was mediated by two guide RNAs that, respectively, target sequences ~100 bp upstream of the most distal mouse V_H1-86P and ~4.3 kb upstream of the human V_H1-2 that replaced V_H5-2 in this modified ES cell line, respectively. In the *Igκ* locus, the mouse V_κ3-2 segment was replaced with human V_κ1-33 segment with an attached CTCF-binding element (CBE) (atccaggaccagcagggggcgcgagagcacaca) inserted 50 bp downstream of human V_κ1-33 segment. The V_κ1-33/CBE replacement of V_κ3-2 was mediated by homologous recombination using a PGKneolox2DTA.2 (Addgene #13449) construct and two guide RNAs that target the mouse V_κ3-2 segment. The Cer/sis regulatory element in this ES cell line was deleted via the use of two guide RNAs that target sequences ~60 bp upstream and ~1 kb downstream of Cer/sis. A human TdT gene was expressed in this mouse model. The human TdT cDNA was cloned into CTV (Addgene #15912) construct in which the TdT expression was driven by CAG promoter and followed by a EGFP expression that mediated by an internal ribosome entry site (IRES) (75). The TdT expression cassette was inserted into the first intron of mouse Rosa26 gene which is on the same chromosome 6 with *Igκ* locus by homologous recombination. The sequences of guide RNAs used for targeting are listed in table S9. The modifications on the chromosome 12 (the *Igh* loci) and chromosome 6 (the *Igκ* and Rosa26 loci) were made independently in two ES clones. Blastocyst injection of these two ES clones was performed, respectively, generating the V_H1-2^{mVHA/IGCR1Δ}-rearranging mouse model and the V_H1-2^{IGCR1Δ}/V_κ1-33^{CSA}-rearranging mouse model. The V_H1-2^{mVHA/IGCR1Δ}/V_κ1-33^{CSA}-rearranging F1 mice

were generated by cross-breeding of these two colonies. Mice were then generated that were homozygous for each modification and used these mice for all experiments.

[00332] All mouse experiments were performed under protocol 20-08-4242R approved by the Institutional Animal Care and Use Committee of Boston Children's Hospital. Mice were maintained on a 14-h light, 10-h dark schedule in a temperature-controlled environment, with food and water provided ad libitum. ES cells were grown on a monolayer of mitotically inactivated mouse embryonic fibroblasts (iMEF) in DMEM medium supplemented with 15% bovine serum, 20mM HEPES, 1x MEM nonessential amino acids, 2mM Glutamine, 100 units of Penicillin/Streptomycin, 100 mM β -mercaptoethanol, 500 units/ml Leukemia Inhibitory Factor (LIF).

[00333] To characterize B cells in the homozygous $V_H1-2^{mVH\Delta/IGCR1\Delta}/V_K1-33^{CSA}$ -rearranging mouse model, splenocytes were isolated from 5-8 week old mice and stained with the following antibodies: APC anti-B220 (eBioscience 17-0452-83); PE anti-Thy1.2 (eBioscience 12-0902-83); Bv605 anti-IgM (BioLegend 406523); Bv711 anti-IgD (BioLegend 405731).

[00334] Immunogens and Immunizations. HexaPro Spike immunogen was produced and purified as previously described (76-79). HexaPro Spike was stabilized by the introduction of six prolines in the S2 region and contained an HRV 3C-cleavable C-terminal twinStrepTagII-8xHis tag to facilitate purification (PMID: 32703906). DNA encoding SARS-CoV-2 HexaPro Spike was synthesized (Genscript) and transiently transfected in FreeStyle 293F cells (Thermo Fisher) using 293Fectin (ThermoFisher). On day 5, cell culture supernatants were harvested by centrifugation of the culture followed by filtering through a 0.8- μ m filter. Supernatant was concentrated down to 400 mL and Spike protein was purified by StrepTactin chromatography (IBA) using the manufacturer's buffers. Trimeric spike protein was further purified by Superose 6 size-exclusion chromatography (GE Healthcare) in 10 mM Tris pH 8, 500 mM NaCl. All purification steps were done at room temperature. Purified proteins were quantified and snap frozen in dry ice mixed with ethanol.

[00335] The VHH7-RBD immunogen was made as previously described (80). Briefly, VHH7-RBD DNA was synthesized (Integrated DNA Technologies) and assembled in the pVRC vector. Constructs were transfected into Expi293 cells (Thermo Fischer Scientific) using Polyethyleneimine "Max" (Polysciences). Cell cultures were maintained in Expi293 Media (Thermo Fischer Scientific) at 37°C for 4 day following transfection. Proteins were harvested by centrifugation at 5,000 $\times$ g for 30 min at 4°C, followed by affinity chromatography with HisPur Ni-NTA Resin (Thermo Fischer Scientific) and size exclusion chromatography with a Hi-Load 16/600 S75 column (Cytivia).

[00336] For each immunization, 200 μ l of immunogen mix, containing 25 μ g of filter-sterilized protein immunogen and 60 μ g of Poly I:C in PBS, was injected into the peritoneum of each mouse. 8 weeks old mice were immunized twice with same immunogen, 4 weeks apart. Blood samples were collected one day before the first immunization and two weeks after each immunization. Spleens were collected 3 weeks after the second immunization.

[00337] Pseudovirus Neutralization Tests. The pseudovirus neutralization assay in 293T/ACE2 cells performed at Duke has been described in detail (81). and is a formally validated adaptation of the assay utilized by the Vaccine Research Center; the Duke assay is FDA approved for D614G. For measurements of neutralization, pseudovirus was incubated with 8 serial 5-fold dilutions of antibody samples (1:20 starting dilution using antibodies diluted to 1.0 mg/ml or 0.5 mg/ml) in duplicate in a total volume of 150 μ l for 1 hr at 37°C in 96-well flat-bottom culture plates. 293T/ACE2-MF cells were detached from T75 culture flasks using TrypLE™ Select Enzyme solution, suspended in growth medium (100,000 cells/ml) and immediately added to all wells (10,000 cells in 100 μ L of growth medium per well). One set of 8 wells received cells + virus (virus control) and another set of 8 wells received cells only (background control). After 71-73 hrs of incubation, medium was removed by gentle aspiration and 30 μ l of Promega 1X lysis buffer was added to all wells. After a 10-minute incubation at room temperature, 100 μ l of Bright-Glo luciferase reagent was added to all wells. After 1-2 minutes, 110 μ l of the cell lysate was transferred to a black/white plate. Luminescence was measured using a GloMax™ Navigator luminometer (Promega). Neutralization titers are the inhibitory dilution (ID) of antibody at which RLUs were reduced by 50% (ID50) compared to virus control wells after subtraction of background RLUs.

[00338] The pseudovirus neutralization assays in 293T/ACE2/TMPRSS2 cells were performed as previously described (82-84). Briefly, human codon-optimized cDNA encoding SARS-CoV-2 spike glycoprotein of the WA-1/2020 and variants were synthesized by GenScript and cloned into eukaryotic cell expression vector pcDNA 3.1 between the *Bam*HI and *Xho*I sites. Pseudovirions were produced by co-transfection Lenti-X 293T cells with psPAX2(gag/pol), pTrip-luc lentiviral vector and pcDNA 3.1 SARS-CoV-2-spike-deltaC19, using Lipofectamine 3000™. The supernatants were harvested at 48h post transfection and filtered through 0.45 μ m membranes and titrated using 293T/ACE2/TMPRSS2 cells (HEK 293T cells that express ACE2 and TMPRSS2 proteins). For the neutralization assay, 50 μ L of SARS-CoV-2 S pseudovirions (counting ~200,000 relative light units) were pre-incubated with an equal volume of medium containing serial dilutions of mAbs at room temperature for 1h. Then 50 μ L of virus-antibody mixtures were added to 293T-ACE2-TMPRSS2 cells (10^4 cells/50 μ L) in a 96-well plate. The input virus with all SARS-CoV-2 strains used in the current study were the same (2×10^5 relative light units/50 μ L/well). After a 3 h incubation, fresh medium was added to the wells. Cells were lysed 24 h later, and luciferase activity was measured using One-Glo™ luciferase assay system (Promega, Cat# E6130). The assay of each serum was performed in duplicate, and the 50% neutralization titer was calculated using Prism 9™ (GraphPad Software). Controls included cells only, virus without any antibody and positive sera.

[00339] Antibody Affinity and Competition Assays by Surface Plasmon Resonance (SPR). SPR screening and affinity measurements of monoclonal Fabs binding to SARS-CoV-2 spike proteins were performed using a Biacore S200™ instrument (Cytiva, formerly GE Healthcare) in HBS-EP+ 1X running buffer and as described previously (76). The SARS-CoV-2 spike (S) proteins with a Strep

tag were first captured onto a Streptavidin sensor chip to a level of 200–400 RU. For screenings, the Fabs were injected over the captured S proteins using the high performance injection mode at a flow rate of 30 μ L/min. Association phase was maintained at 180s injections of each Fab followed by a dissociation of 720s. After each dissociation phase, the S proteins and bound Fabs were removed from the sensor surface using a 30s regeneration pulse of Glycine pH1.5. For affinity measurement, the Fabs were injected over the captured S proteins using single cycle kinetics with the high performance injection mode at a flow rate of 50 μ L/min. Association phase was maintained at 120s injections of each Fab at increasing concentrations followed by a dissociation of 900s. After each dissociation phase, the CoV-S proteins and bound Fabs were removed from the sensor surface using a 30s regeneration pulse of Glycine pH1.5. Results were analyzed using the Biacore S200™ Evaluation software (Cytiva). A blank streptavidin surface along with blank buffer binding were used for double reference subtraction to account for non-specific protein binding and signal drift. Subsequent curve fitting analyses were performed using a 1:1 Langmuir model with a local Rmax for the Fabs or using the heterogeneous ligand model with local Rmax.

[00340] Antibody binding competition and blocking were measured by SPR following immobilization by amine coupling of monoclonal antibodies to CM5 and CM3 sensor chips (BIAcore/Cytiva) (76). Antibody blocking assays were performed by sequential high performance injections of Spike protein (20 μ M) over mAb immobilized surfaces for 3 minutes at 30 μ L/min immediately followed by a test Ab (200 μ M) for 3 minutes at 30 μ L/min. The dissociation of the antibody sandwich complex with the spike protein was monitored for 10 minutes with buffer flow and then a 24 second injection of Glycine pH2.0 for regeneration. Double reference subtraction was used to account for signal drift. Data analyses were performed with BIA-evaluation™ 4.1 software (BIAcore/Cytiva). The reported competition experiments are representative of two data sets.

[00341] NSEM Methods. To form the Fab-spike complex, Fab was mixed with spike at a 9:1 molar ratio and incubated at 37 °C for 1 h. The complex was then cross-linked for 5 min by diluting to 200 μ g/ml spike concentration with 8 mM glutaraldehyde in HBS buffer (20 mM HEPES, 150 mM NaCl, 5 g/dL glycerol, pH 7.4), and then quenched for 5 min by addition of sufficient 1 M Tris stock to give 80 mM final Tris concentration. For negative stain, a portion of the sample was diluted in HBS buffer to 100 or 50 μ g/ml and 5 μ l applied to a glow-discharged carbon-coated EM grid for 10–12 second, then blotted, and stained with 2 g/dL uranyl formate for 1 min, and then blotted and air-dried. Grids were examined on a Philips EM420™ electron microscope operating at 120 kV and nominal magnification of 49,000x, and ~100 images were collected on a 76 Mpix CCD camera at 2.4 Å/pixel. Images were analyzed by 2D class averages and 3D reconstructions calculated using standard protocols with Relion™ 3.0 (85).

[00342] Cryo-EM single particle analysis. Cryo-EM images were acquired on a Titan Krios™ electron microscope equipped with a Gatan™ K3 direct electron detector. Cryo-SPARC (86) was used for particle picking, two-dimensional (2D) classification, three dimensional (3D)

classification and refinement. To prepare cryo EM grids, the full-length G614 spike trimer (3.1 mg/ml)(87) and the SP1-77 Fab (7 mg/ml) were mixed at a molar ratio of 1:9 and incubated at room temperature for one hour. The complex was purified by gel filtration chromatography on a Superose 6 10/300 column (GE Healthcare, Chicago, IL) in a buffer containing 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.02% DDM. Both the mixed complex and purified complex were used for cryo grid preparation. 4.0 μ l of the complex was applied to a 1.2/1.3 Quantifoil™ gold grid (Quantifoil Micro Tools GmbH), which had been glow discharged with a PELCO easiGlow™ Glow Discharge Cleaning system (Ted Pella, Inc.) for 60 s at 15 mA. Grids were immediately plunge-frozen in liquid ethane using a Vitrobot™ Mark IV (ThermoFisher Scientific), and excess protein was blotted away by using grade 595 filter paper (Ted Pella, Inc.) with a blotting time of 4 s, a blotting force of -12 at 4°C with 100% humidity. The grids were first screened for ice thickness and particle distribution. Selected grids were used to acquire images by a Titan Krios™ transmission electron microscope (ThermoFisher Scientific) operated at 300 keV and equipped with a BioQuantum™ GIF/K3 direct electron detector. Automated data collection was carried out using SerialEM version 3.8.6 (88) at a nominal magnification of 105,000 $\times$ and the K3 detector in counting mode (calibrated pixel size, 0.83 Å) at an exposure rate of 13.761 electrons per pixel per second. Each movie add a total accumulated electron exposure of $\sim$ 53.853 e-/Å², fractionated in 50 frames. Data sets were acquired using a defocus range of 0.5-2.2 μ m.

[00343] Cryo-EM images were first processed in cryoSPARC™ v.3.3.1.(86) with the drift correction performed using the patch mode, and contrast transfer function (CTF) estimated by the patch mode as well. Motion corrected sums with dose-weighting were used for subsequent image processing. Templates for particle-picking were generated from a small number of manually picked particles and the template-based particle picking was then performed for all 28,899 recorded images with 7,657,522 particles extracted in total (box size 672Å, downsizing to 128Å). The particles were subjected to four rounds of 2D classification in cryoSPARC, giving 3,894,421 good particles. A low-resolution negative-stain reconstruction of the Wuhan-Hu-1 (D614) sample was low-pass filtered to 40Å resolution and used as an initial model for 3D classification (89). The selected good particles were subjected to two rounds of heterogeneous 3D classification with six copies of the initial model as references in C1 symmetry. One major class (12.8%) with clear structural features was re-extracted to a smaller boxsize (480Å) and subjected to another two rounds of heterogeneous refinement with four copies of the initial model as references in C1 symmetry. There were two major classes, one representing the three-RBD-down S trimer bound with three SP1-77 Fabs and another representing the one-RBD-up S trimer in complex with three SP1-77 Fabs. The first class was subjected to one round of non-uniform refinement in C3 symmetry, giving a map at 2.9Å resolution from 250,319 particles. The second class was refined in C1 symmetry instead, giving a map at 3.1Å resolution from 209,249 particles. To further improve the overall resolution, both classes were subjected to local CTF refinement, resulting in a 2.7Å

map for the complex with the S trimer in the three-RBD-down conformation, and a 2.9Å map for a 2.7Å map for the complex with the S trimer in the one-RBD-up conformation. To improve the density for the Fab and RBD, several different local refinements were performed with a soft mask covering a single RBD and the bound Fab. From the three-RBD-down complex, the local refinement gave a map at 3.2Å resolution; from the one-RBD-up complex, the local refinements gave a 3.3Å map for the Fab bound to the RBD in the down conformation, and a 4.8Å map for the Fab bound to the RBD in the up conformation. The best density maps were used for model building.

[00344] All resolutions were reported from the gold-standard Fourier shell correlation (FSC) using the 0.143 criterion. Density maps were corrected from the modulation transfer function of the K3 detector and sharpened by applying a temperature factor that was estimated using Sharpening Tools in cryoSPARC™. Local resolution was also determined using cryoSPARC.

[00345] For model building, G614 S trimer structures were used (PDB ID: 7KRQ and PDB ID: 7KRR (90)) as the initial templates for the spike protein, and the S309 antibody structure (PDB ID: 6WS6 (91)) for SP1-77 antibody. The SP1-77 Fab structure was predicted by AlphaFold (92), largely confirming our model building and the predicted structure also helped further improving some details of the final model. Several rounds of manual building were performed in Coot (93). The models were refined in Phenix™ (94) against the 2.7Å (three-RBD-down) and 2.9Å (one-RBD-up) maps, respectively, as well as the 3.2Å map from the masked local refinement using the model containing a single RBD and the SP1-77 Fab. Iteratively, refinement was performed in both Phenix™ (real space refinement) and ISOLDE™ (95), and the Phenix refinement strategy included minimization_global, local_grid_search, and adp, with rotamer, Ramachandran, and reference-model restraints, using 7KRQ and 7KRR as the reference model. Structural biology applications used in this project were compiled and configured by SBGrid™ (96).

[00346] Generation of VSV-SARS-CoV-2 Atto 565. The generation of recombinant VSV chimeras expressing eGFP where the glycoprotein G was replaced with spike (S) protein Wuhan-Hu-1 strain (VSV-SARS-CoV-2) was previously described (97). VSV-SARS-CoV-2 chimera were obtained by infection of MA104 cells. Cells were grown in 15 to 20 150-mm dishes and infected at a multiplicity of infection (MOI) of 0.01. The supernatant containing the virus was collected 72 hours post infection and clarified by centrifugation at 1,000 x g for 10 min at 4°C. A pellet with virus and extracellular particles was obtained by centrifugation in a Ti45 fixed-angle rotor at 72,000 x g (25,000 rpm) for 2 hours at 4°C, then resuspended overnight in 0.5 mL PBS at 4°C. This solution was layered on top of then pelleted through 15% sucrose-PBS solution by centrifugation in a SW55 swinging-bucket rotor at 148,000 x g (35,000 rpm) for 2 hours at 4°C. The pellet was then resuspended overnight in 0.4 mL PBS at 4°C, layered on top of a 15 to 45% sucrose-PBS linear gradient and subjected to centrifugation in a SW55 swinging-bucket rotor at 194,000 x g (40,000 rpm) for 1.5 hours at 4°C. The predominant light scattering band located in

the lower one-third of the gradient and containing the virions was removed by side puncture of the gradient tube. Approximately 0.3 ml of this solution was mixed with 25 ml of PBS and subjected to centrifugation in a Ti60 fixed-angle rotor at 161,000 x g (40,000 rpm) for 2 hours at 4°C. The final pellet was resuspended overnight in 0.2 - 0.5 mL PBS aliquots and stored at -80°C.

[00347] Stock concentration of VSV-SARS-CoV-2 at a concentration of ~150 µg/mL viral RNA was conjugated with Atto565-NHS ester (Sigma-Aldrich, cat.72464) as previously described (98). Virus stocks in 200 µL were adjusted to 0.1 M NaHCO₃ (pH 8.3). Atto565 NHS esters were resuspended in anhydrous DMSO (Sigma) and then aliquoted to and dried down into 0.25 µg/mL stocks under house vacuum and then stored at -20°C. Virus stock was added to dried Atto565 NHS ester and incubated at room temperature for 1 hour in the dark. After 1 hour, Tris pH 8.0 to a final concentration of 200 mM was added to quench the reaction. Free label was then separated from labeled virus using a 0.5 mL Piece Zeba spin desalting column with a 40 kDa molecular weight cut off. Labeled virus was stored at 4°C and used within 1 week of labeling.

[00348] Infection assays were done with cells plated one day before the experiment and used for the infection assays with a final density of ~ 80% confluency as previously described (97). VSV - SARS-CoV-2-Atto 565 was incubated with the indicated concentration of antibody for 1 hour at 37°C. Virus was then added to the cells with indicated concentration of antibody for 1 hour, followed by 3 washes with cell culture media (DMEM, 10% FBS, 25 mM HEPES, pH 7.4), and then incubation for 7 hours at 37°C and 10% CO₂ in DMEM containing 25 mM HEPES, pH 7.4. At the end of the 7-hr incubation, cells were treated for 30 sec with 5 µg/mL WGA-Alexa647 in PBS to define the cell outline, then fixed with 4% PFA in PBS for 15 minutes at room temperature and imaged within 24 hours using a spinning-disk confocal microscope equipped with a 40x oil NA=1.4 objective (1 pixel = 0.33 µm) to acquire random fields, each containing a Z-stack of 20 consecutive optical planes taken 1 µm apart. Cells were scored as infected if the cytosolic eGFP fluorescence was 1.4 times that of the background of uninfected cells from the control experiment without virus.

[00349] REFERENCES

1. Y. Cao, et al, BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature*, (2022).
2. Q. Wang, et al, Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, & BA.5. *Nature*, , (2022).
3. J. E. Bowen, et al, Omicron spike function and neutralizing activity elicited by a comprehensive panel of vaccines. *Science*, eabq0203 (2022).
4. D. Yamasoba, I. Kimura, Y. Kosugi, K. Uriu, S. Fujita, J. Ito, K. Sato, the Genotype to Phenotype Japan (G2P-Japan) Consortium. Neutralization sensitivity of Omicron BA.2.75 to therapeutic monoclonal antibodies. *bioRxiv* 2022.07.14.500041 (2022).

5. Y. Cao, et al, Neutralizing antibody evasion and receptor binding features of SARS-CoV-2 Omicron BA.2.75. *bioRxiv* 2022.07.18.500332 (2022).
6. D. J. Sheward, C. Kim, J. Fischbach, S. Muschiol, R. A. Ehling, N. K. Björkström, G. B. K. Hedestam, S. T. Reddy, J. Albert, T. P. Peacock, B. Murrell, Evasion of neutralizing antibodies by Omicron sublineage BA.2.75. *bioRxiv* 2022.07.19.500716 (2022).
7. E. Cameroni, et al, Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. *Nature* 602, 664–670 (2022).
8. Y. Cao, et al, Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* 602, 657–663 (2022).
9. L. Liu, S. Iketani, Y. Guo, J. F. Chan, M. Wang, L. Liu, Y. Luo, H. Chu, Y. Huang, M. S. Nair, J. Yu, K. K. Chik, T. T. Yuen, C. Yoon, K. K. To, H. Chen, M. T. Yin, M. E. Sobieszczyk, Y. Huang, H. Wang, Z. Sheng, K. Y. Yuen, D. D. Ho, Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* 602, 676–681 (2022).
10. M. McCallum et al, Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *Science* 375, 864–868 (2022).
11. W. Dejnirattisai, et al, SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* 185, 467–484.e15 (2022).
12. T. Zhou, et al, Structural basis for potent antibody neutralization of SARS-CoV-2 variants including B.1.1.529. *Science* 376, eabn8897 (2022).
13. C. Dacon, et al., Broadly neutralizing antibodies target the coronavirus fusion peptide. *Science* , eabq3773 (2022).
14. K. Westendorf, et al, LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2 variants. *Cell Rep.* 39, 110812 (2022).
15. Y. J. Park, et al, Antibody-mediated broad sarbecovirus neutralization through ACE2 molecular mimicry. *Science* 375, 449–454 (2022).
16. P. Zhou, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273 (2020).
17. M. Hoffmann, H. Kleine-Weber, S. Pohlmann, A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol. Cell* 78, 779–784.e5 (2020).
18. D. Wrapp, N. Wang, K. S. Corbett, J. A. Goldsmith, C. L. Hsieh, O. Abiona, B. S. Graham, J. S. McLellan, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260–1263 (2020).
19. M. Hoffmann, et al., SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8 (2020).
20. C. B. Jackson, M. Farzan, B. Chen, H. Choe, Mechanisms of SARS-CoV-2 entry into cells. *Nat. Rev. Mol. Cell Biol.* 23, 3–20 (2022).

21. J. Hansen, et al., Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* 369, 1010–1014 (2020).
22. C. O. Barnes, et al, SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* 588, 682–687 (2020).
23. D. Pinto, et al, Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* 583, 290–295 (2020).
24. P. A. Nikitin, et al, IMM-BCP-01, a patient-derived anti-SARS-CoV-2 antibody cocktail, is active across variants of concern including Omicron BA.1 and BA.2. *Sci. Immunol.*, eabl9943 (2022).
25. D. Sun, et al, Potent neutralizing nanobodies resist convergent circulating variants of SARS-CoV-2 by targeting diverse and conserved epitopes. *Nat. Commun.* 12, 4676 (2021).
26. K. J. Jackson, et al, The shape of the lymphocyte receptor repertoire: Lessons from the B cell receptor. *Front. Immunol.* 4, 263 (2013).
27. J. Glanville, et al, Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. *Proc. Natl. Acad. Sci. U.S. A.* 106, 20216–20221 (2009).
- 28 W. C. Chen, C. M. Murawsky, Strategies for generating diverse antibody repertoires using transgenic animals expressing human antibodies. *Front. Immunol.* 9, 460 (2018).
29. P. Ren, et al, Potent and specific human monoclonal antibodies against SARS-CoV-2 Omicron variant by rapid mRNA immunization of humanized mice. *bioRxiv* 2022.03.17.484817 (2022).
30. J. L. Xu, M. M. Davis, Diversity in the CDR3 region of VH is sufficient for most antibody specificities. *Immunity* 13, 37–45 (2000).
31. M. Rapp, et al, Modular basis for potent SARS-CoV-2 neutralization by a prevalent VH1-2-derived antibody class. *Cell Rep.* 35, 108950 (2021).
32. G. D. Yancopoulos, et al, Preferential utilization of the most JH-proximal VH gene segments in pre-B-cell lines. *Nature* 311, 727–733 (1984).
33. M. Tian, et al, Induction of HIV neutralizing antibody lineages in mice with diverse precursor repertoires. *Cell* 166, 1471–1484.e18 (2016).
34. S. Jain, Z. Ba, Y. Zhang, H. Q. Dai, F. W. Alt, CTCF-binding elements mediate accessibility of RAG substrates during chromatin scanning. *Cell* 174, 102–116.e14 (2018).
35. C. Guo, et al, CTCF-binding elements mediate control of V(D)J recombination. *Nature* 477, 424–430 (2011).
36. Z. Ba, et al, CTCF orchestrates long-range cohesin-driven V(D)J recombinational scanning. *Nature* 586, 305–310 (2020).
37. Y. Xiang, S. K. Park, W. T. Garrard, A major deletion in the V κ –J κ intervening region results in hyperelevated transcription of proximal V κ genes and a severely restricted repertoire. *J. Immunol.* 193, 3746–3754 (2014).

38. N. Pishesha, et al, A class II MHC-targeted vaccine elicits immunity against SARS-CoV-2 and its variants. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2116147118 (2021).
39. N. K. Routhu, et al, SARS-CoV-2 RBD trimer protein adjuvanted with Alum-3M-052 protects from SARS-CoV-2 infection and immune pathology in the lung. *Nat. Commun.* 12, 3587 (2021).
40. L. Piccoli, et al, Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. *Cell* 183, 1024–1042.e21 (2020).
41. L. Liu, et al, Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* 584, 450–456 (2020).
42. M. A. Tortorici, et al, Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* 370, 950–957 (2020).
43. D. F. Robbiani, et al, Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 584, 437–442 (2020).
44. M. Yuan, et al, Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. *Science* 373, 818–823 (2021).
45. W. Dejnirattisai, et al, The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell* 184, 2183–2200.e22 (2021).
46. A. Punjani, J. L. Rubinstein, D. J. Fleet, M. A. Brubaker, cryoSPARC: Algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* 14, 290–296 (2017).
47. Y. Watanabe, J. D. Allen, D. Wrapp, J. S. McLellan, M. Crispin, Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* 369, 330–333 (2020).
48. S. Iketani, et al, Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature* 604, 553–556 (2022).
49. J. B. Case, et al., Resilience of S309 and AZD7442 monoclonal antibody treatments against infection by SARS-CoV-2 Omicron lineage strains. *Nat. Commun.* 13, 3824 (2022).
50. Organization, W.H. Tracking SARS-CoV-2 variants. [who.int/en/activities/tracking-SARS-CoV-2-variants](https://www.who.int/en/activities/tracking-SARS-CoV-2-variants) (2021)
51. J. Zhang, et al, Structural and functional impact by SARS-CoV-2 Omicron spike mutations. *Cell Rep.* 39, 110729 (2022).
52. A. J. B. Kreutzberger, et al, SARS-CoV-2 requires acidic pH to infect cells. *bioRxiv* 2022.06.09.495472 (2022).
53. S. Belouzard, V. C. Chu, G. R. Whittaker, Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5871–5876 (2009).
54. F. Muecksch, et al. Nussenzweig, Increased memory B cell potency and breadth after a SARS-CoV-2 mRNA boost. *Nature* 607, 128–134 (2022).

55. H. Q. Dai, et al, Loop extrusion mediates physiological Igh locus contraction for RAG scanning. *Nature* 590, 338–343 (2021).
56. H. Chen, et al, BCR selection and affinity maturation in Peyer's patch germinal centres. *Nature* 582, 421–425 (2020).
57. S. G. Lin, Z. Ba, Z. Du, Y. Zhang, J. Hu, F. W. Alt, Highly sensitive and unbiased approach for elucidating antibody repertoires. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7846–7851 (2016).
58. D. Li, et al, In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. *Cell* 184, 4203–4219.e32 (2021).
59. M. Bianchi, et al, Electron-microscopy-based epitope mapping defines specificities of polyclonal antibodies elicited during HIV-1 BG505 envelope trimer immunization. *Immunity* 49, 288–300.e8 (2018).
60. M. Tian, et al, An in vivo method for diversifying the functions of therapeutic antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2025596118 (2021).
61. P. B. Gilbert, et al, Coronavirus Vaccine Prevention Network (CoVPN)/Coronavirus Efficacy (COVE) Team§, United States Government (USG)/CoVPN Biostatistics Team§, Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 375, 43–50 (2022).
62. J. D. Berry, et al, Development and characterisation of neutralising monoclonal antibody to the SARS-coronavirus. *J. Virol. Methods* 120, 87–96 (2004).
63. C. M. Coleman, M. B. Frieman, Growth and quantification of MERS-CoV infection. *Curr. Protoc. Microbiol.* 37, 15E.12 11–19 (2015).
64. J. Kint, H. J. Maier, E. Jagt, Quantification of infectious bronchitis coronavirus by titration in vitro and in ovo. *Methods Mol. Biol.* 1282, 89–98 (2015).
65. J. Zhang, Y. Cai, T. Xiao, J. Lu, H. Peng, S. M. Sterling, R. M. Walsh Jr., S. Rits-Volloch, H. Zhu, A. N. Woosley, W. Yang, P. Sliz, B. Chen, Structural impact on SARS-CoV-2 spike protein by D614G substitution. *Science* 372, 525–530 (2021).
66. J. Zhang, et al, Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. *Science* 374, 1353–1360 (2021).
67. D. N. Mastronarde, Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol.* 152, 36–51 (2005).
68. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 66, 486–501 (2010).
69. P. D. Adams, et al, PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 66, 213–221 (2010).
70. T. I. Croll, ISOLDE: A physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallogr. Sect. D Struc. Biol.* 74, 519–530 (2018).

71. A. J. B. Kreutzberger, et al, Synergistic block of SARS-CoV-2 infection by combined drug inhibition of the host entry factors PIKfyve kinase and TMPRSS2 protease. *J. Virol.* 95, e0097521 (2021).
72. F. Aguet, et al, Membrane dynamics of dividing cells imaged by lattice light-sheet microscopy. *Mol. Biol. Cell* 27, 3418–3435 (2016).
73. M. Tian, et al, Induction of HIV neutralizing antibody lineages in mice with diverse precursor repertoires. *Cell* 166, 1471–1484.e18 (2016).
74. H. Q. Dai, et al, Loop extrusion mediates physiological Igh locus contraction for RAG scanning. *Nature* 590, 338–343 (2021).
75. T. H. Thai, et al, Regulation of the germinal center response by microRNA-155. *Science* 316, 604–608 (2007).
76. D. Li, et al, In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. *Cell* 184, 4203–4219.e32 (2021).
77. K. O. Saunders, et al, Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses. *Nature* 594, 553–559 (2021).
78. D. Wrapp, et al, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260–1263 (2020).
79. P. Zhou, et al., Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 588, E6 (2020).
80. N. Pishesha, et al, A class II MHC-targeted vaccine elicits immunity against SARS-CoV-2 and its variants. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2116147118 (2021).
81. P. B. Gilbert, et al, Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 375, 43–50 (2022).
82. J. Tang, S. Ravichandran, Y. Lee, G. Grubbs, E. M. Coyle, L. Klenow, H. Genser, H. Golding, S. Khurana, Antibody affinity maturation and plasma IgA associate with clinical outcome in hospitalized COVID-19 patients. *Nat. Commun.* 12, 1221 (2021).
83. S. Ravichandran, et al, Antibody signature induced by SARS-CoV-2 spike protein immunogens in rabbits. *Sci. Transl. Med.* 12, eabc3539 (2020).
84. S. N. Neerukonda, R. Vassell, R. Herrup, S. Liu, T. Wang, K. Takeda, Y. Yang, T. L. Lin, W. Wang, C. D. Weiss, Establishment of a well-characterized SARS-CoV-2 lentiviral pseudovirus neutralization assay using 293T cells with stable expression of ACE2 and TMPRSS2. *PLOS ONE* 16, e0248348 (2021).
85. J. Zivanov, T. Nakane, B. O. Forsberg, D. Kimanius, W. J. Hagen, E. Lindahl, S. H. Scheres, New tools for automated high-resolution cryo-EM structure determination in RELION-3. *eLife* 7, e42166 (2018).
86. A. Punjani, J. L. Rubinstein, D. J. Fleet, M. A. Brubaker, cryoSPARC: Algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* 14, 290–296 (2017).

87. J. Zhang, Y. Cai, T. Xiao, J. Lu, H. Peng, S. M. Sterling, R. M. Walsh Jr., S. Rits-Volloch, H. Zhu, A. N. Woosley, W. Yang, P. Sliz, B. Chen, Structural impact on SARS-CoV-2 spike protein by D614G substitution. *Science* 372, 525–530 (2021).
88. D. N. Mastronarde, Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol.* 152, 36–51 (2005).
89. Y. Cai, J. Zhang, T. Xiao, H. Peng, S. M. Sterling, R. M. Walsh Jr., S. Rawson, S. Rits-Volloch, B. Chen, Distinct conformational states of SARS-CoV-2 spike protein. *Science* 369, 1586–1592 (2020).
90. J. Zhang, et al, Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. *Science* 374, 1353–1360 (2021).
91. D. Pinto, et al, Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* 583, 290–295 (2020).
92. J. Jumper, et al, Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589 (2021).
93. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. Sect. D Biol. Crystallogr.* 66, 486–501 (2010).
94. P. D. Adams, et al, PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. Sec. D Biol. Crystallogr.* 66, 213–221 (2010).
95. T. I. Croll, ISOLDE: A physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallogr. Sec. D Struc. Biol.* 74, 519–530 (2018).
96. A. Morin, B. Eisenbraun, J. Key, P. C. Sanschagrin, M. A. Timony, M. Ottaviano, P. Sliz, Collaboration gets the most out of software. *eLife* 2, e01456 (2013).
97. A. J. B. Kreutzberger, A. Sanyal, R. Ojha, J. D. Pyle, O. Vapalahti, G. Balistreri, T. Kirchhausen, Synergistic block of SARS-CoV-2 infection by combined drug inhibition of the host entry factors PIKfyve kinase and TMPRSS2 protease. *J. Virol.* 95, e0097521 (2021).
98. A. J. B. Kreutzberger, et al, SARS-CoV-2 requires acidic pH to infect cells. *bioRxiv* 2022.06.09.495472 (2022).
- 99 F. Madeira, M. Pearce, A. R. N. Tivey, P. Basutkar, J. Lee, O. Edbali, N. Madhusoodanan, A. Kolesnikov, R. Lopez, Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res.* 50, W276–W279 (2022).

[00350] Table 3: Sequence features of Anti-SARS-CoV-2 antibodies isolated from single human VH-rearranging mouse model

mAb ID	Alternative mAb ID	B cell lineage	Immu nogen	Human VH	Mouse JH	HC CDR3	SEQ ID NO	VH-SHM (Amino Acid)	Human VK	Mouse JK	LC CDR3	SEQ ID NO	VK-SHM (Amino Acid)	
SP1-77	SPIKE-BC1-7-7	SP1	SARS-CoV2 spike	hVH1-2	mJH1	ARDRVLYGR SFGWYFDV		8	hVK1-33	mJK2	QQYDN LPT		3	
SP1-13	SPIKE-BC1-1-3			hVH1-2	mJH1	ARDRVLYGR SYGWYFDV		6	hVK1-33	mJK2	QQYDN LPT		3	
SP1-62	SPIKE-BC1-6-2			hVH1-2	mJH1	ARDRVLYGR SYGWYFDV		7	hVK1-33	mJK2	QQYDN LPT		4	
SP1-73	SPIKE-BC1-7-3			hVH1-2	mJH1	ARDRVLYGR SYGWYFDV		2	hVK1-33	mJK2	QQYDN LPT		5	
SP1-96	SPIKE-BC1-9-6			hVH1-2	mJH1	ARDRVLYGR SYGWYFDV		6	hVK1-33	mJK2	QQYDN LPT		2	
SP2-16	SPIKE-BC2-1-6	SP2		hVH1-2	mJH4	ARERRVIHYS GILYALDY		7	hVK1-33	mJK2	QQYDN LHMFT		2	
SP2-122	SPIKE-BC2-12-2			hVH1-2	mJH4	ARERRVIHY Y GILYALDY		5	hVK1-33	mJK2	QQYDN LHMFT		1	
SP2-125	SPIKE-BC2-12-5			hVH1-2	mJH4	ARERRVIHYS GILYALDY		3	hVK1-33	mJK2	QQYDN LHMFT		2	
SP2-48	SPIKE-BC2-4-8			hVH1-2	mJH4	ARERRVIHY Y GILYAMDY		3	hVK1-33	mJK2	QQYDD LHMFT		1	
SP3-101	SPIKE-BC3-10-1	SP3		hVH1-2	mJH3	AITGRRPWFP F			11	hVK1-33	mJK2	QQYDN LPYT		7
SP3-42	SPIKE-BC3-4-2			hVH1-2	mJH3	AITGRRPWFP Y			6	hVK1-33	mJK2	QQYDY LPYT		7
SP3-46	SPIKE-BC3-4-6			hVH1-2	mJH3	AITGRRPWFA N			8	hVK1-33	mJK2	QQYDY LPYT		6
SP3-98	SPIKE-BC3-9-8			hVH1-2	mJH3	TITGRRPFPY			7	hVK1-33	mJK2	QQYDN LPYT		5
SP4-102	SPIKE-BC4-10-2	SP4		hVH1-2	mJH3	ARGERRSWF TY			10	hVK1-33	mJK2	QQYDN LPYT		10
SP4-113	SPIKE-BC4-11-3			hVH1-2	mJH3	ARGERRSWF TY			10	hVK1-33	mJK2	QQYDN LPYT		7
SP4-73	SPIKE-BC4-7-3			hVH1-2	mJH3	ARGERRSWF TY			10	hVK1-33	mJK2	QQYDN LPYT		5

SP4-15	SPIKE-BC4-1-5		VHH7-3	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH3	ARGGRRTWFA		9	hVK1-33	mJK2	QQYDLPYT	4
SP4-65	SPIKE-BC4-6-5				hVH1-2	mJH3	ARGARRTWFA		10	hVK1-33	mJK2	QQYDNL	2
SP4-87	SPIKE-BC4-8-7				hVH1-2	mJH3	ARGGRRTWFA		9	hVK1-33	mJK2	QQYDLPYT	3
VHH7-3-37	VHH7-BC3-37	VHH7-5	VHH7-3	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH3	ARDLGDYDAWFA		0	hVK1-33	mJK1	QQYDNL	6
VHH7-3-66	VHH7-BC3-66				hVH1-2	mJH3	ARDLGDYDAWFA		0	hVK1-33	mJK1	QQYDNL	3
VHH7-5-29	VHH7-BC5-29				hVH1-2	mJH3	ARDRVGQRFVY		9	hVK1-33	mJK1	QQYDNL	7
VHH7-5-75	VHH7-BC5-75	VHH7-7	VHH7-5	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH3	ARDRVGQRFVY		9	hVK1-33	mJK1	QQYDNL	3
VHH7-5-82	VHH7-BC5-82				hVH1-2	mJH3	ARDRVGQRFVY		5	hVK1-33	mJK1	QQYDNL	4
VHH7-7-53	VHH7-BC7-53				hVH1-2	mJH2	ARRNCDY		5	hVK1-33	mJK2	QQYDNL	1
VHH7-7-76	VHH7-BC7-76	VHH7-8	VHH7-7	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH2	ARRNCDY		4	hVK1-33	mJK2	QQYDNL	1
VHH7-7-79	VHH7-BC7-79				hVH1-2	mJH2	ARRNCDY		4	hVK1-33	mJK2	QQYDNL	3
VHH7-7-85	VHH7-BC7-85				hVH1-2	mJH2	ARRNCDN		4	hVK1-33	mJK2	QQYDNL	3
VHH7-8-5	VHH7-BC8-5	VHH7-10	VHH7-8	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH3	ARGRGGTSWLA		6	hVK1-33	mJK2	QQYDNL	3
VHH7-8-59	VHH7-BC8-59				hVH1-2	mJH3	ARGKGGTSWFAY		3	hVK1-33	mJK2	QQYDNL	1
VHH7-8-62	VHH7-BC8-62				hVH1-2	mJH3	ARGKGGTSWFAY		3	hVK1-33	mJK2	QQYDNL	1
VHH7-8-7	VHH7-BC8-7	VHH7-10	VHH7-8	VHH7-RBD (VHH7) nanobody target MHCII)	hVH1-2	mJH3	ARGRGGTSWFAY		4	hVK1-33	mJK2	QQYDNL	1
VHH7-10-19	VHH7-BC10-19				hVH1-2	mJH2	ARDPLTGIFY		6	hVK1-33	mJK2	QQYDNL	1

CLAIMS

WHAT IS CLAIMED IS:

1. An antibody, antibody reagent, chimeric antigen receptor (CAR), or antigen-binding fragment thereof, that specifically binds a SARS-COV-2 polypeptide, said antibody, antibody reagent, CAR, or antigen-binding fragment thereof comprising at least one heavy or light chain complementarity determining region (CDR) selected from:
 - a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
 - b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
 - c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
 - d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
 - e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
 - f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
 - g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
 - h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
 - i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
 - j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
 - k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
 - l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;

- m) the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n) the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
- o) the heavy chain CDRs of SEQ ID NOs: 107-109 and the light chain CDRs of SEQ ID NOs: 110-112;
- p) the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- q) the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- r) the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;
- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t) the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u) the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224

- dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
 - ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
 - ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
 - gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
 - hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
 - ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
 - jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
 - kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
 - ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
 - mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
 - nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
 - oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
a conservative substitution variant thereof.
2. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof, of claim 1, which comprises three heavy chain CDRs or three light chain CDRs having amino acid sequences selected from:
- a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
 - b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain

CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;

- c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
- d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
- e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
- f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
- g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;
- h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
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- p) the heavy chain CDRs of SEQ ID NOs: 115-117 and the light chain CDRs of SEQ ID NOs: 118-120;
- q) the heavy chain CDRs of SEQ ID NOs: 123-125 and the light chain CDRs of SEQ ID NOs: 126-128;
- r) the heavy chain CDRs of SEQ ID NOs: 131-133 and the light chain CDRs of SEQ ID NOs: 134-136;

- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
- t) the heavy chain CDRs of SEQ ID NOs: 147-149 and the light chain CDRs of SEQ ID NOs: 150-152;
- u) the heavy chain CDRs of SEQ ID NOs: 155-157 and the light chain CDRs of SEQ ID NOs: 158-160;
- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;
- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
- ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;

- jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
 - kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
 - ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
 - mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
 - nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
 - oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or
a conservative substitution variant thereof.
3. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of claim 1, which comprises three heavy chain CDRs and three light chain CDRs having amino acid sequences selected from:
- a) the heavy chain CDRs of SEQ ID NOs: 3-5 and the light chain CDRs of SEQ ID NOs: 6-8;
 - b) the heavy chain CDR1 of SEQ ID NO: 3, the heavy chain CDR2 of SEQ ID NO: 4, the heavy chain CDR of one of SEQ ID NO: 5, 321, 323, 325, 327, and 329, the light chain CDR1 of SEQ ID NO: 6, the light chain CDR of SEQ ID NO: 7, and the light chain CDR3 of SEQ ID NO: 8;
 - c) the heavy chain CDRs of SEQ ID NOs: 11-13 and the light chain CDRs of SEQ ID NOs: 14-16;
 - d) the heavy chain CDRs of SEQ ID NOs: 19-21 and the light chain CDRs of SEQ ID NOs: 22-24;
 - e) the heavy chain CDRs of SEQ ID NOs: 27-29 and the light chain CDRs of SEQ ID NOs: 30-32;
 - f) the heavy chain CDRs of SEQ ID NOs: 35-37 and the light chain CDRs of SEQ ID NOs: 38-40;
 - g) the heavy chain CDRs of SEQ ID NOs: 43-45 and the light chain CDRs of SEQ ID NOs: 46-48;

- h) the heavy chain CDRs of SEQ ID NOs: 51-53 and the light chain CDRs of SEQ ID NOs: 54-56;
- i) the heavy chain CDRs of SEQ ID NOs: 59-61 and the light chain CDRs of SEQ ID NOs: 62-64;
- j) the heavy chain CDRs of SEQ ID NOs: 67-69 and the light chain CDRs of SEQ ID NOs: 70-72;
- k) the heavy chain CDRs of SEQ ID NOs: 75-77 and the light chain CDRs of SEQ ID NOs: 78-80;
- l) the heavy chain CDRs of SEQ ID NOs: 83-85 and the light chain CDRs of SEQ ID NOs: 86-88;
- m) the heavy chain CDRs of SEQ ID NOs: 91-93 and the light chain CDRs of SEQ ID NOs: 94-96;
- n) the heavy chain CDRs of SEQ ID NOs: 99-101 and the light chain CDRs of SEQ ID NOs: 102-104;
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- s) the heavy chain CDRs of SEQ ID NOs: 139-141 and the light chain CDRs of SEQ ID NOs: 142-144;
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- v) the heavy chain CDRs of SEQ ID NOs: 163-165 and the light chain CDRs of SEQ ID NOs: 166-168;
- w) the heavy chain CDRs of SEQ ID NOs: 171-173 and the light chain CDRs of SEQ ID NOs: 174-176;
- x) the heavy chain CDRs of SEQ ID NOs: 179-181 and the light chain CDRs of SEQ ID NOs: 182-184;

- y) the heavy chain CDRs of SEQ ID NOs: 187-189 and the light chain CDRs of SEQ ID NOs: 190-192;
- z) the heavy chain CDRs of SEQ ID NOs: 195-197 and the light chain CDRs of SEQ ID NOs: 198-200;
- aa) the heavy chain CDRs of SEQ ID NOs: 203-205 and the light chain CDRs of SEQ ID NOs: 206-208;
- bb) the heavy chain CDRs of SEQ ID NOs: 211-213 and the light chain CDRs of SEQ ID NOs: 214-216;
- cc) the heavy chain CDRs of SEQ ID NOs: 219-221 and the light chain CDRs of SEQ ID NOs: 222-224
- dd) the heavy chain CDRs of SEQ ID NOs: 227-229 and the light chain CDRs of SEQ ID NOs: 230-232;
- ee) the heavy chain CDRs of SEQ ID NOs: 235-237 and the light chain CDRs of SEQ ID NOs: 238-240;
- ff) the heavy chain CDRs of SEQ ID NOs: 243-245 and the light chain CDRs of SEQ ID NOs: 246-248;
- gg) the heavy chain CDRs of SEQ ID NOs: 251-253 and the light chain CDRs of SEQ ID NOs: 254-256;
- hh) the heavy chain CDRs of SEQ ID NOs: 259-261 and the light chain CDRs of SEQ ID NOs: 262-264;
- ii) the heavy chain CDRs of SEQ ID NOs: 267-269 and the light chain CDRs of SEQ ID NOs: 270-272;
- jj) the heavy chain CDRs of SEQ ID NOs: 275-277 and the light chain CDRs of SEQ ID NOs: 278-280;
- kk) the heavy chain CDRs of SEQ ID NOs: 283-285 and the light chain CDRs of SEQ ID NOs: 286-288;
- ll) the heavy chain CDRs of SEQ ID NOs: 291-293 and the light chain CDRs of SEQ ID NOs: 294-296;
- mm) the heavy chain CDRs of SEQ ID NOs: 299-301 and the light chain CDRs of SEQ ID NOs: 302-304;
- nn) the heavy chain CDRs of SEQ ID NOs: 307-309 and the light chain CDRs of SEQ ID NOs: 310-312;
- oo) the heavy chain CDRs of SEQ ID NOs: 315-317 and the light chain CDRs of SEQ ID NOs: 318-320; or

a conservative substitution variant thereof.

4. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of claim 1, which comprises VH and/or VL amino acid sequences selected from:

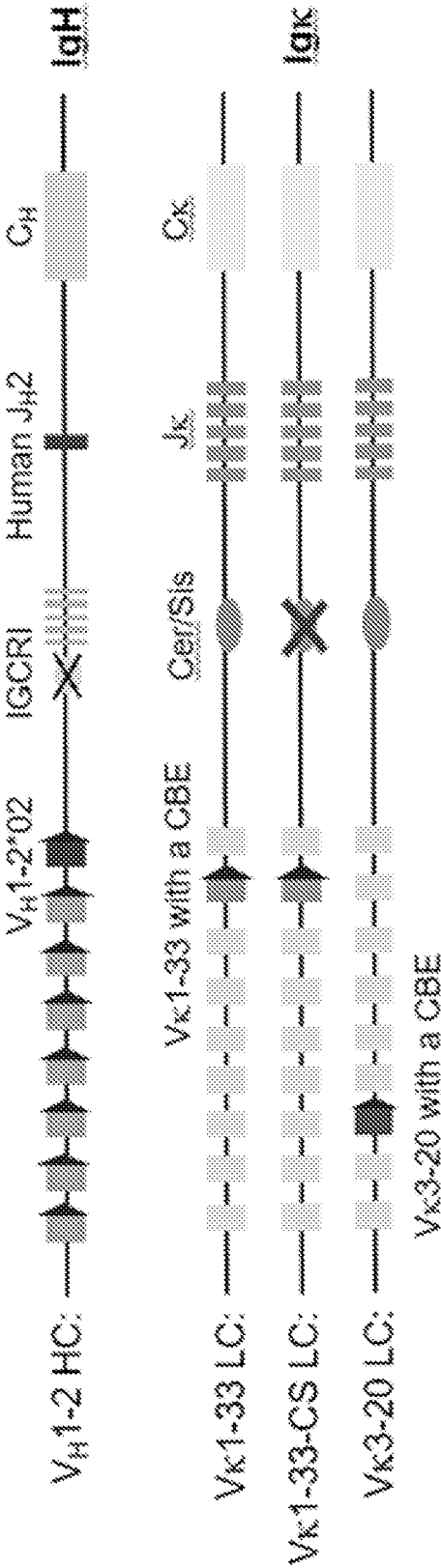
- a) SEQ ID NOs: 1 and 2;
- b) the VH sequences of one of SEQ ID NOs: 1, 322, 324, 326, 328, and 330-348 and the VL sequences of SEQ ID NO: 2 and 349;
- c) SEQ ID NOs: 9 and 10;
- d) SEQ ID NOs: 17 and 18;
- e) SEQ ID NOs: 25 and 26;
- f) SEQ ID NOs: 33 and 34;
- g) SEQ ID NOs: 41 and 42;
- h) SEQ ID NOs: 49 and 50;
- i) SEQ ID NOs: 57 and 58;
- j) SEQ ID NOs: 65 and 66;
- k) SEQ ID NOs: 73 and 74;
- l) SEQ ID NOs: 81 and 82;
- m) SEQ ID NOs: 89 and 90;
- n) SEQ ID NOs: 97 and 98;
- o) SEQ ID NOs: 105 and 106;
- p) SEQ ID NOs: 113 and 114;
- q) SEQ ID NOs: 121 and 122;
- r) SEQ ID NOs: 129 and 130;
- s) SEQ ID NOs: 137 and 138;
- t) SEQ ID NOs: 145 and 146;
- u) SEQ ID NOs: 153 and 154;
- v) SEQ ID NOs: 161 and 162;
- w) SEQ ID NOs: 169 and 170;
- x) SEQ ID NOs: 177 and 178;
- y) SEQ ID NOs: 185 and 186;
- z) SEQ ID NOs: 193 and 194;
- aa) SEQ ID NOs: 201 and 2025;
- bb) SEQ ID NOs: 209 and 210;
- cc) SEQ ID NOs: 217 and 218;

- dd) SEQ ID NOs: 225 and 226;
 - ee) SEQ ID NOs: 233 and 234;
 - ff) SEQ ID NOs: 241 and 242;
 - gg) SEQ ID NOs: 249 and 250;
 - hh) SEQ ID NOs: 257 and 258;
 - ii) SEQ ID NOs: 265 and 266;
 - jj) SEQ ID NOs: 273 and 274;
 - kk) SEQ ID NOs: 281 and 282;
 - ll) SEQ ID NOs: 289 and 290;
 - mm) SEQ ID NOs: 297 and 298;
 - nn) SEQ ID NOs: 305 and 306;
 - oo) SEQ ID NOs: 313 and 314; or
a conservative substitution variant thereof.
5. A first antibody, antibody reagent, CAR, or antigen-binding fragment thereof that specifically binds a SARS-COV-2 polypeptide, and can compete for binding of SARS-COV-2 with a second antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-4.
6. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-5, further comprising a conservative substitution in a sequence not comprised by a CDR.
7. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is fully human or fully humanized.
8. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-7 wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is fully humanized except for the CDR sequences.
9. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-8 wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof comprises one or more of:
- a) a heavy chain CDR3 in which the sequence "YGRS" of the heavy chain CDR3 is mutated to comprise "YGSS" or "YGGS";

- b) a VH in which the sequence "FDVW" of the VH is mutated to comprise "FDLW";
 - c) a VH in which the sequence "WGAG" of the VH is mutated to comprise "WGRG";
 - d) a VH in which the sequence "GTTV" of the VH is mutated to comprise "GTLV"; and
 - e) a VL in which the sequence "FGGG" of the VL is mutated to comprise "FGQG."
10. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-9, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is selected from the group consisting of:
- an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, and a bispecific antibody.
11. A nucleic acid sequence encoding the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10.
12. A cell comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10 or the nucleic acid sequence of claim 11.
13. A pharmaceutical composition comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10; or the cell of claim 12, and a pharmaceutically acceptable carrier.
14. The pharmaceutical composition of claim 13, comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof and at least one of casrivcimab, imdevimab, or sotrovimab.
15. A solid support comprising the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10.
16. The solid support of claim 15, wherein the antibody, antibody reagent, CAR, or antigen-binding fragment thereof is detectably labeled.

17. The solid support of any one of claims 15-16, wherein the solid support comprises a particle, a bead, a polymer, or a substrate.
18. A kit for the detection of a SARS-COV-2 polypeptide in a sample, the kit comprising at least a first antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10 immobilized on a solid support and comprising a detectable label.
19. A molecular complex comprising at least one antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10 bound to a SARS-COV-2 polypeptide.
20. A method of treating a coronavirus infection in a subject in need thereof, the method comprising administering
 - (i) the antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10;
 - (ii) the composition of claim 13 or 14; and/or
 - (iii) the cell of claim 12, to the subject.
21. The antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10; the composition of claim 13 or 14; and/or the cell of claim 12 for use in a method of treating a coronavirus infection in a subject in need thereof, the method comprising administering antibody, antibody reagent, CAR, or antigen-binding fragment thereof of any one of claims 1-10; the composition of claim 13 or 14; and/or the cell of claim 12 to the subject.
22. The method or antibody, antibody reagent, CAR, or antigen-binding fragment thereof of of claim 20 or 21, wherein the coronavirus infection is a SARS-CoV-2 infection.
23. The method or antibody, antibody reagent, CAR, or antigen-binding fragment thereof of of claim 20 or 21, wherein the the coronavirus infection is a B.1.1.7, Q lineage, B.1.351, P.1, B.1.617.2, AY lineage, B.1.427, B.1.429, B.1.525, B.1.526, B.1.617.1, 1.617.3, B.1.621, B.1.621.1, P.2, B.1.1.529, BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.4/5, BA.2.12.1, or BA.5 SARS-CoV-2 infection.

Fig. 1A



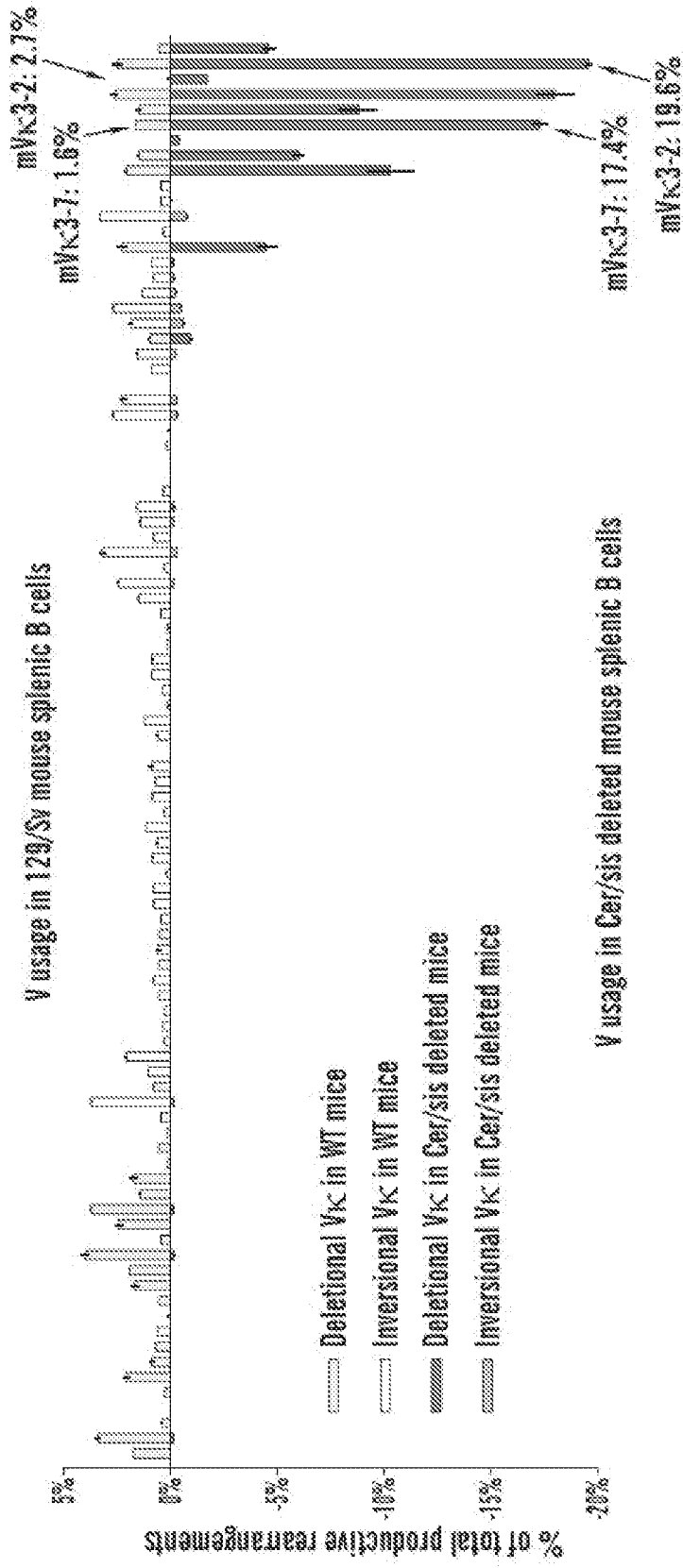
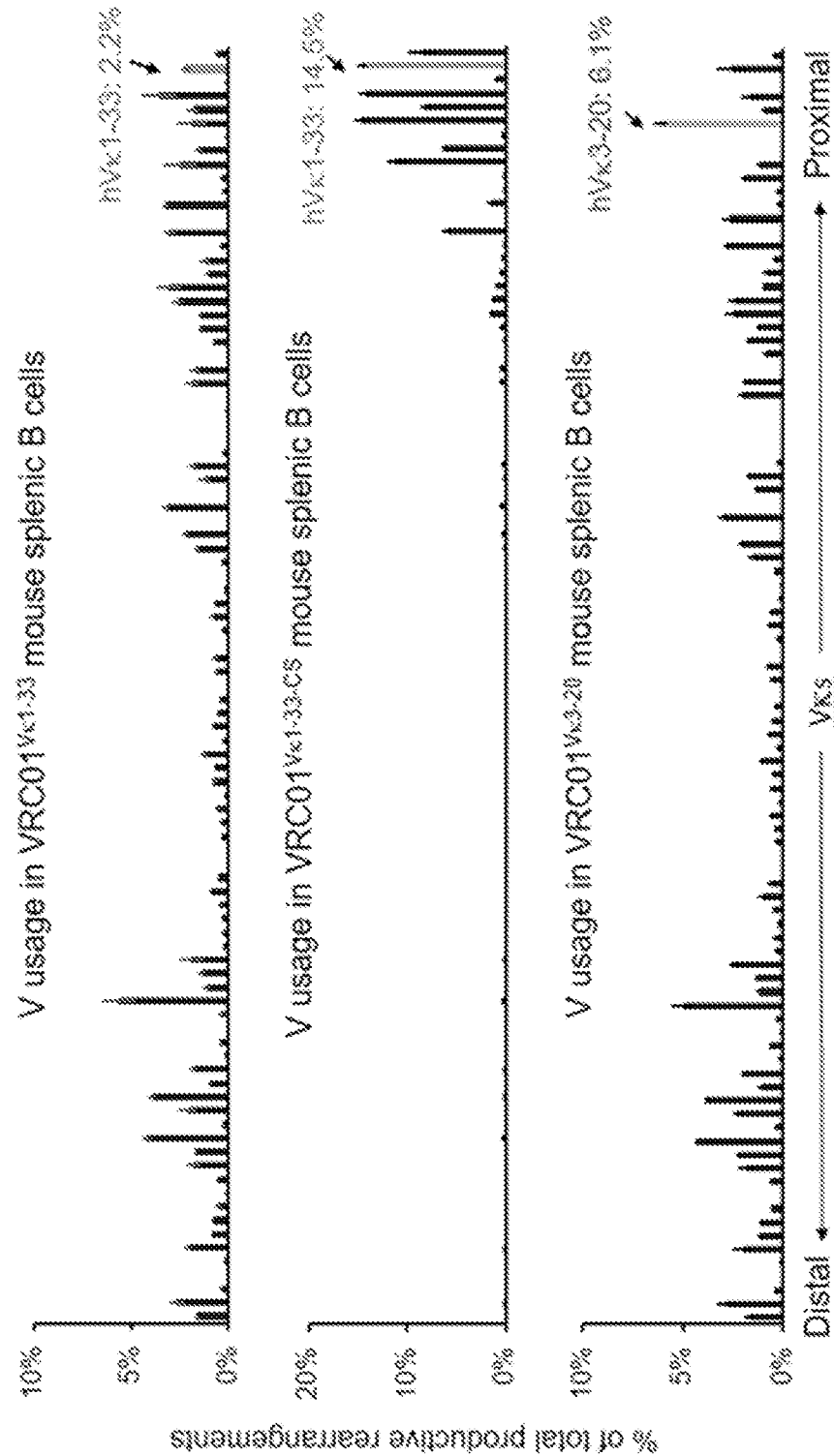


FIG. 1B

Fig. 1C



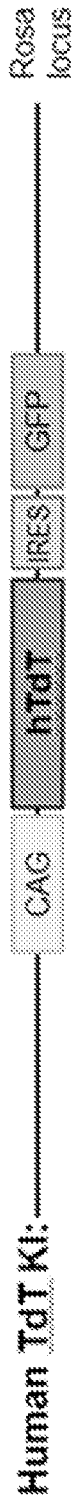


Fig. 2A

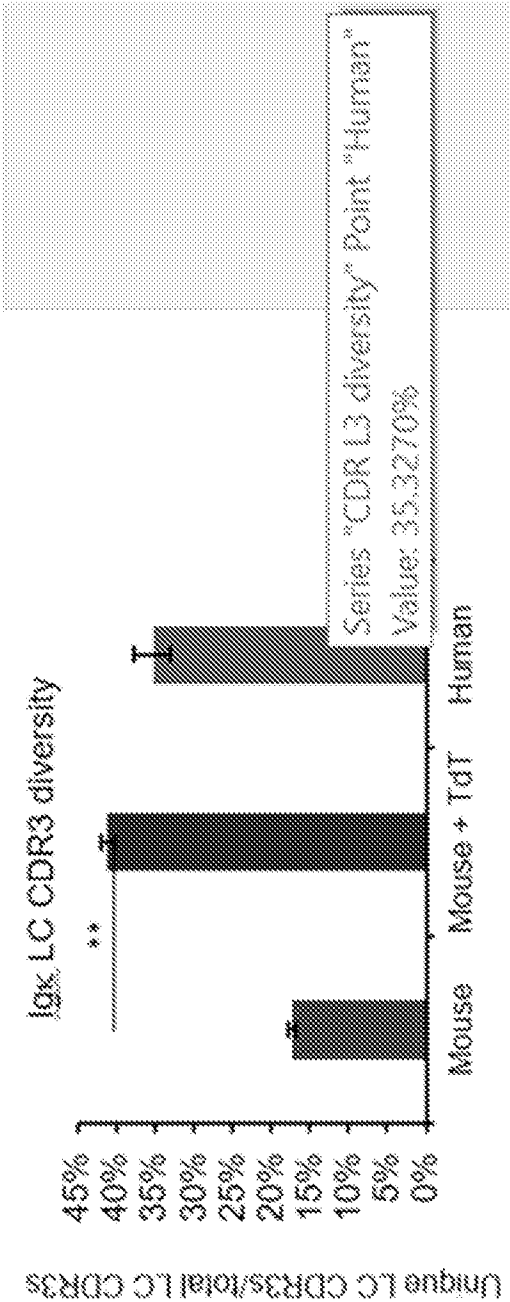


Fig. 2C

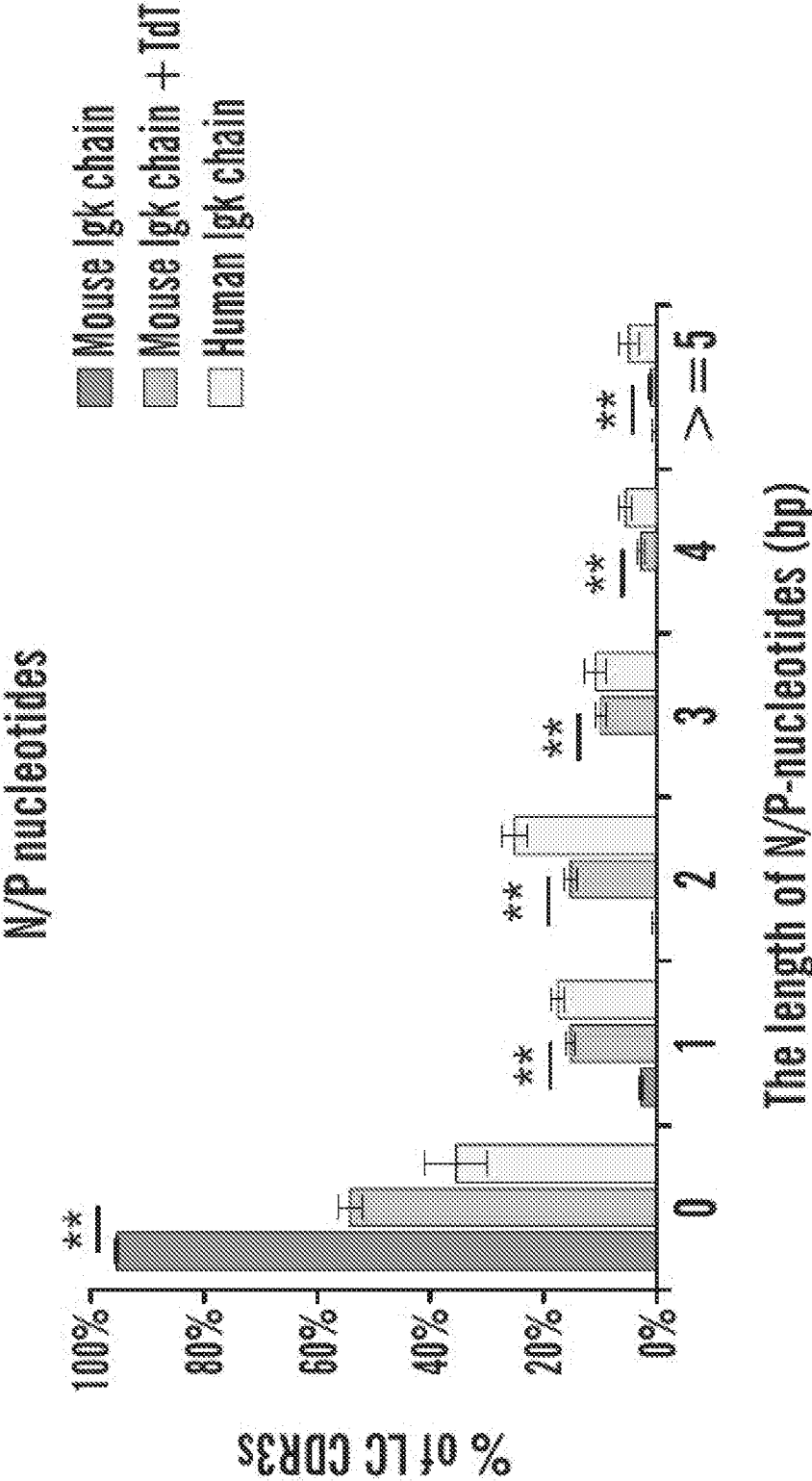


FIG. 2B

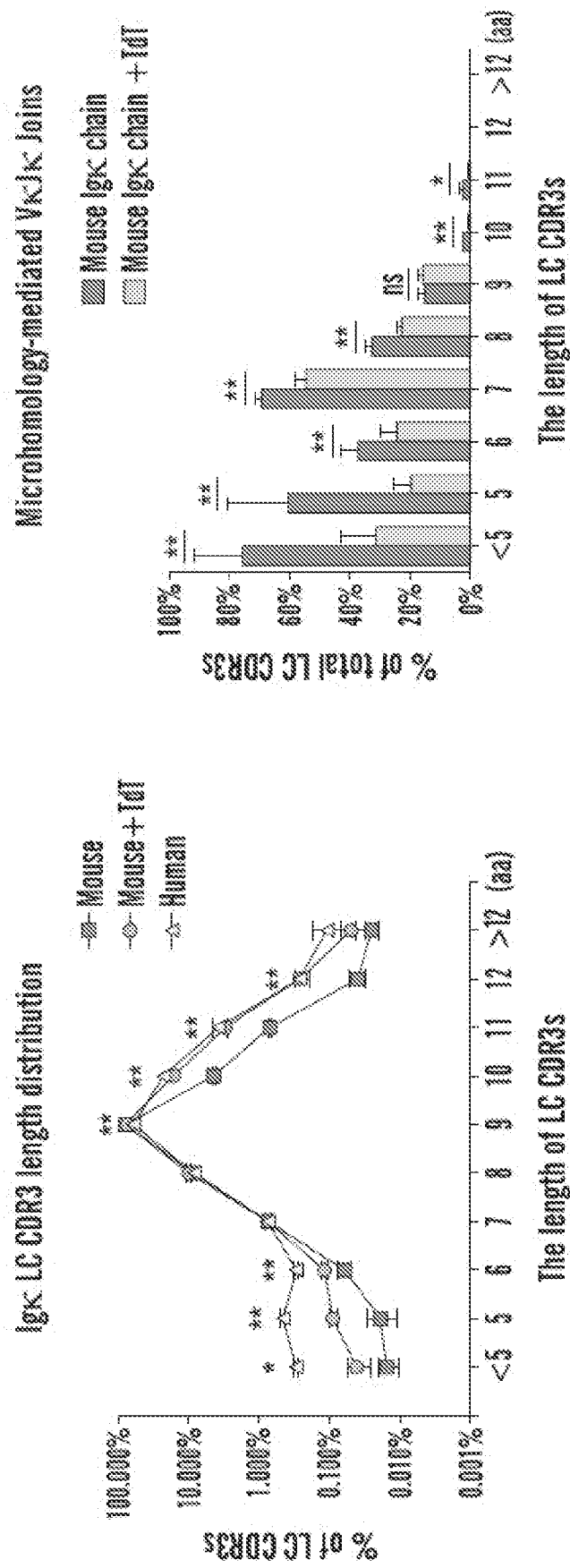
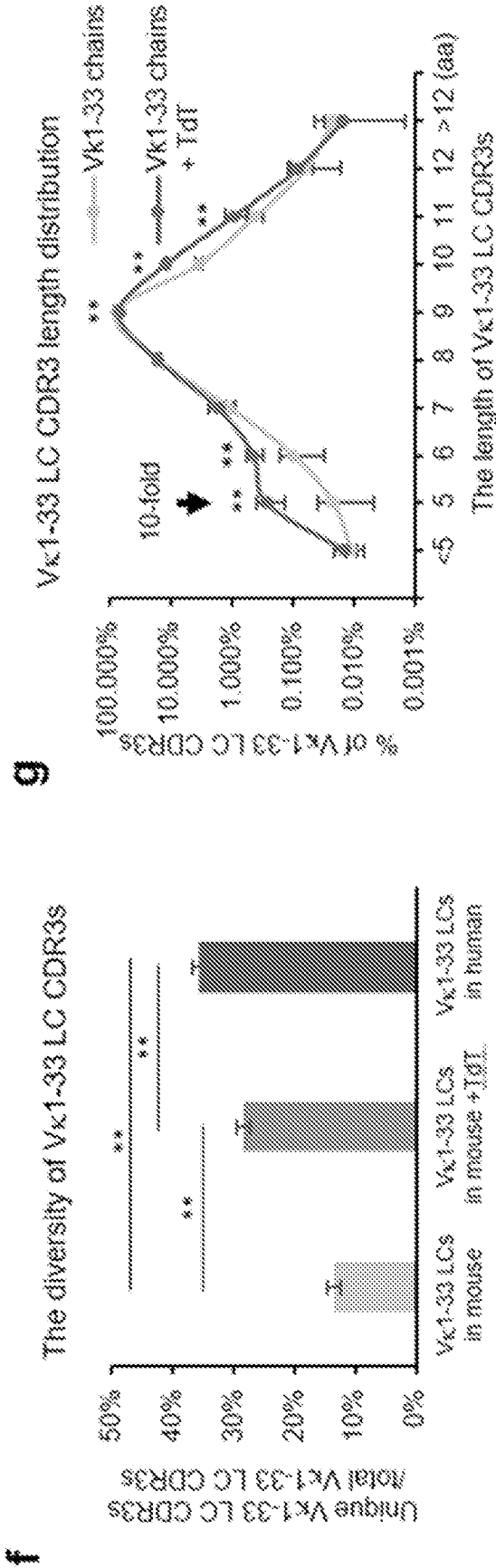


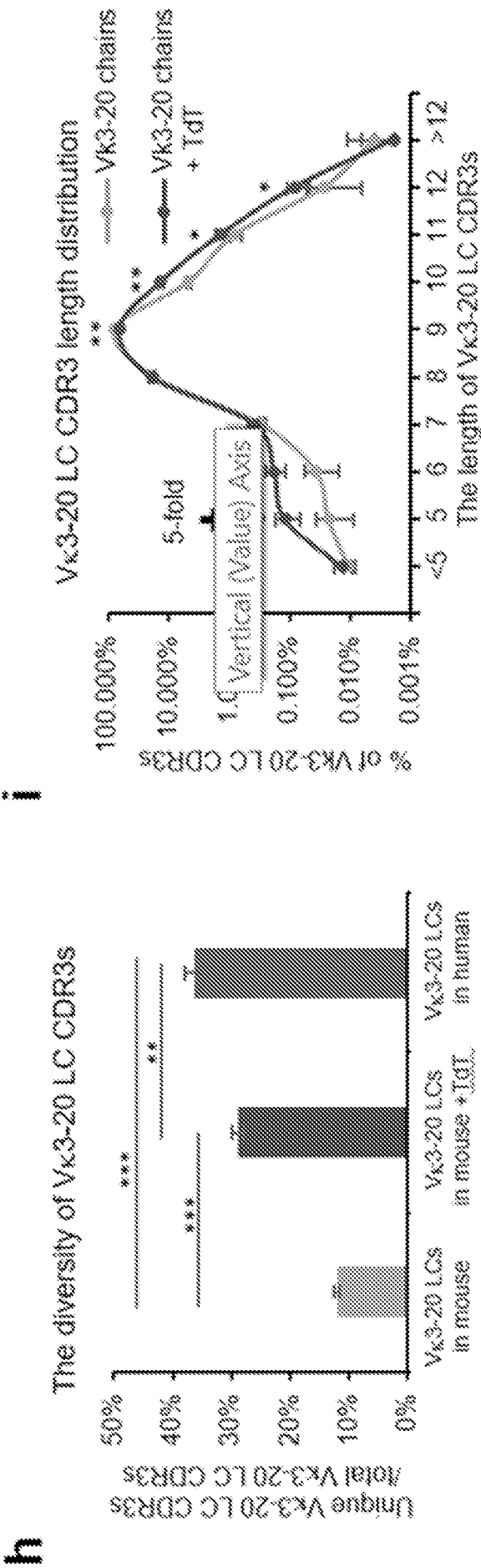
FIG. 2E

FIG. 2D

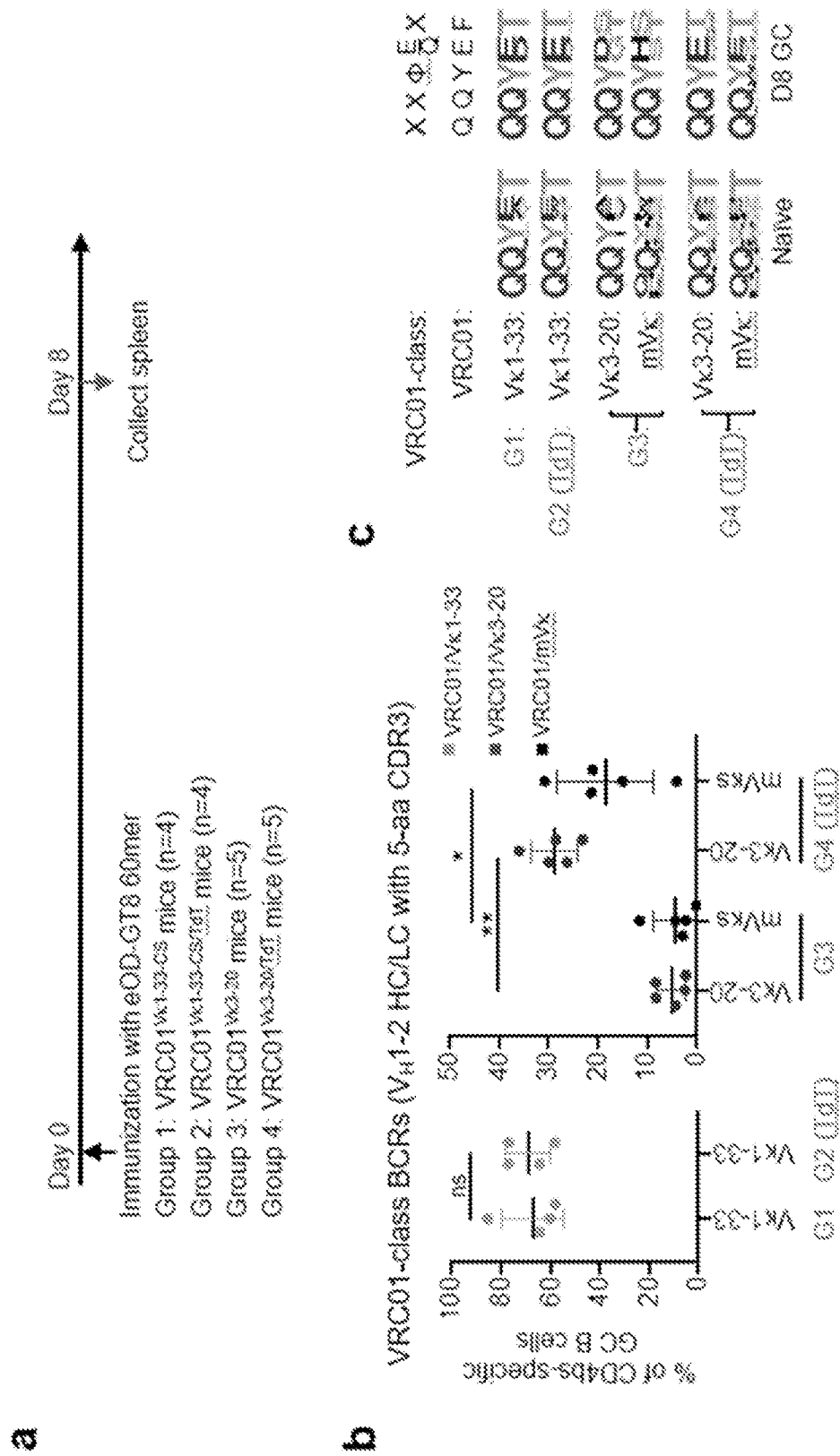
Figs. 2F-2G



Figs. 2H-2I



Figs. 3A-3C



Figs. 3D-3E

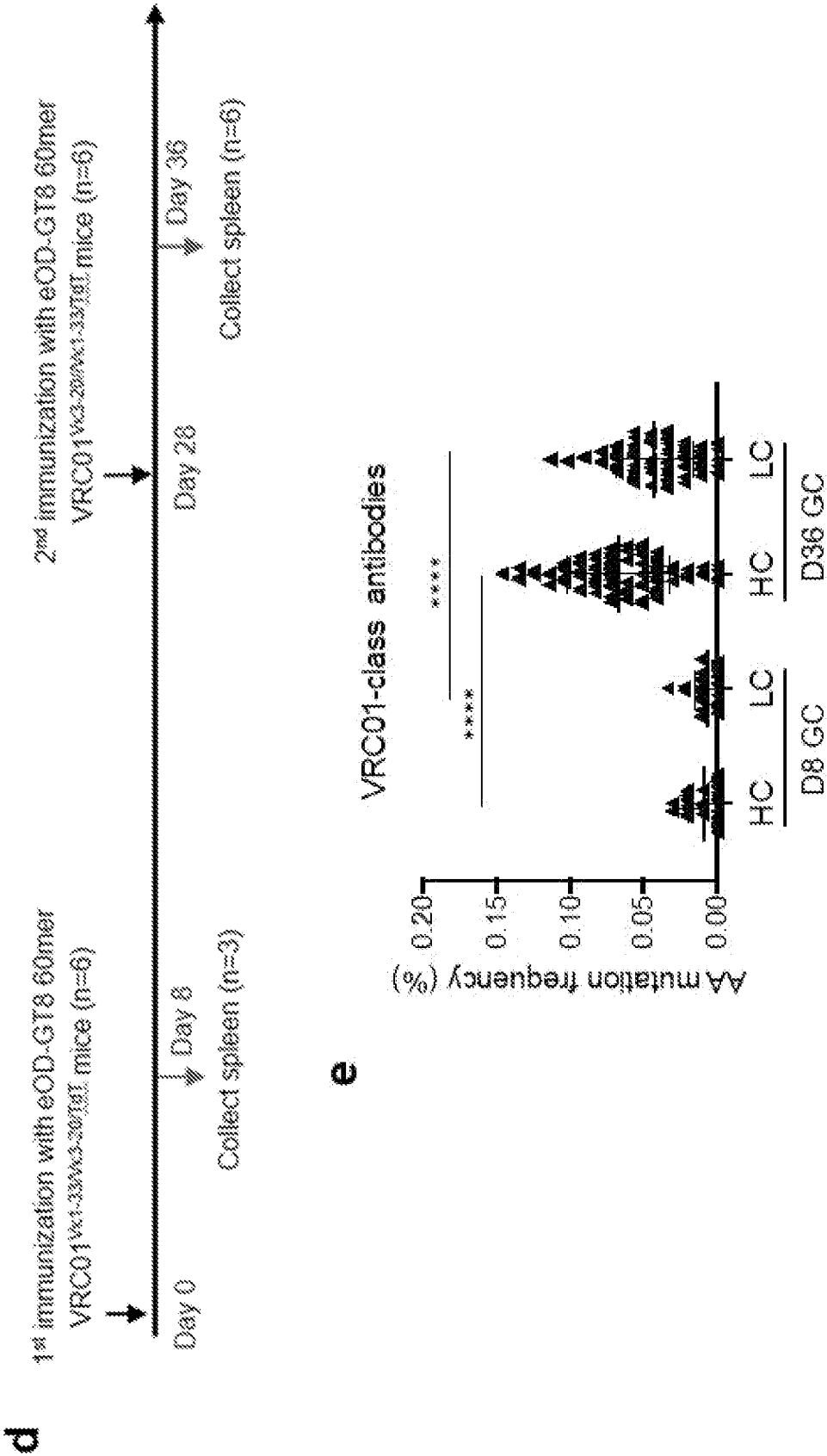
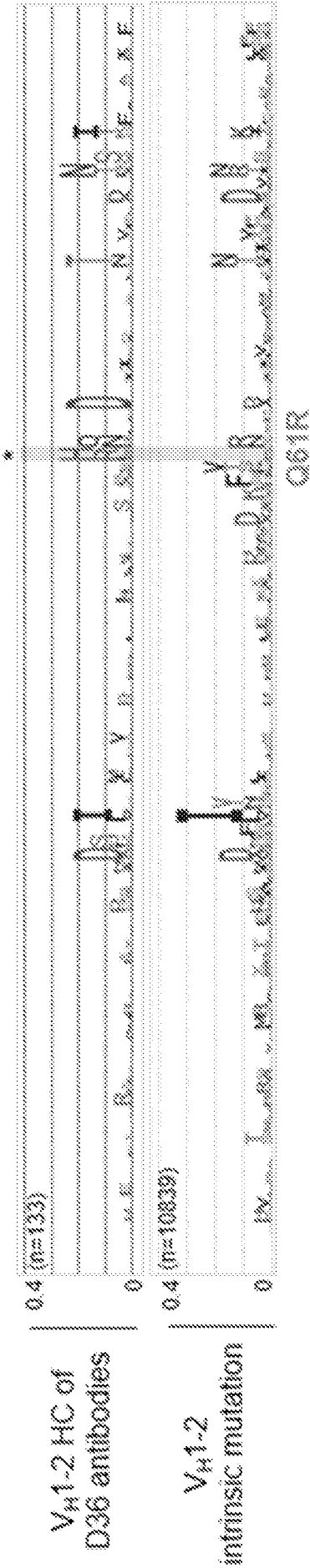


Fig. 3F



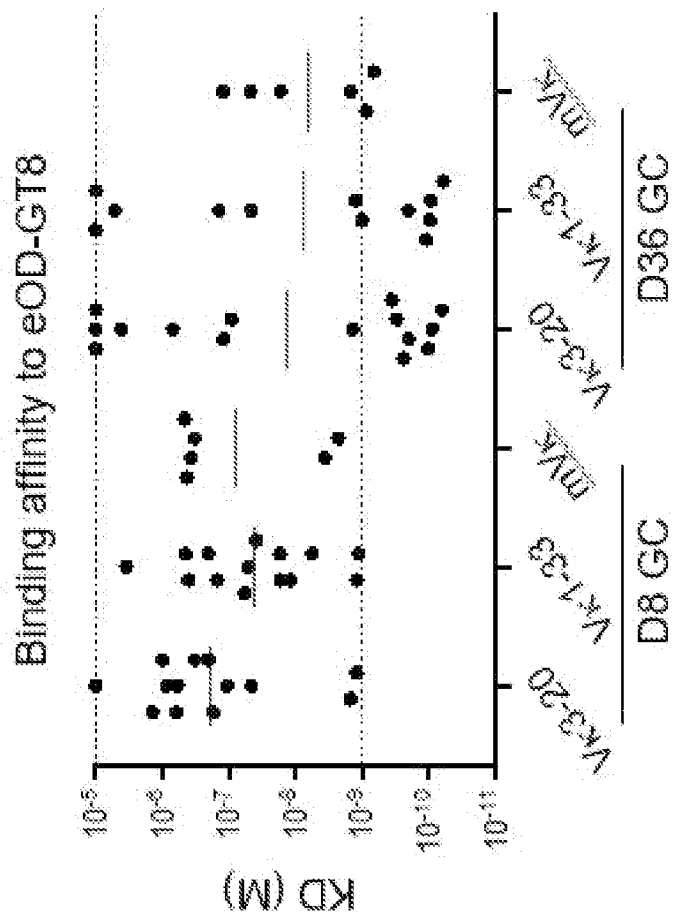
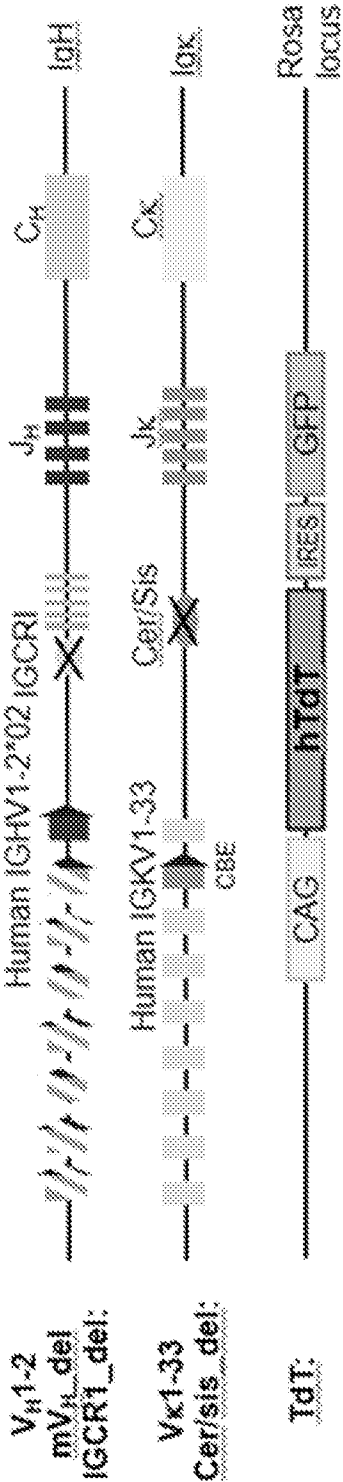


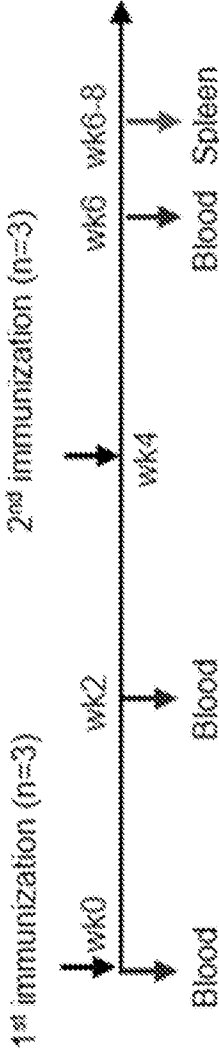
Fig. 3G

Figs. 4A-4B

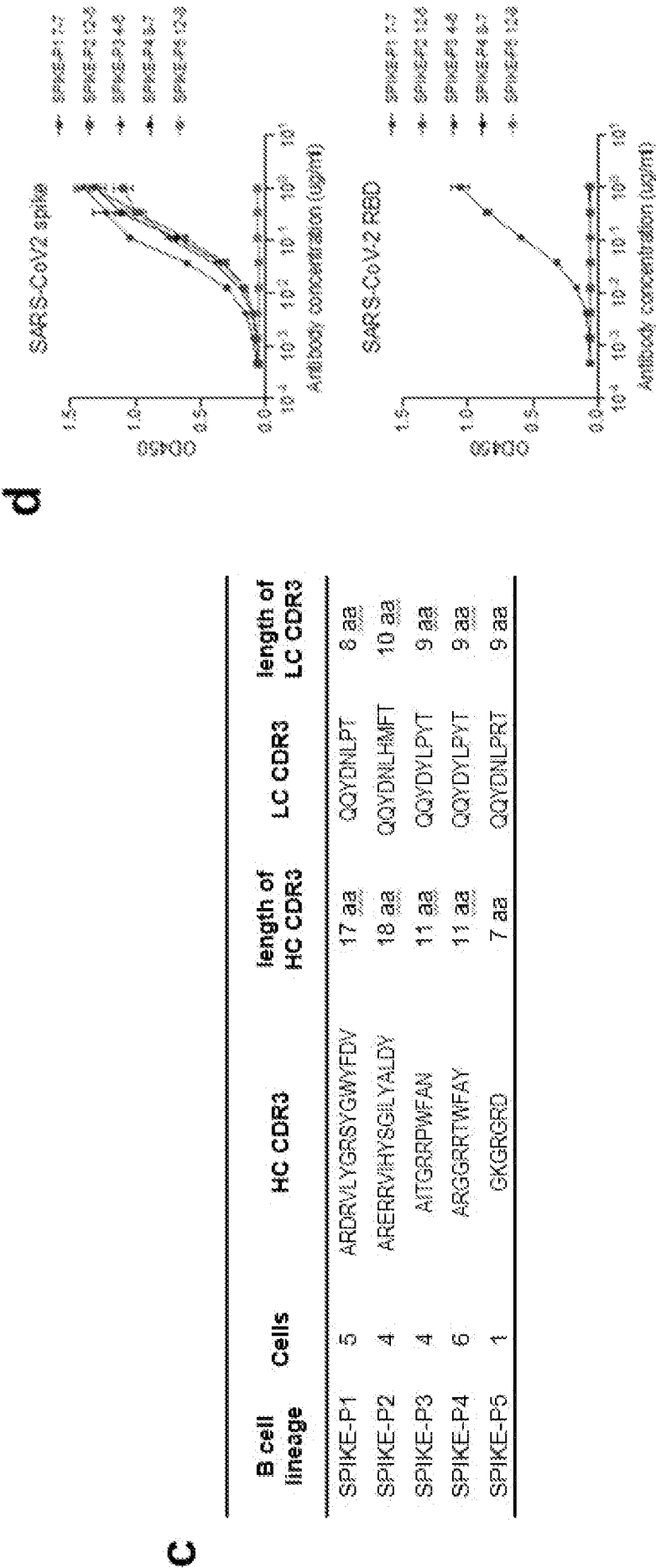
a



b



Figs. 4C-4D



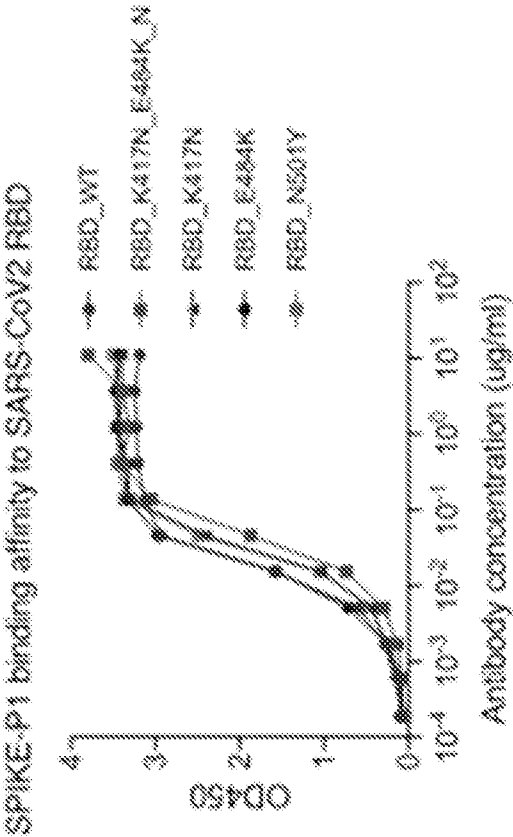


Fig. 4E

Neutralization assays in 293T/ACE2 Cells (µg/ml)						
Pseudotyped viruses	D614G	B.1.1.7	B.1.351	P.1	B.1.429	B.1.526. E484K
IC ₅₀	0.022	0.027	0.010	0.012	0.026	0.005
IC ₉₀	0.055	0.841	0.030	0.045	0.074	0.025

Fig. 4F

PRNT live virus assay (µg/mL)				
Lived virus	WA1	B.1.1.7	B.1.351	P.1
PRNT ₅₀	0.01	0.01	0.0010	0.0100
IC ₅₀	0.0011	0.0011	0.0008	0.0008
IC ₉₀	0.0024	0.0013	0.0011	0.0012

Fig. 4G

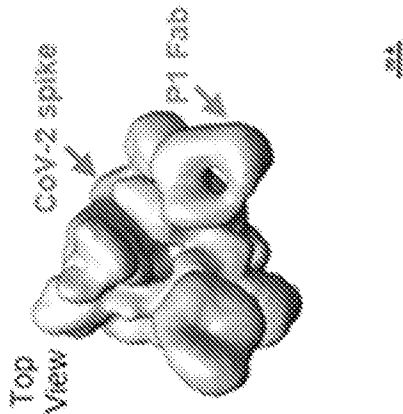
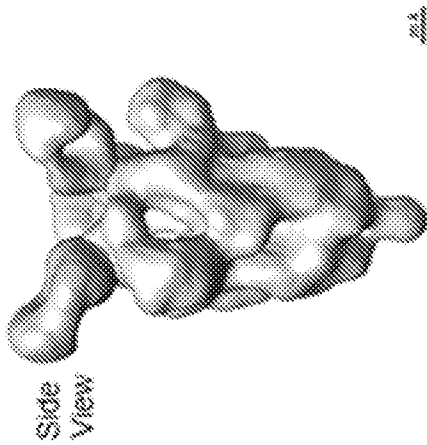
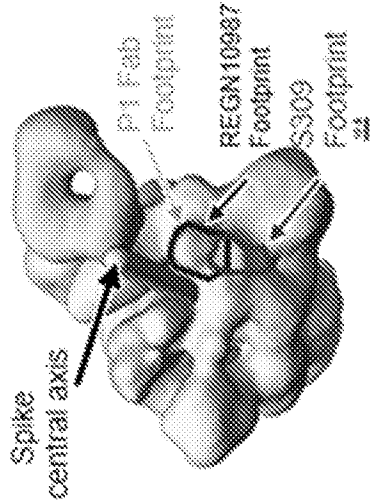
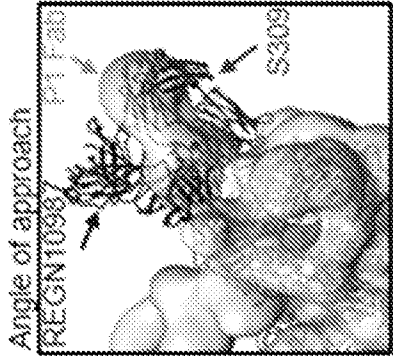


Fig. 4I

Fig. 4H

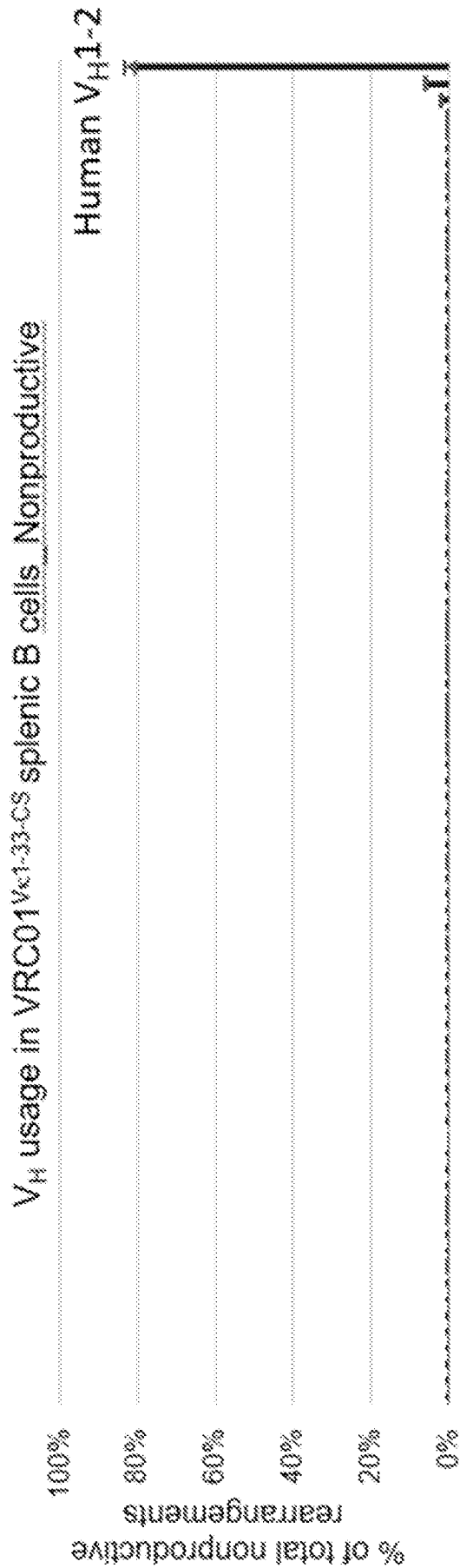


Fig. 5A

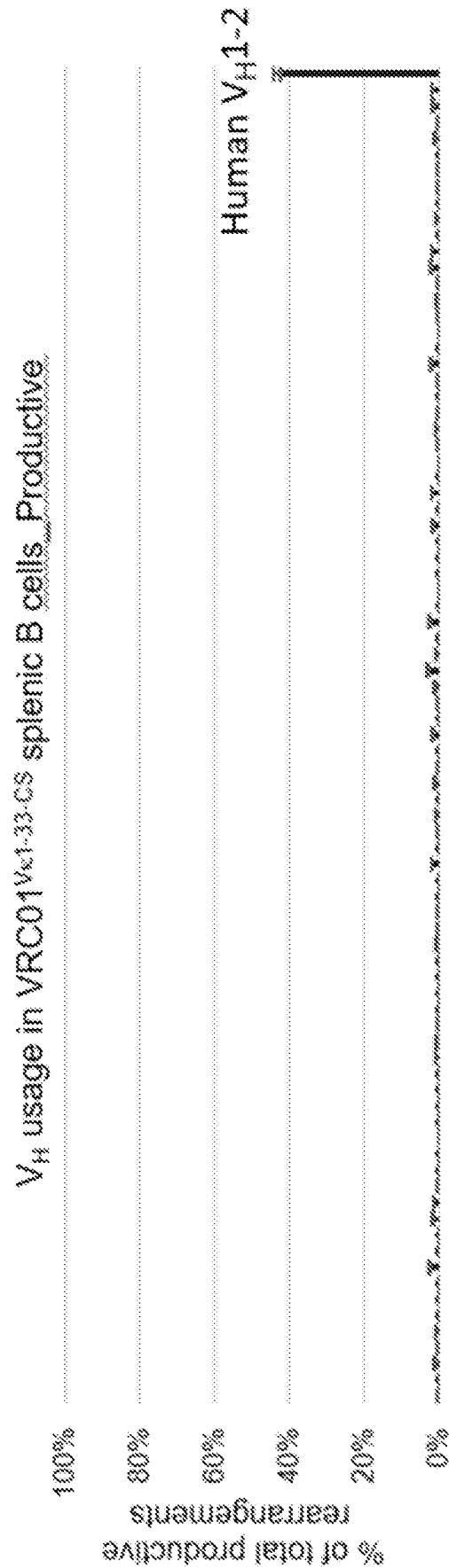


Fig. 5B

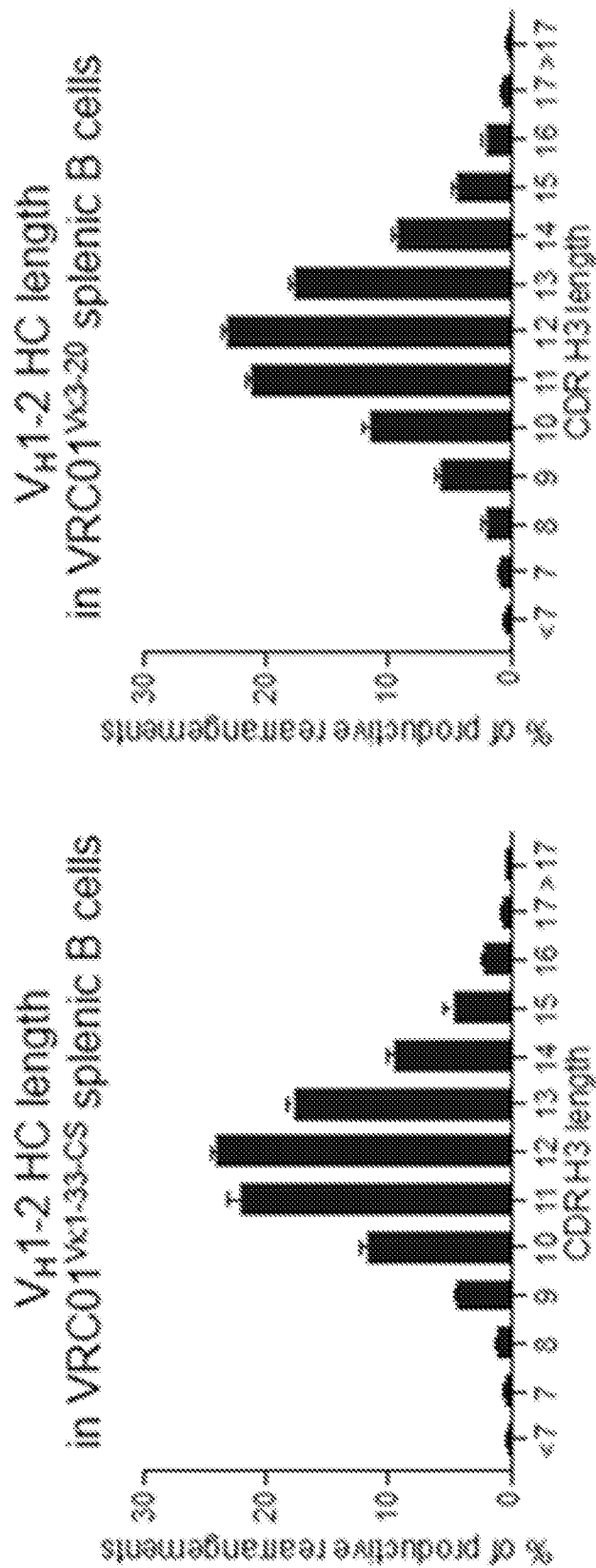


Fig. 5C

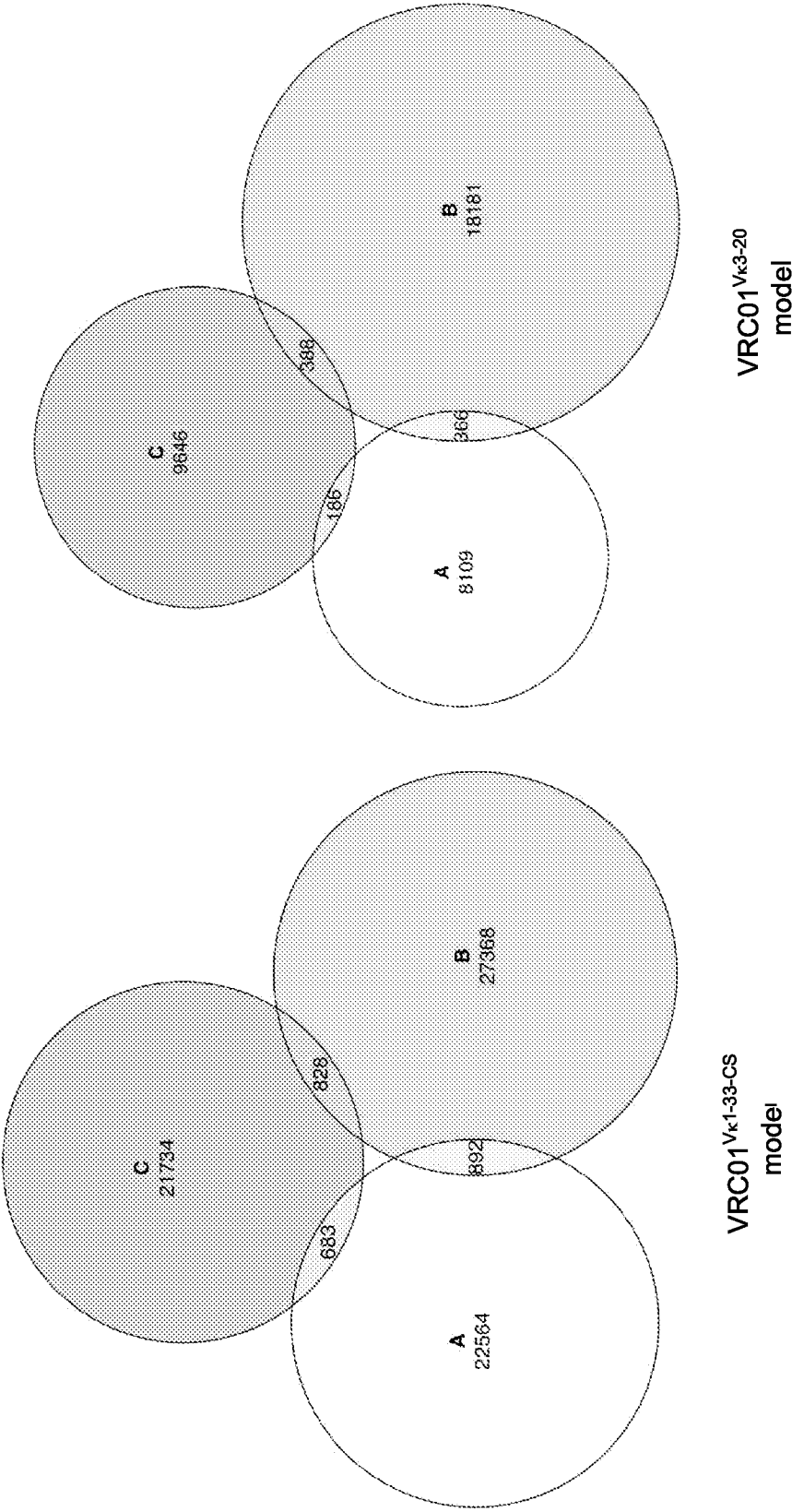


Fig. 5D

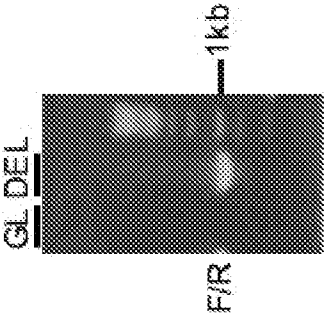


Fig. 6A

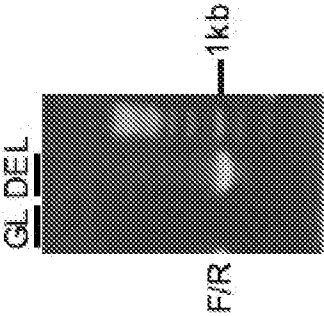


Fig. 6B

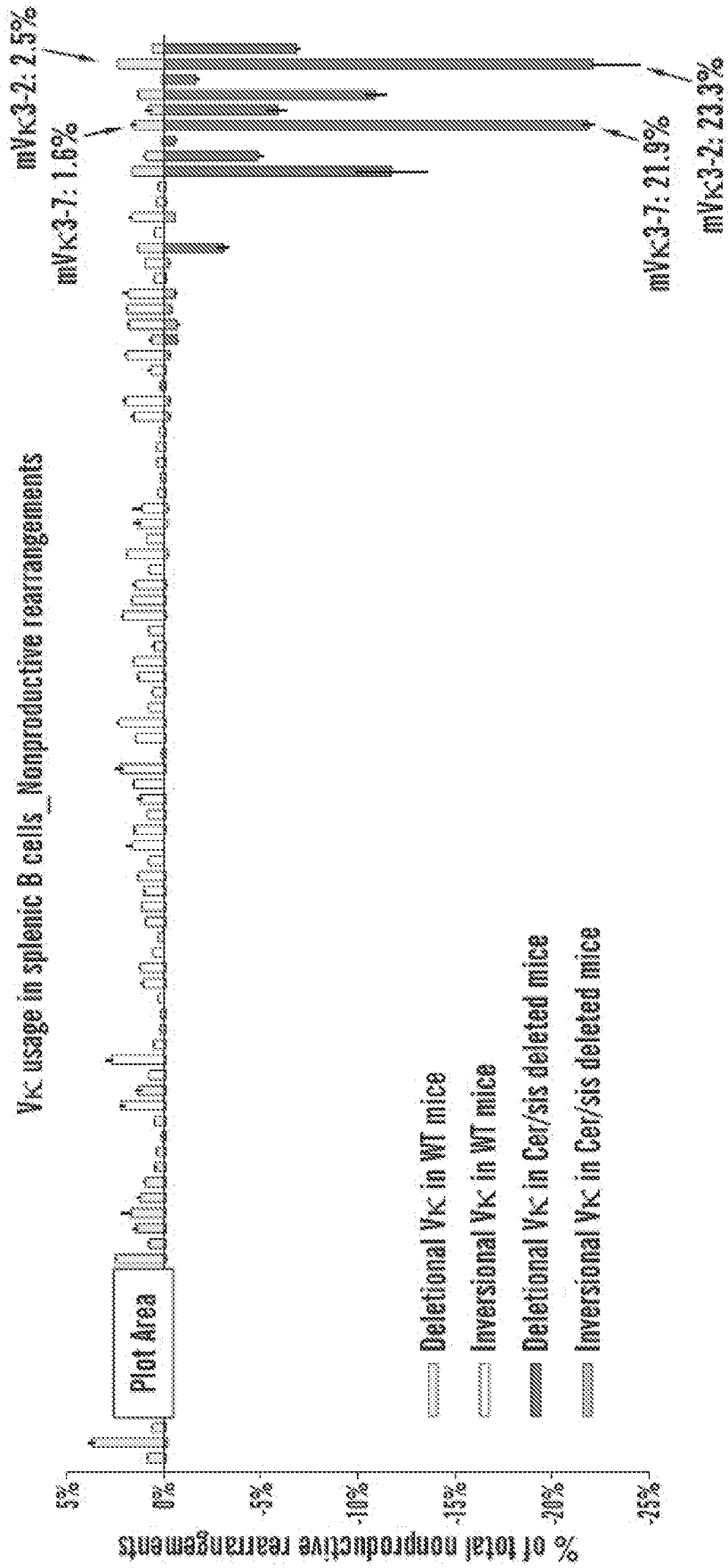
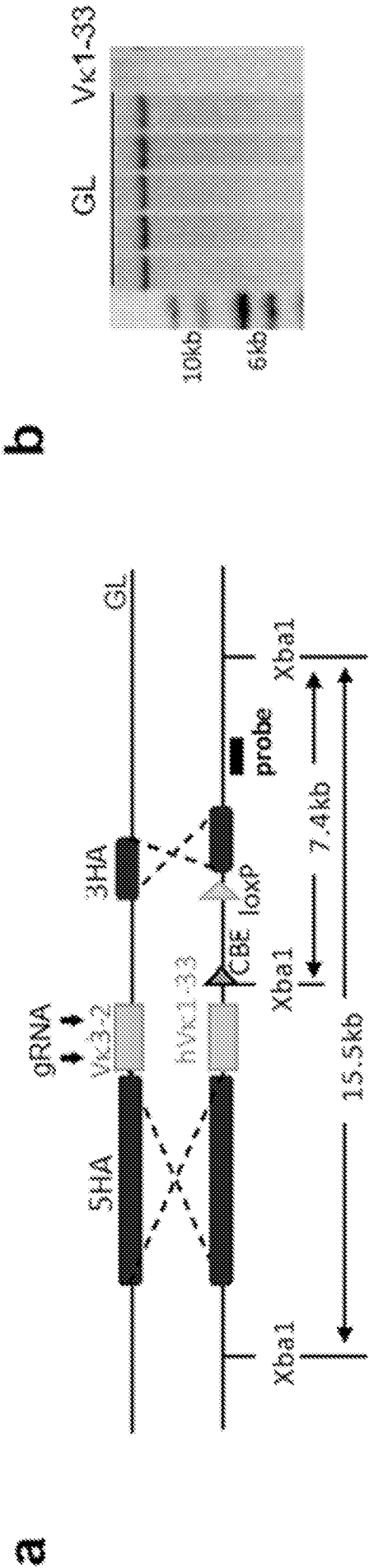


FIG. 6C

Figs. 7A-7B



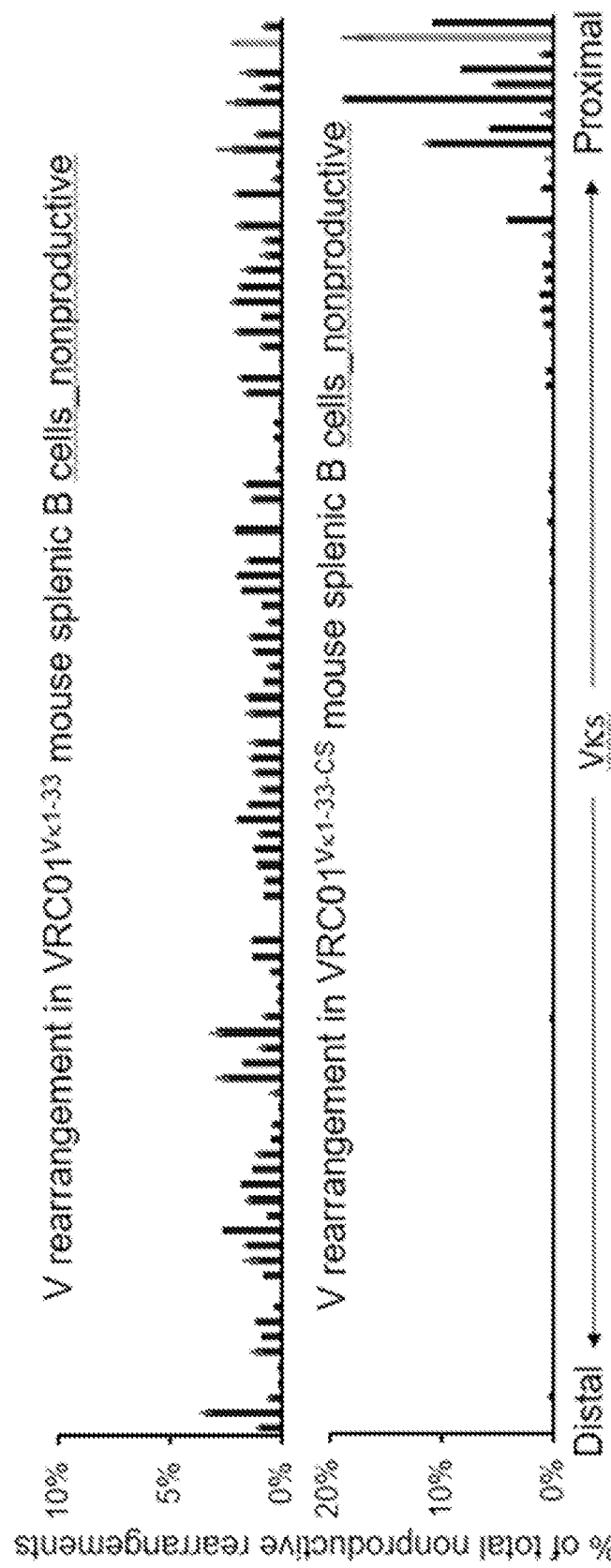
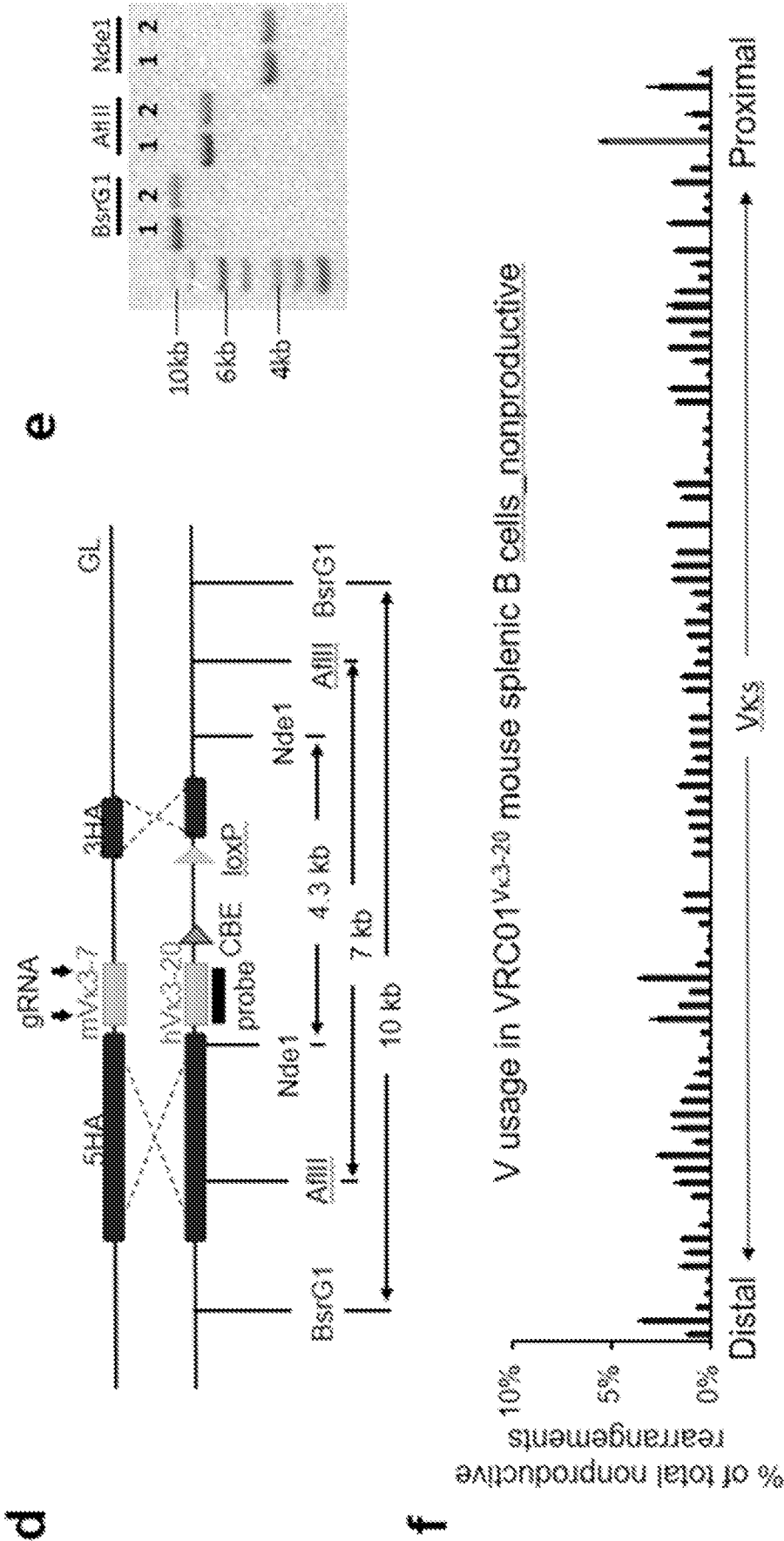


Fig. 7C

Figs. 7D-7F



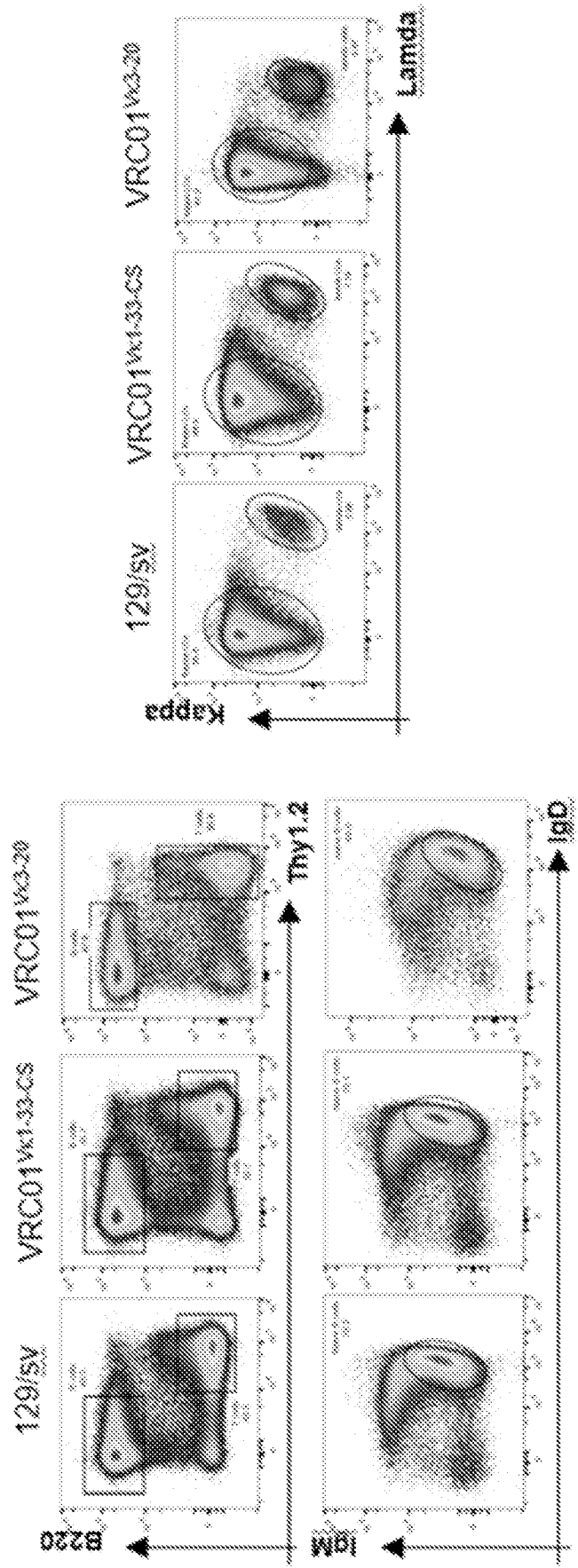
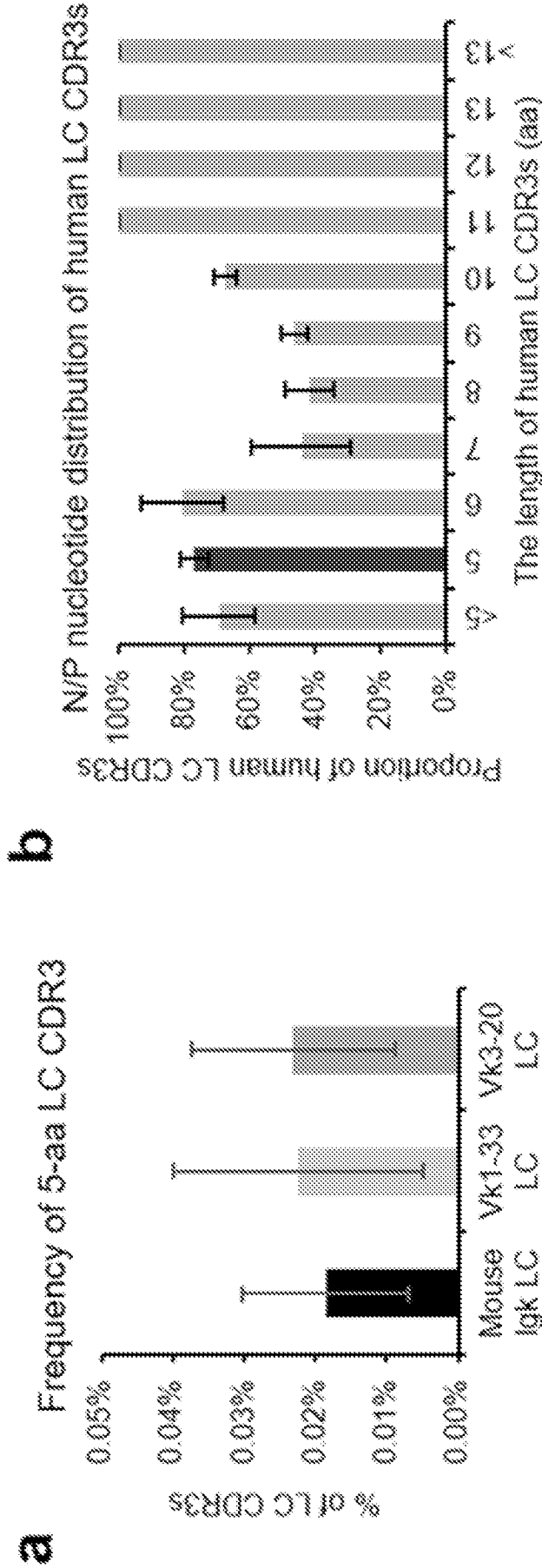
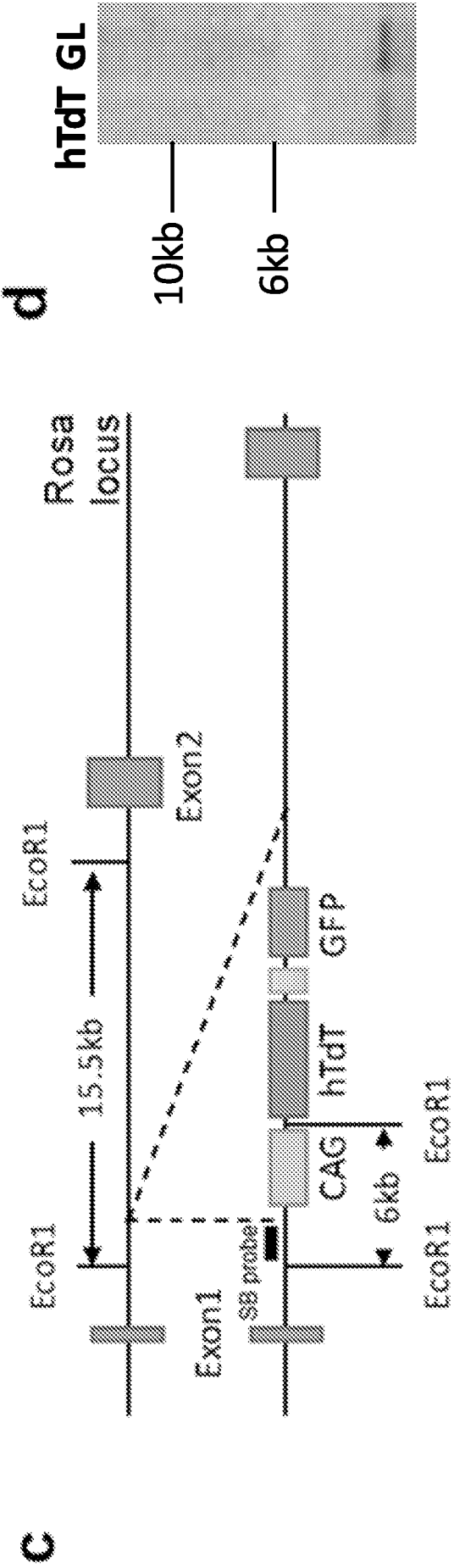


Fig. 7G

Figs. 8A-8B

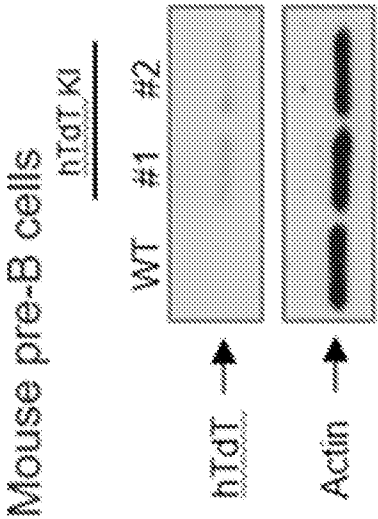


Figs. 8C-8D

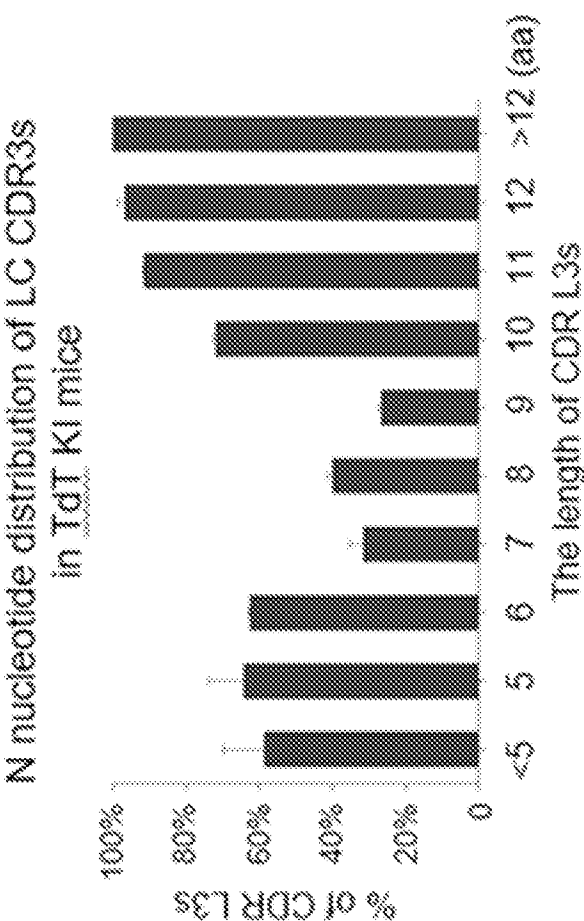


Figs. 8E-8F

e



f



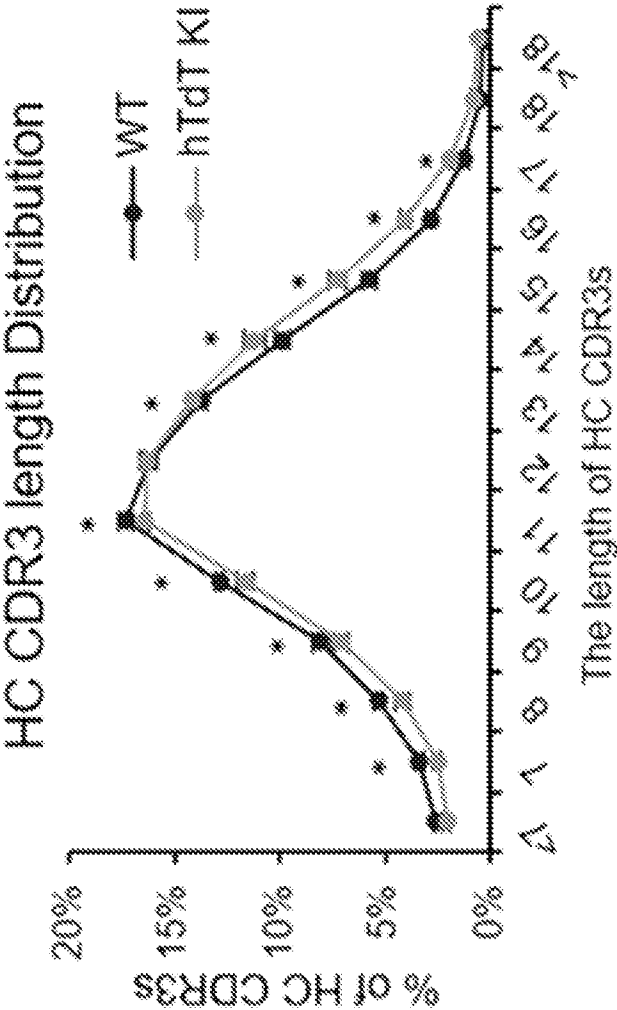
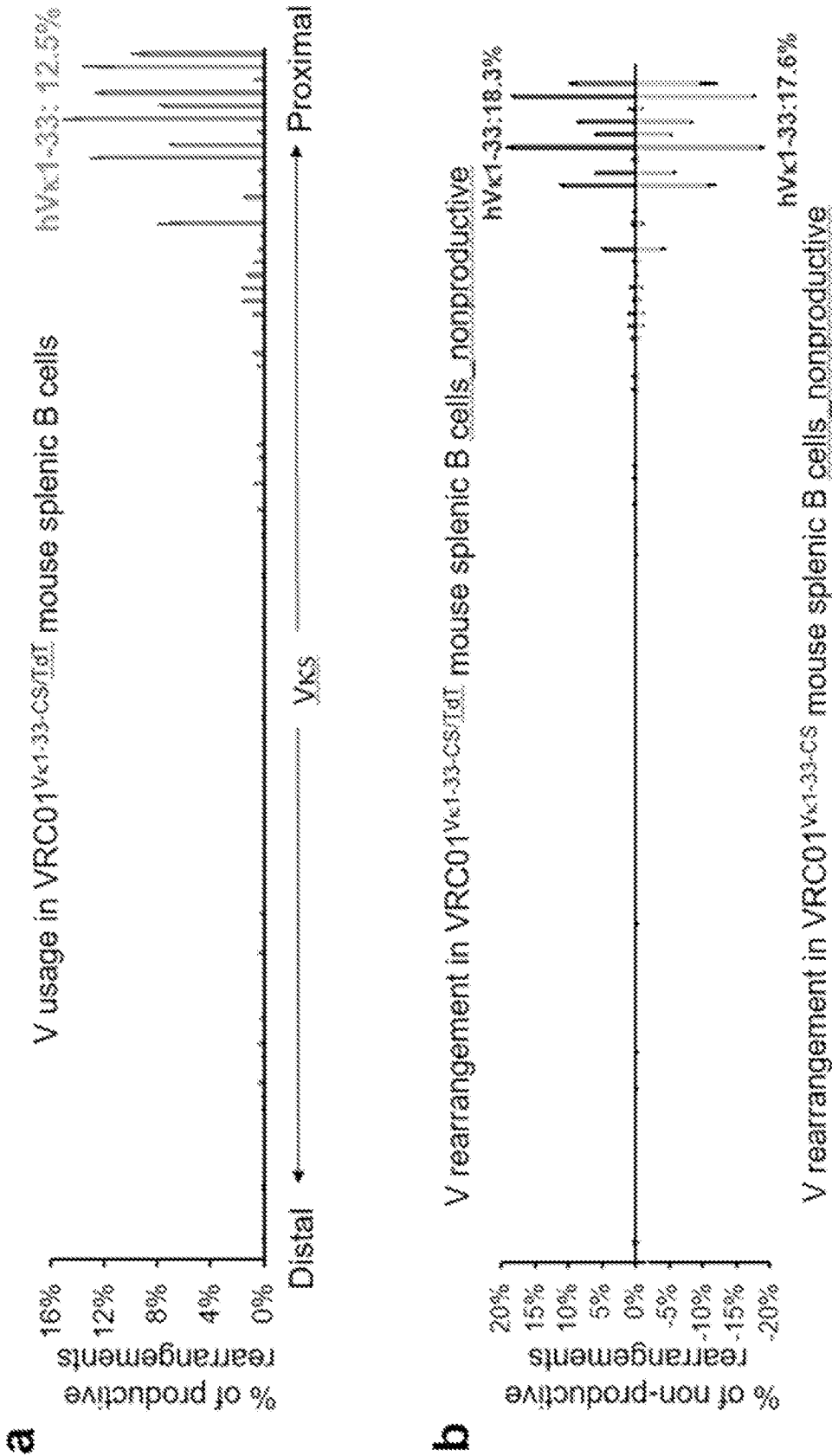
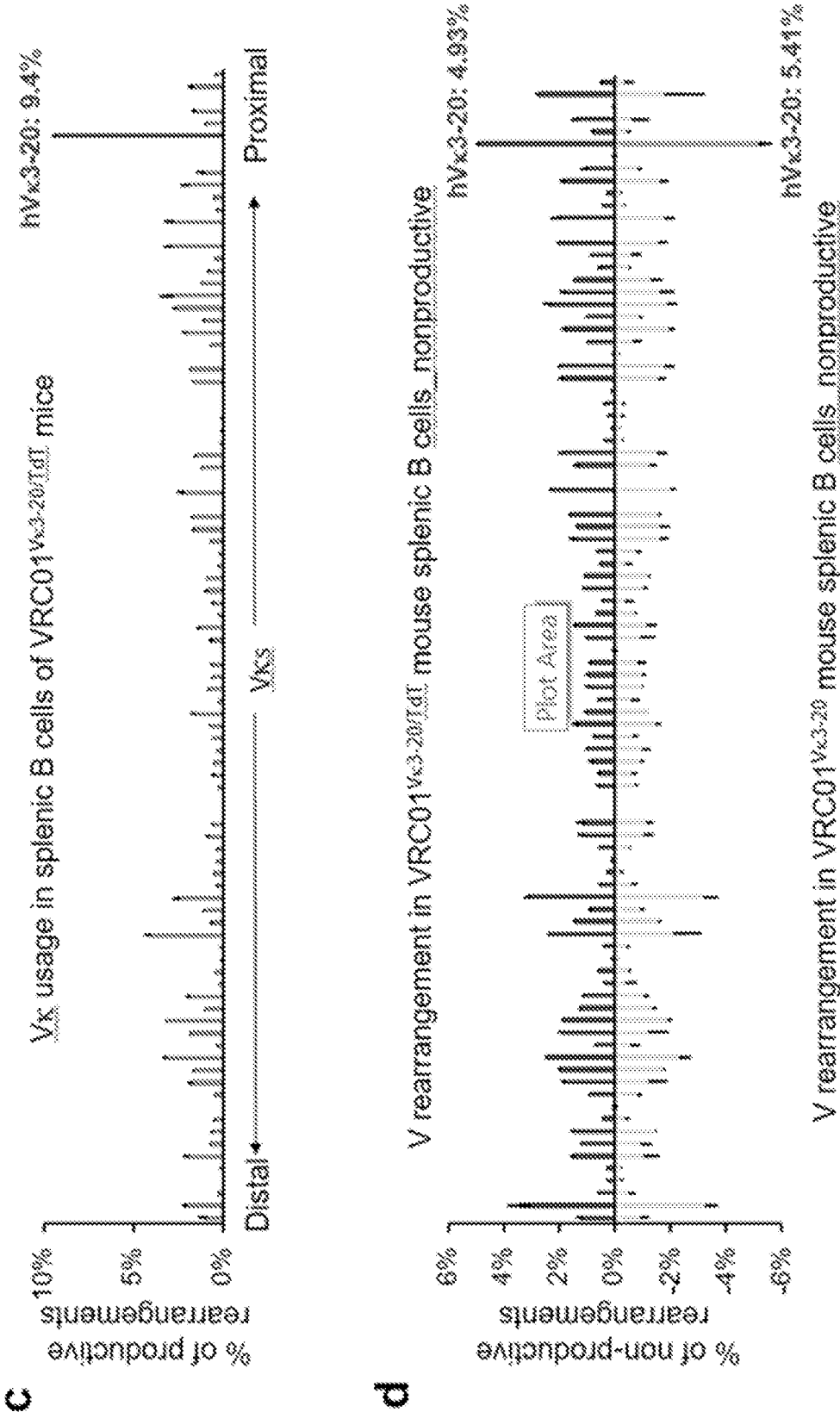


Fig. 8G

Figs. 9A-9B



Figs. 9C-9D



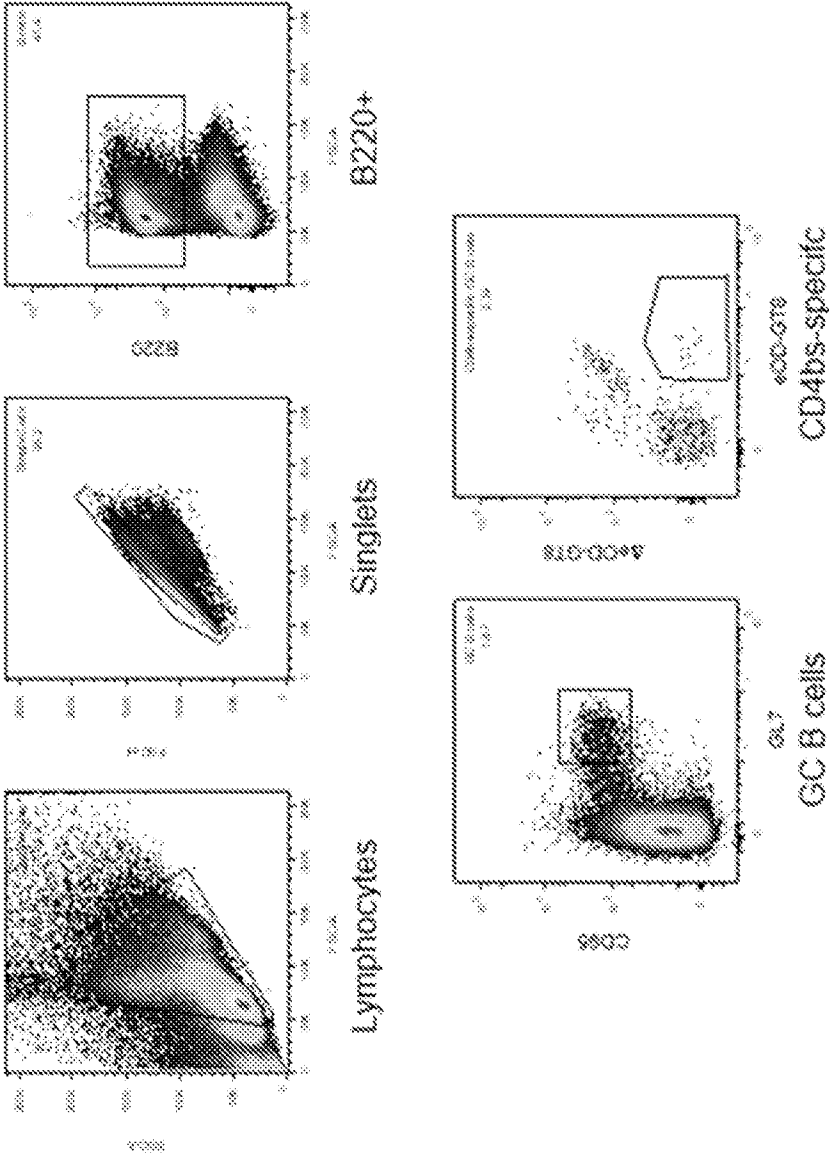


Fig. 10A

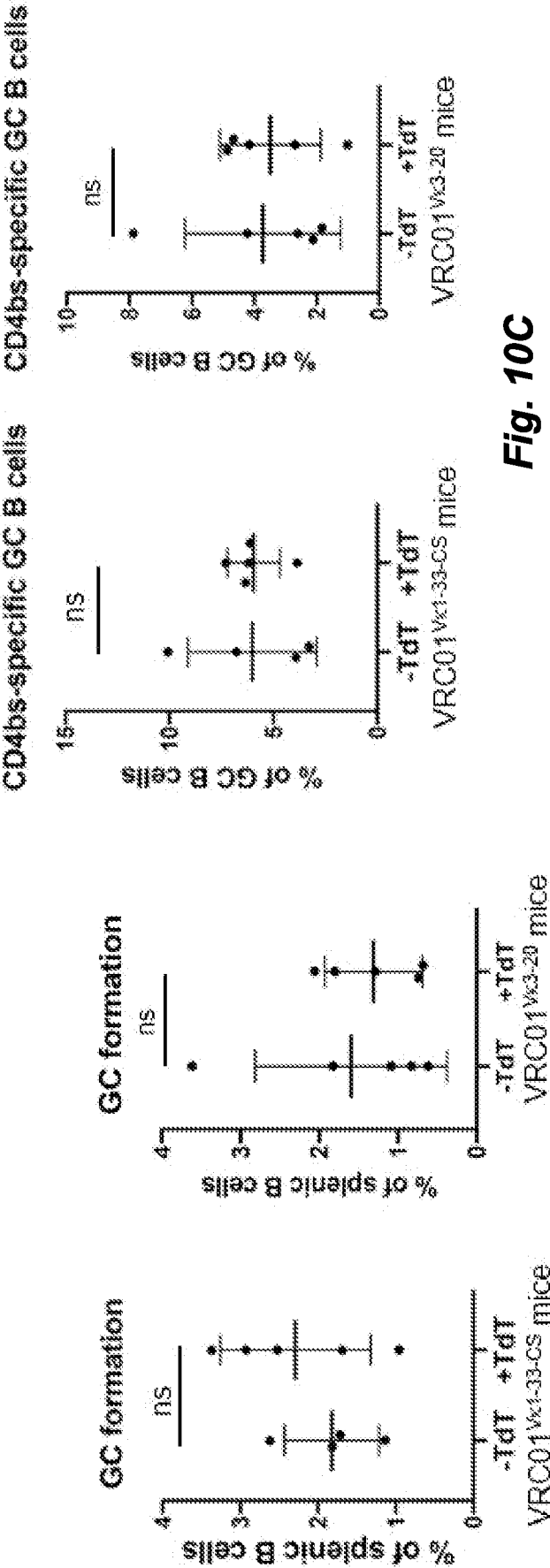


Fig. 10C

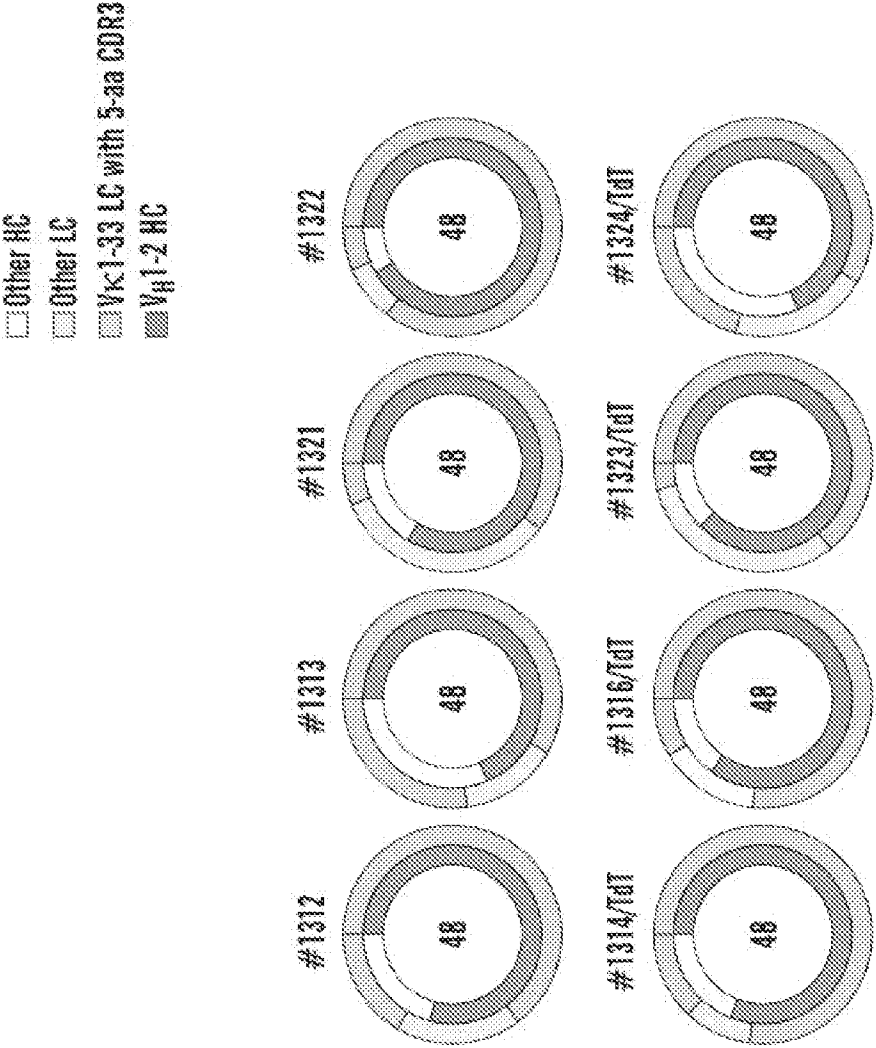


FIG. 10D

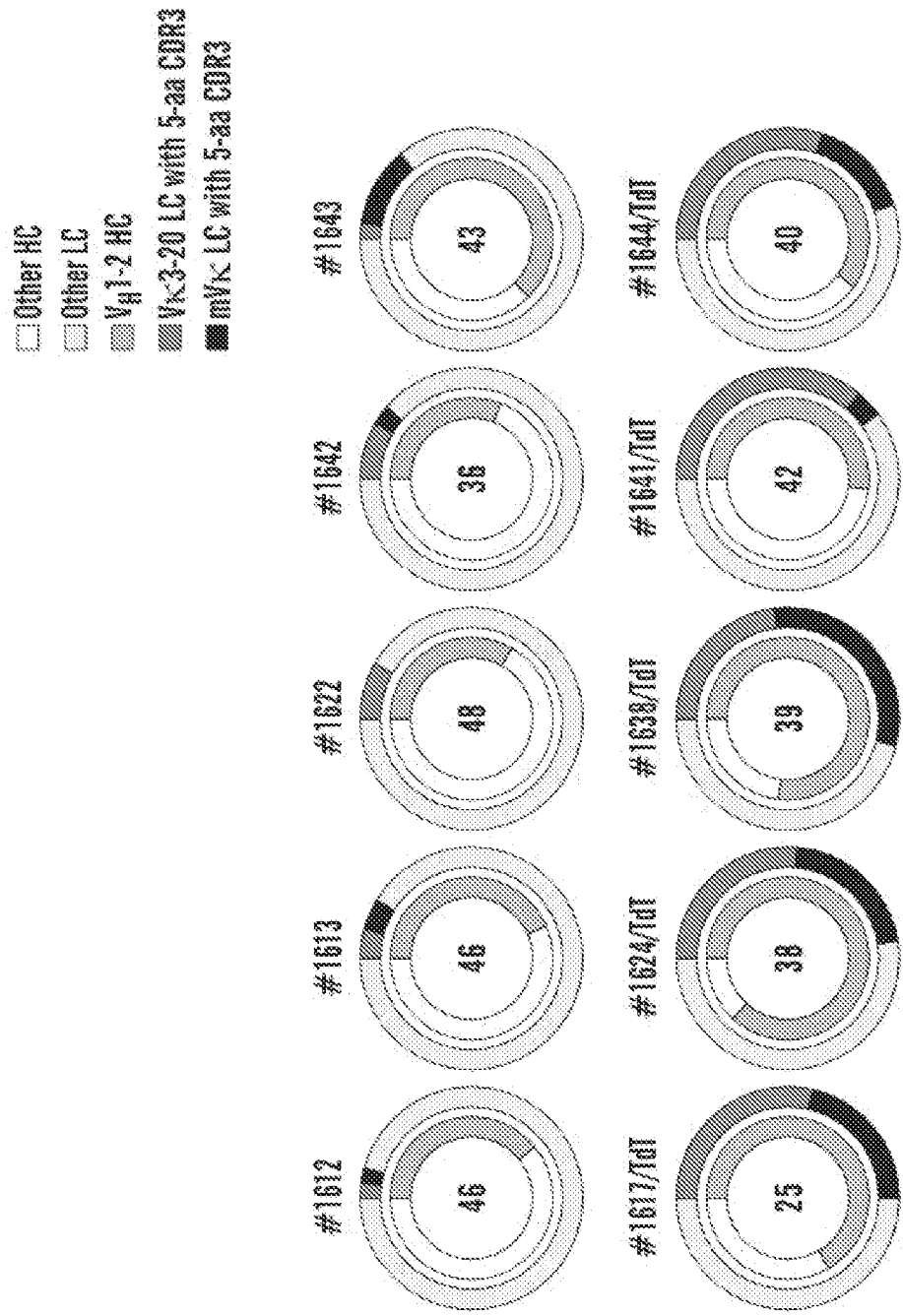
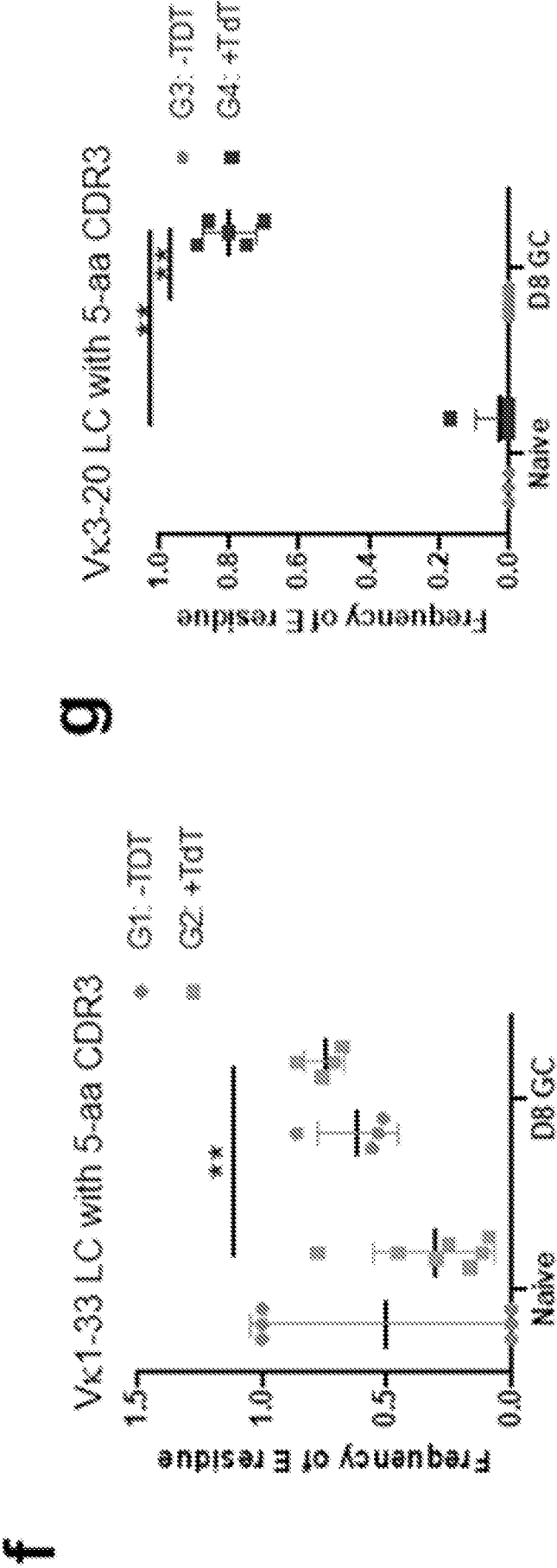


FIG. 10E

Fig. 10F-10G



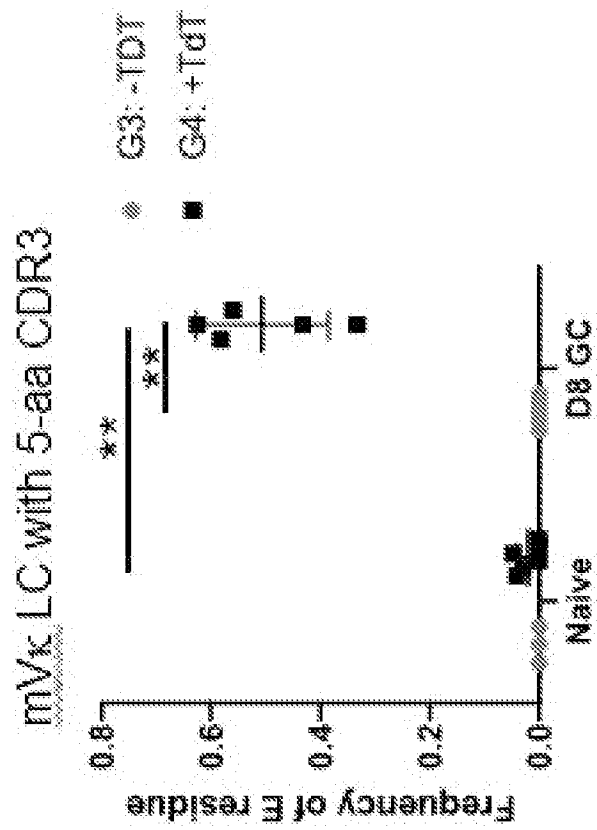
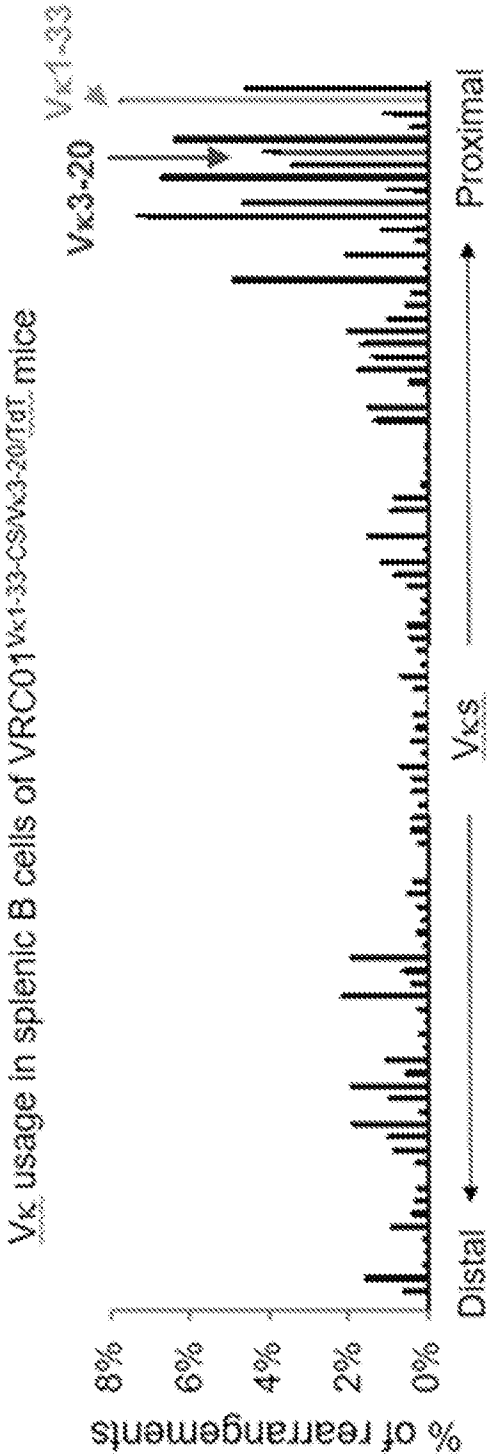


Fig. 10H

Figs. 11A-11B

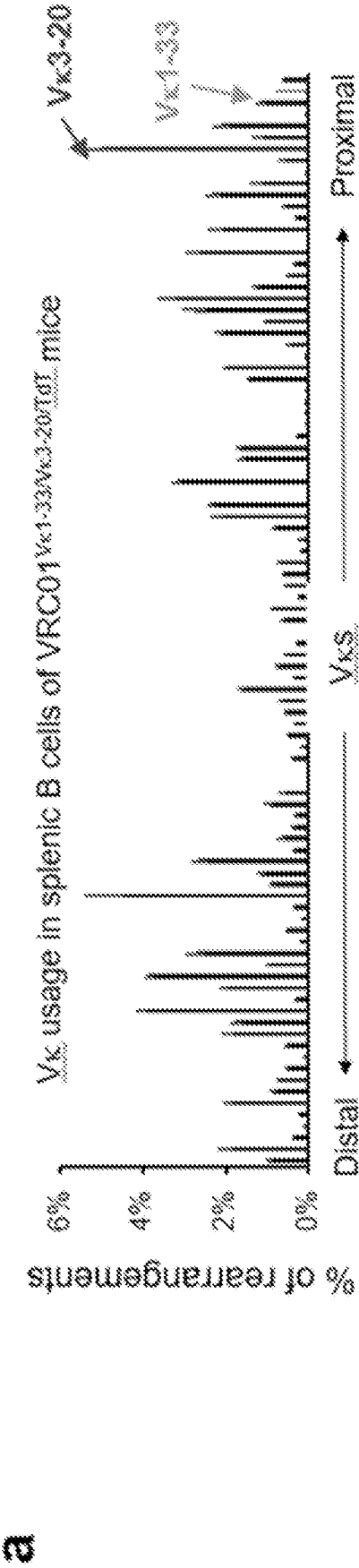
a



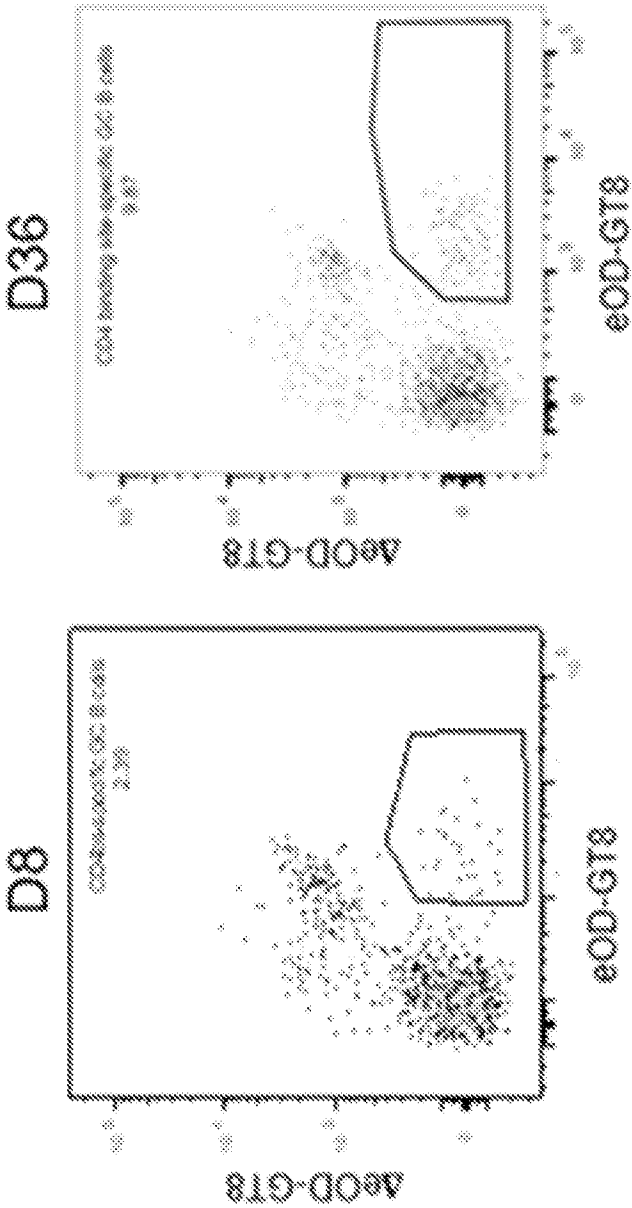
b

	#0131601	#131602	#131603
CD4bs-specific GC B cells	48	48	48
VRC01V _K 1-33 B cells	18	26	28
VRC01V _K 3-20 B cells	1	0	0

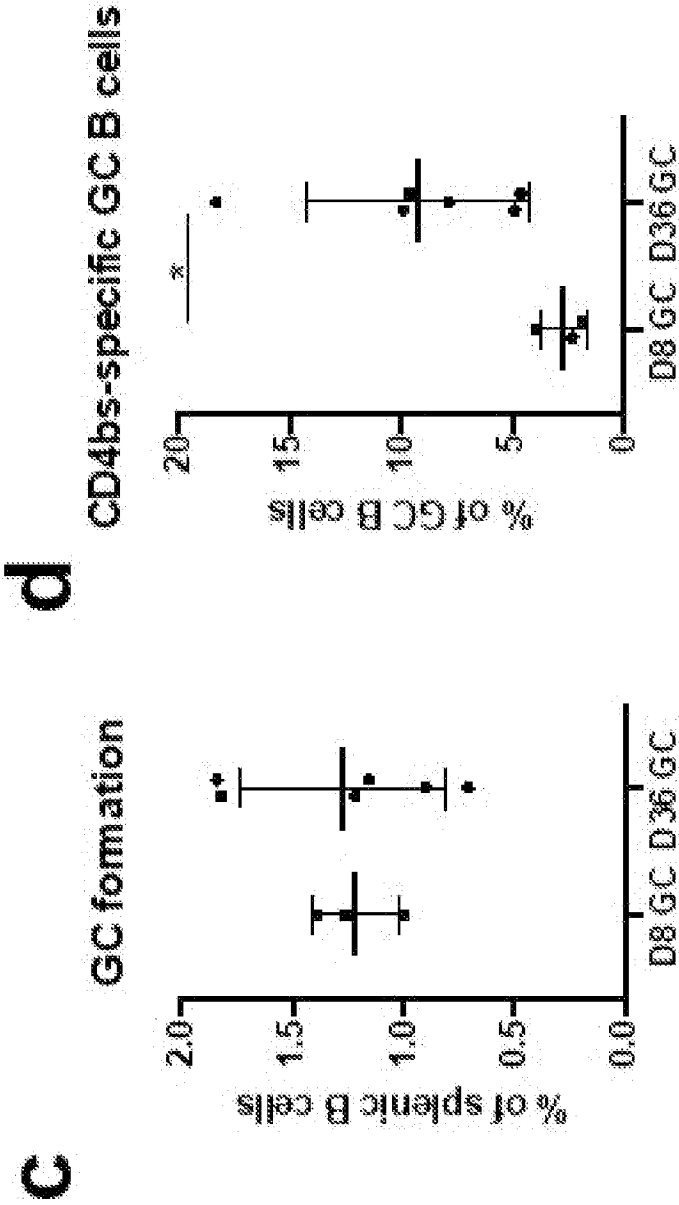
Fig. 12A



b



Figs. 12C-12D



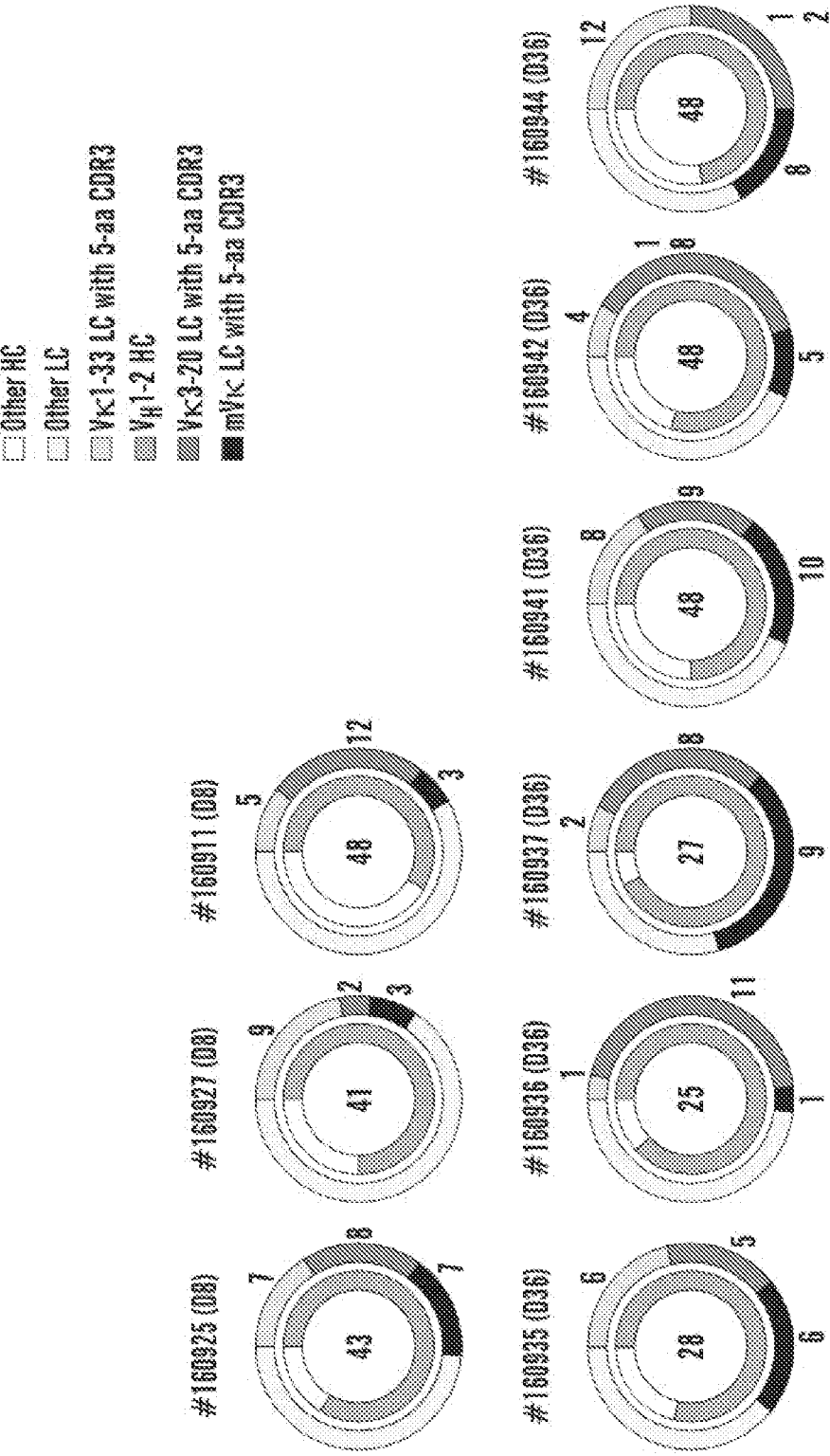


FIG. 12E

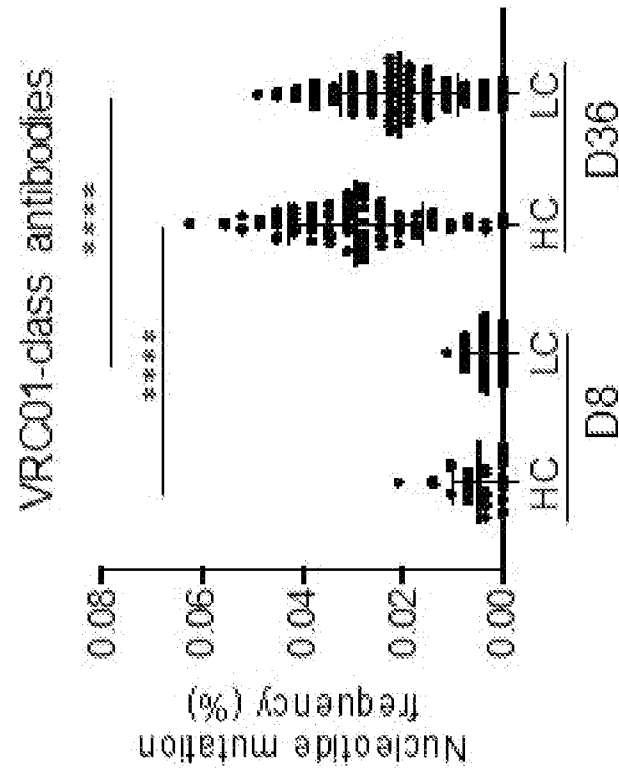


Fig. 12F

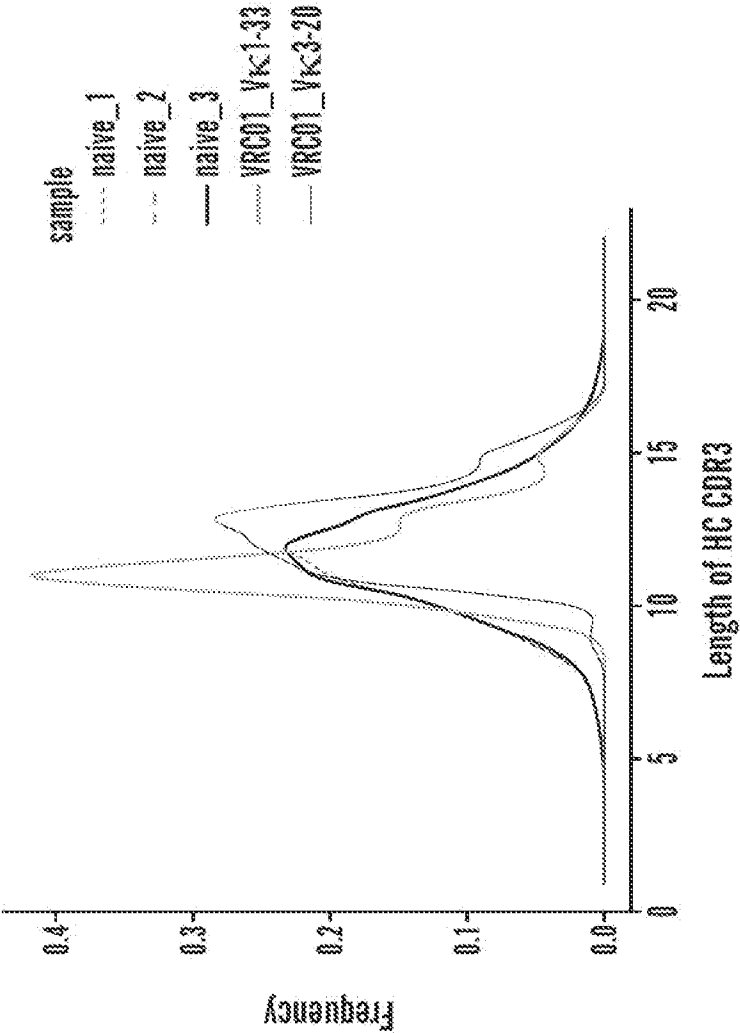


FIG. 12H

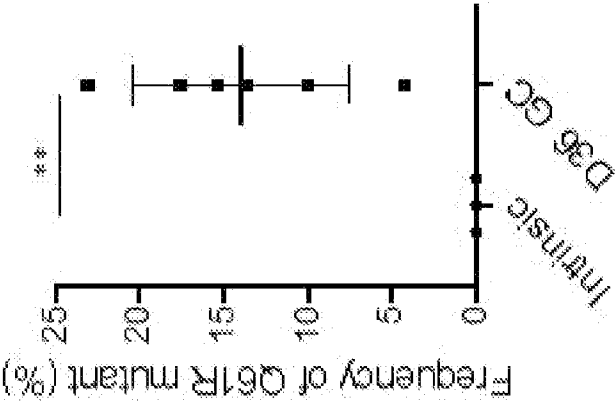


Fig. 12G



Fig. 12I

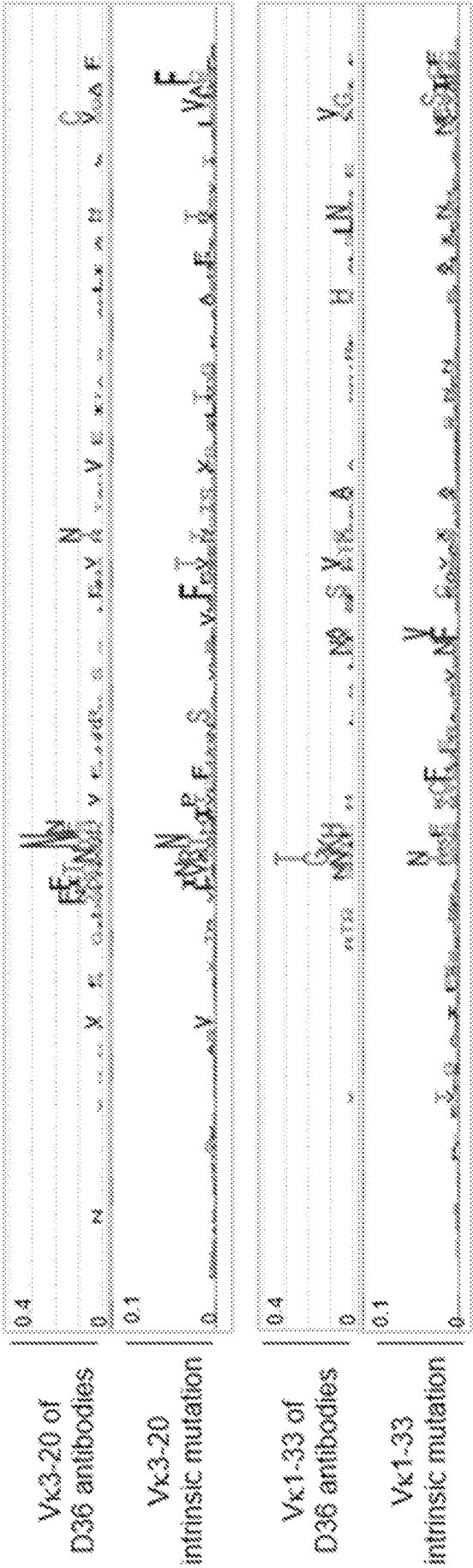
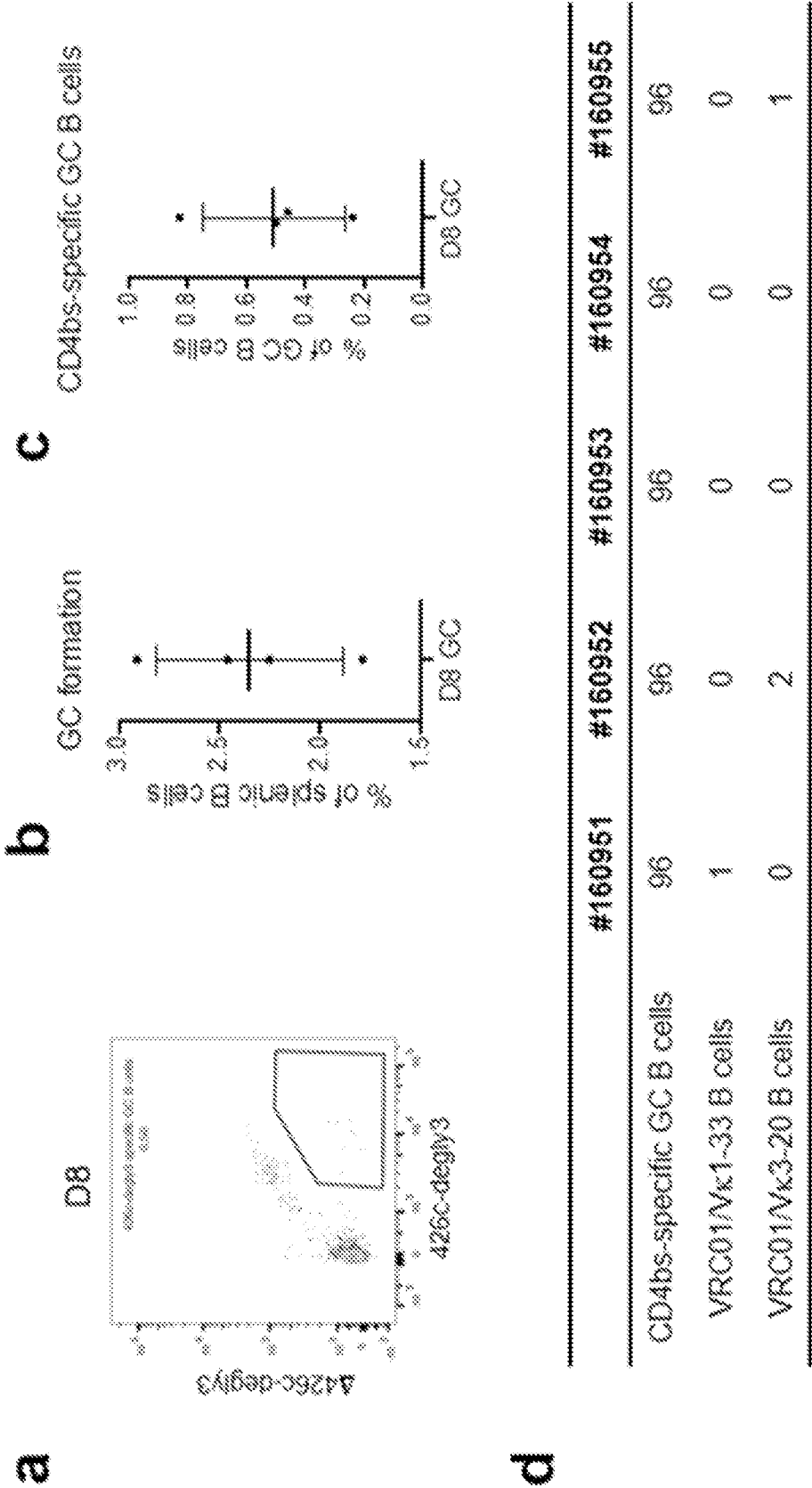


Fig. 12J

Figs. 13A-13D



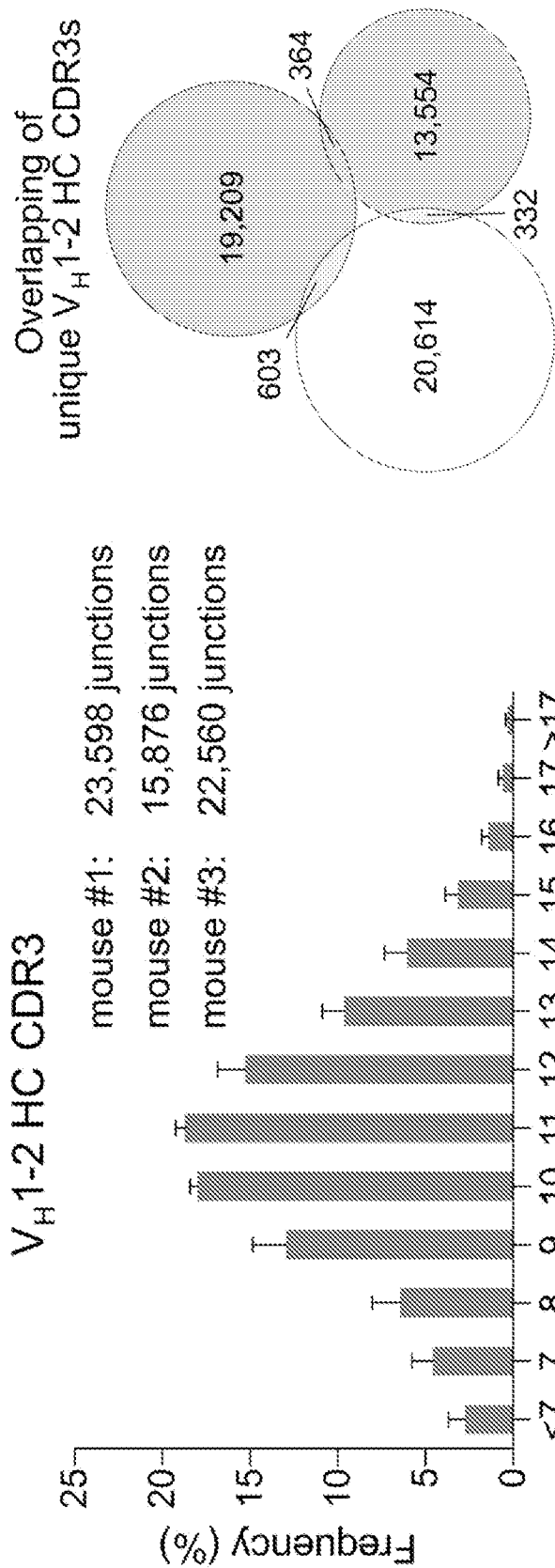


Fig. 14A

Fig. 14B

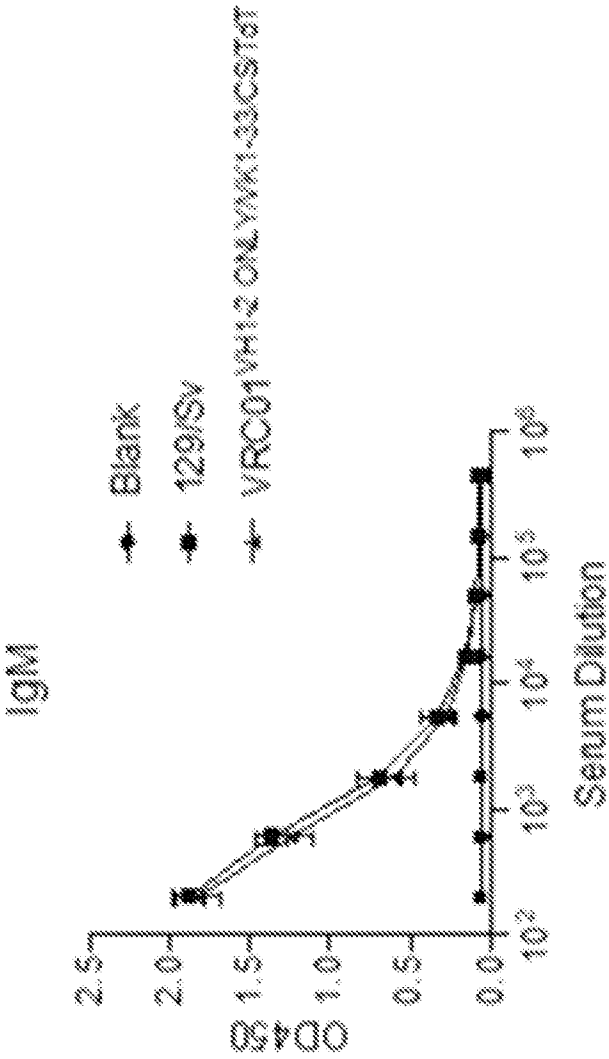
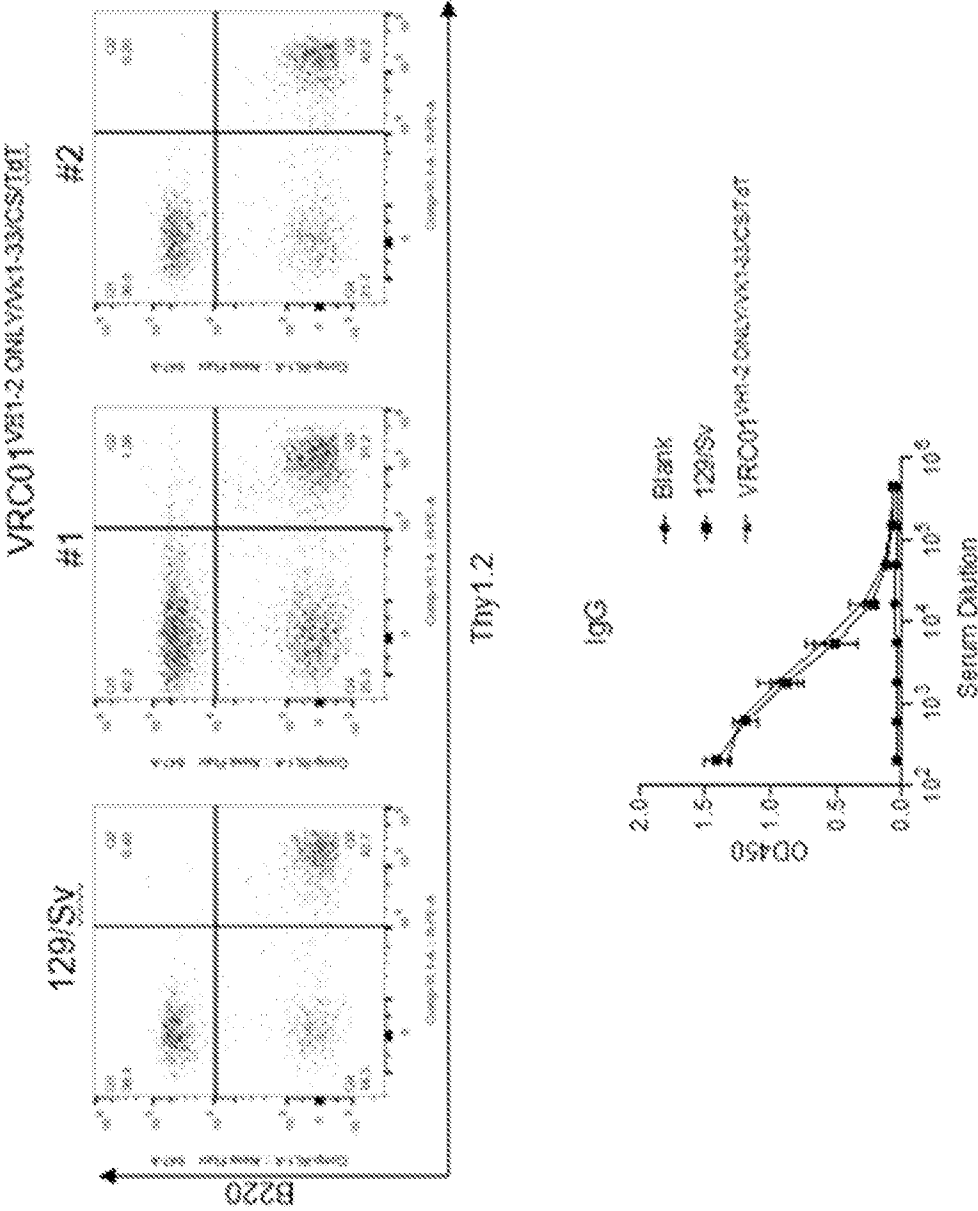


Fig. 14C

Fig. 14D



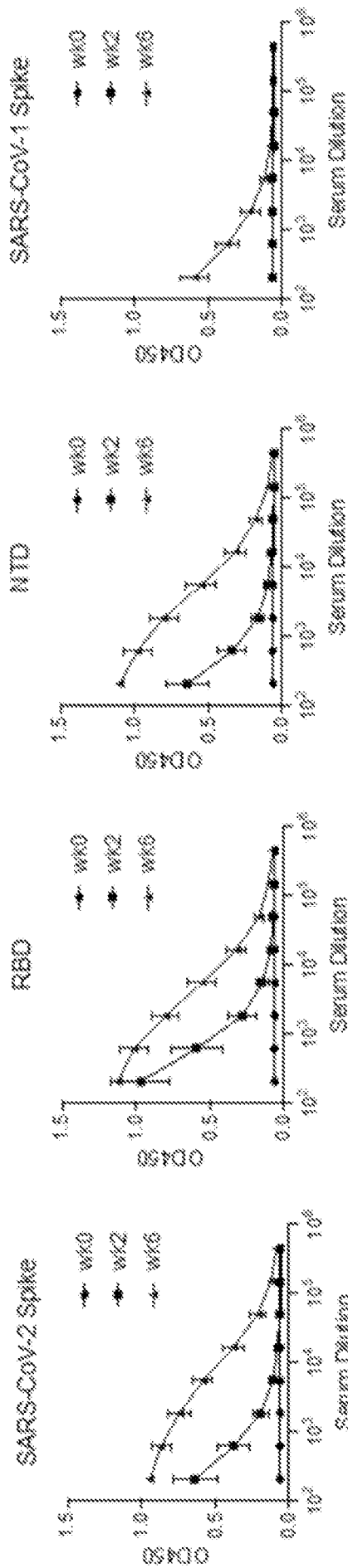


Fig. 14E

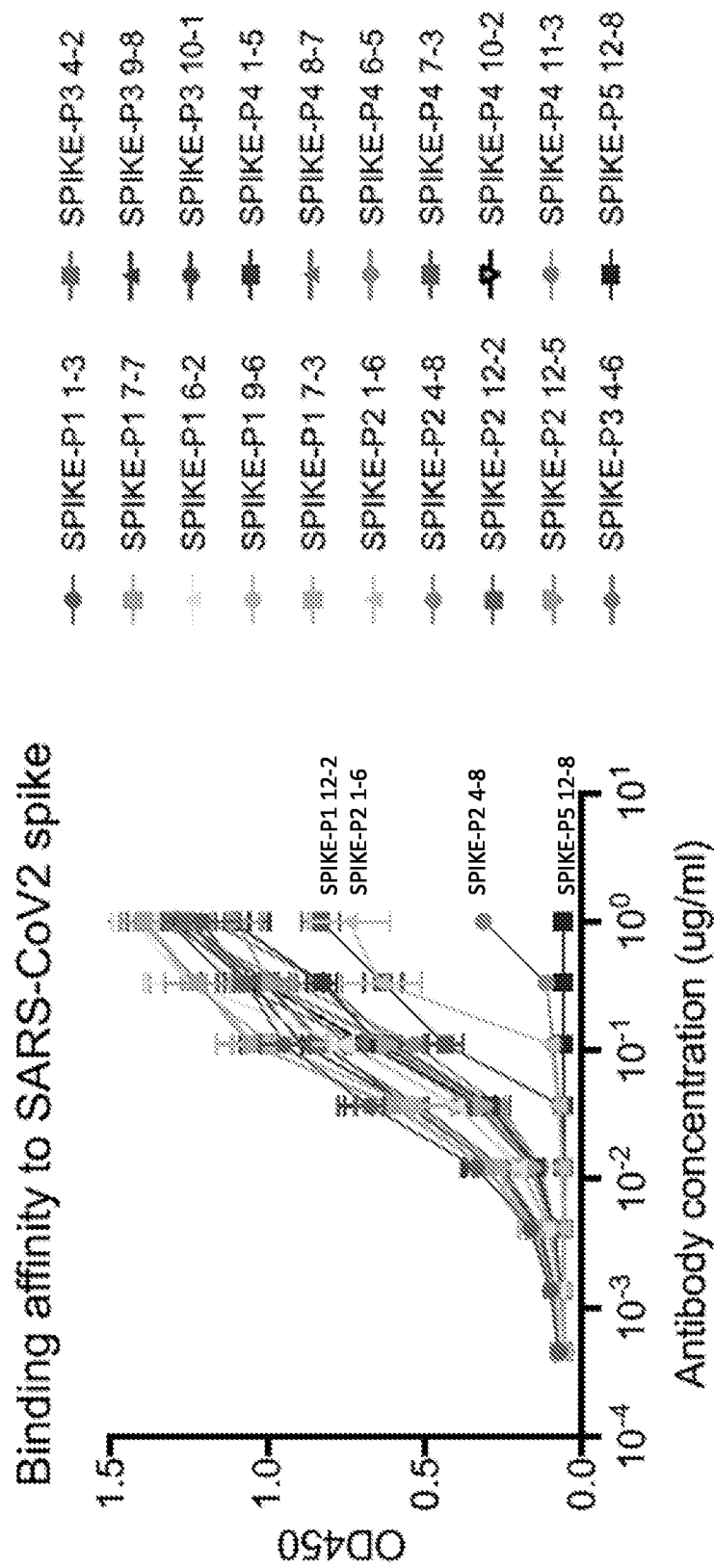


Fig. 14F

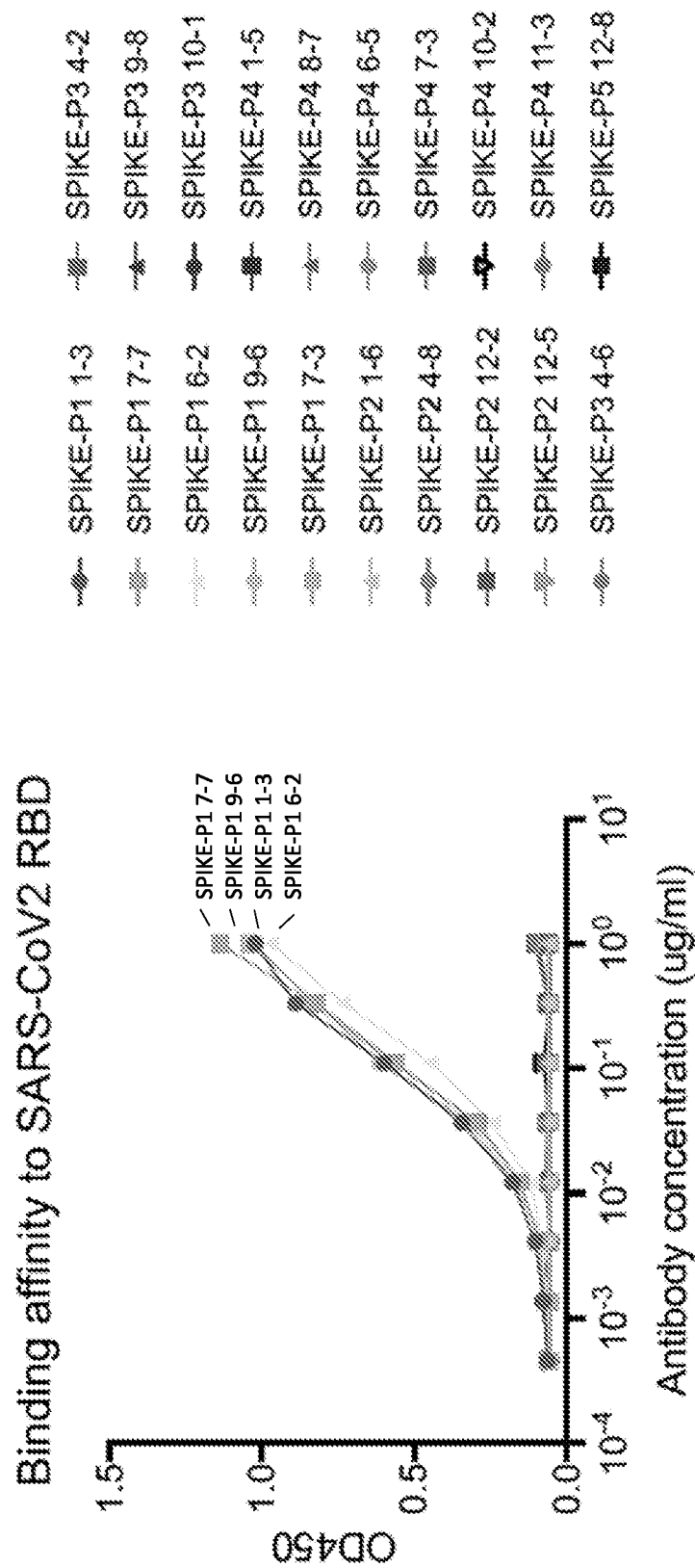


Fig. 14G

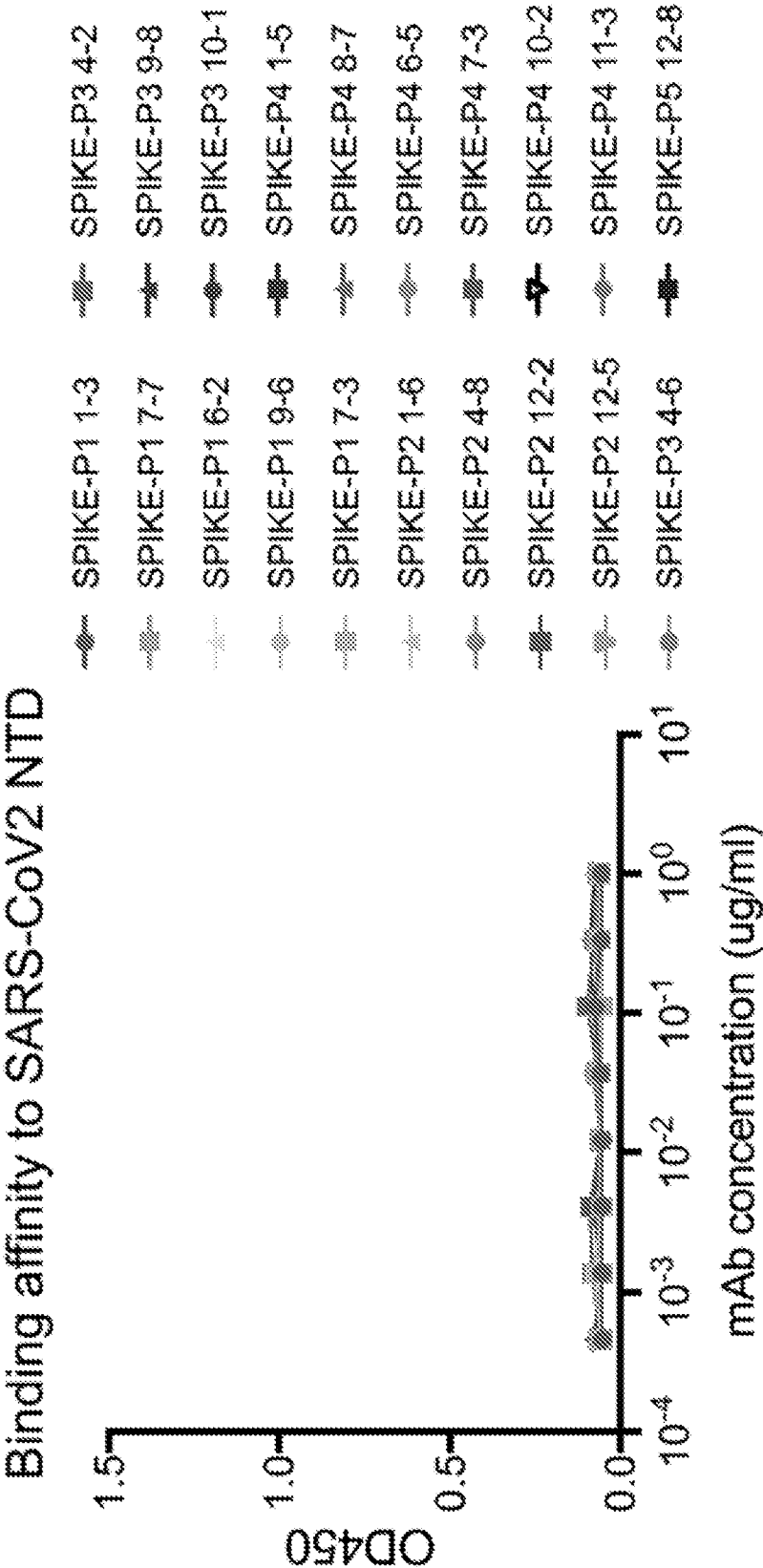


Fig. 14H

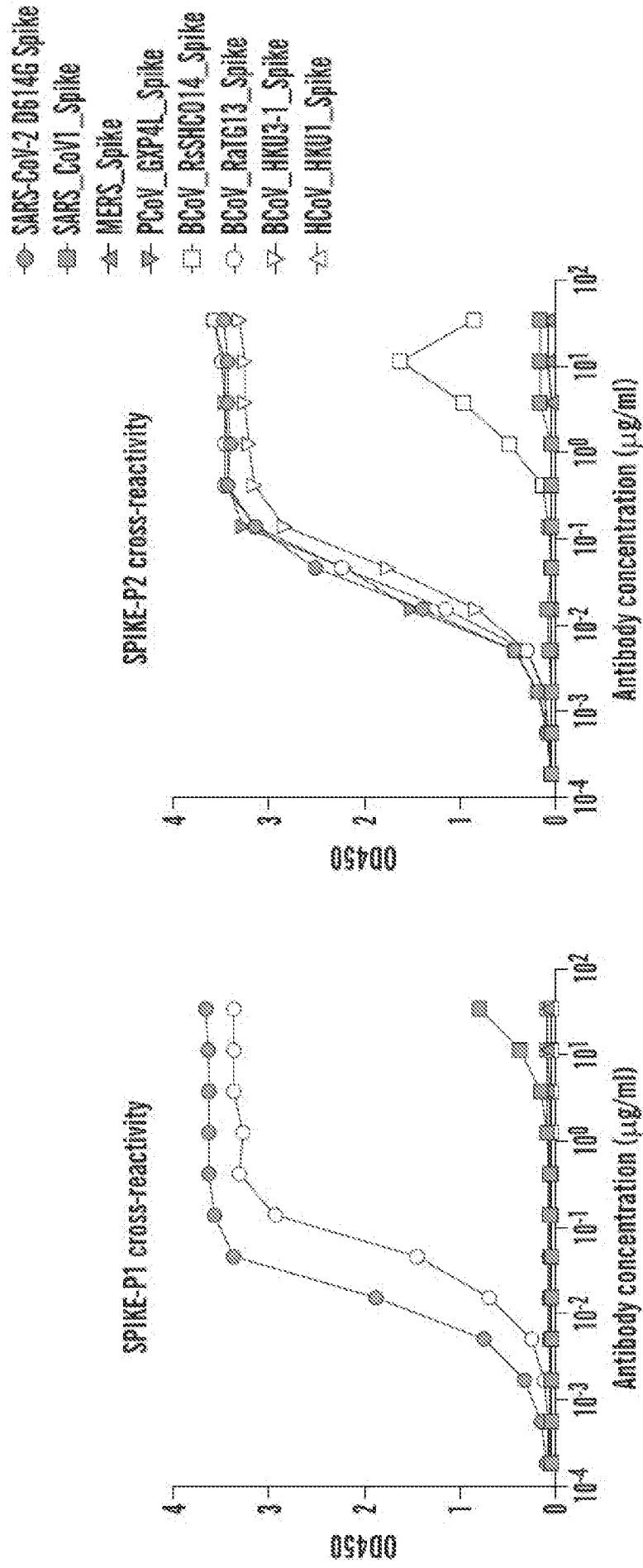


Fig. 14I

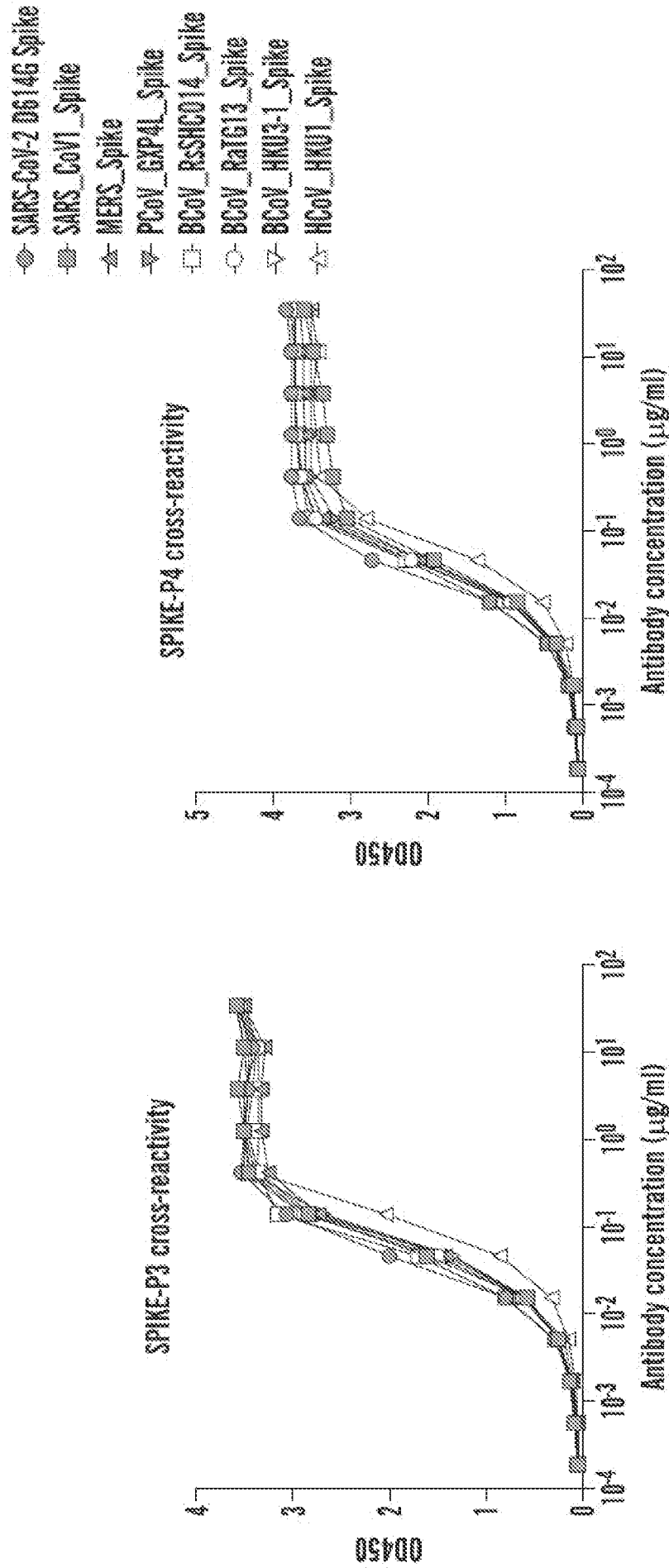


Fig. 14J

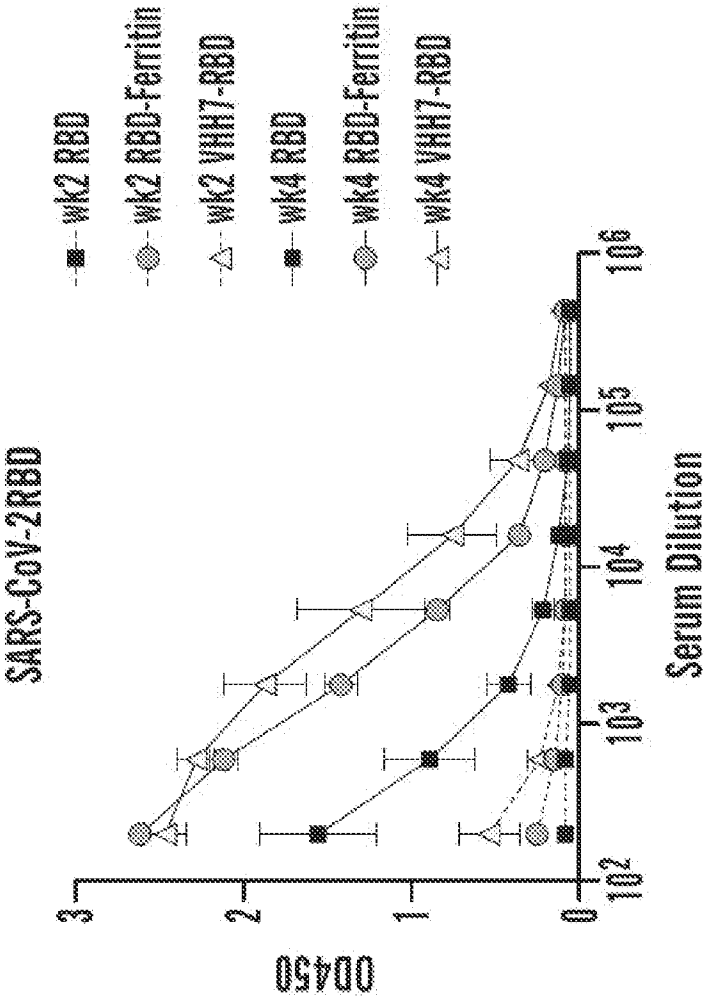


FIG. 15B

	MW	IP
RBD	27kD	13.5ug
RBD-Ferritin	46kD	20ug
VHH7-RBD	43kD	20ug

Fig. 15A

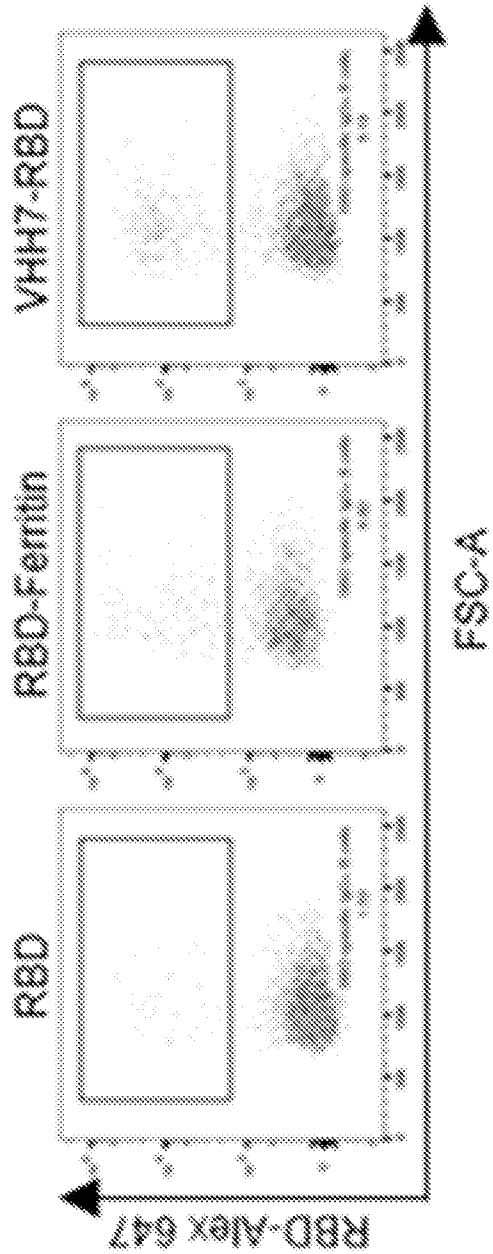


Fig. 15C

B cell lineage	Immunogen	Cells	CDR H3	length of CDR H3	CDR L3	length of CDR L3
SPIKE-P1	CoV2 spike	5	ARDRLVLYGRSYGWYFDV	17 aa	QQYDNLPT	8 aa
R8DNP-P1	R8D-Ferritin	2	ARRERODECTWFAY	14 aa	QQYANLLYT	9 aa
R8DNP-P2	R8D-Ferritin	1	ARPLFDI	7 aa	QQYDALSWT	9 aa
R8DNP-P3	R8D-Ferritin	1	ARDFFNWDGAYFFDY	15 aa	QHYDNLRT	8 aa
R8DNP-P4	R8D-Ferritin	1	ARERGMVTFPDY	12 aa	QQYDNLRT	8 aa
R8DNP-P5	R8D-Ferritin	1	ARRGSPNWGFAY	12 aa	QOHDNLRT	8 aa
R8DNP-P6	R8D-Ferritin	1	AREGGYDGGYGGFY	15 aa	QQYDNLRT	8 aa
R8DNP-P7	R8D-Ferritin	1	ARSYSRYDPYFFDY	14 aa	QQYDNPFSYT	9 aa
R8DNP-P8	R8D-Ferritin	1	ARDRGYGGFAY	11 aa	QQYDNLRT	8 aa
R8DNP-P9	R8D-Ferritin	1	ARCKGNFAWFAY	12 aa	QQYDNLFWT	9 aa
R8DNP-P10	R8D-Ferritin	1	ARDRNSGYG	10 aa	QQYDNLWT	8 aa
R8DNP-P11	R8D-Ferritin	7	ARDRVGGRFFY	11 aa	QQYDNLWT	8 aa
VHH7-P1	VHH7-R8D	1	ARERELTFAY	11 aa	QQYDNLWT	8 aa
VHH7-P3	VHH7-R8D	2	ARDLGQYDAWFAY	13 aa	QQYDNLRWY	9 aa
VHH7-P4	VHH7-R8D	1	ARETMITTFAY	11 aa	QQYDNLWLT	9 aa
VHH7-P5	VHH7-R8D	4	ARDRVGGRFFY	11 aa	QQYDNLWT	8 aa
VHH7-P6	VHH7-R8D	1	ARSYGYDYIAMDY	14 aa	QQYDNLWT	8 aa
VHH7-P7	VHH7-R8D	4	ARRNCDY	7 aa	QQYDNLPLYT	9 aa
VHH7-P8	VHH7-R8D	4	ARRGGGTSWFAY	12 aa	QQYDNLTY	8 aa
VHH7-P9	VHH7-R8D	1	VRRGGNWDERYFDV	15 aa	QQYDNLPLT	9 aa
VHH7-P10	VHH7-R8D	1	ARDPLTGFDY	11 aa	QQYDNLPLYMYT	11 aa

Fig. 15D

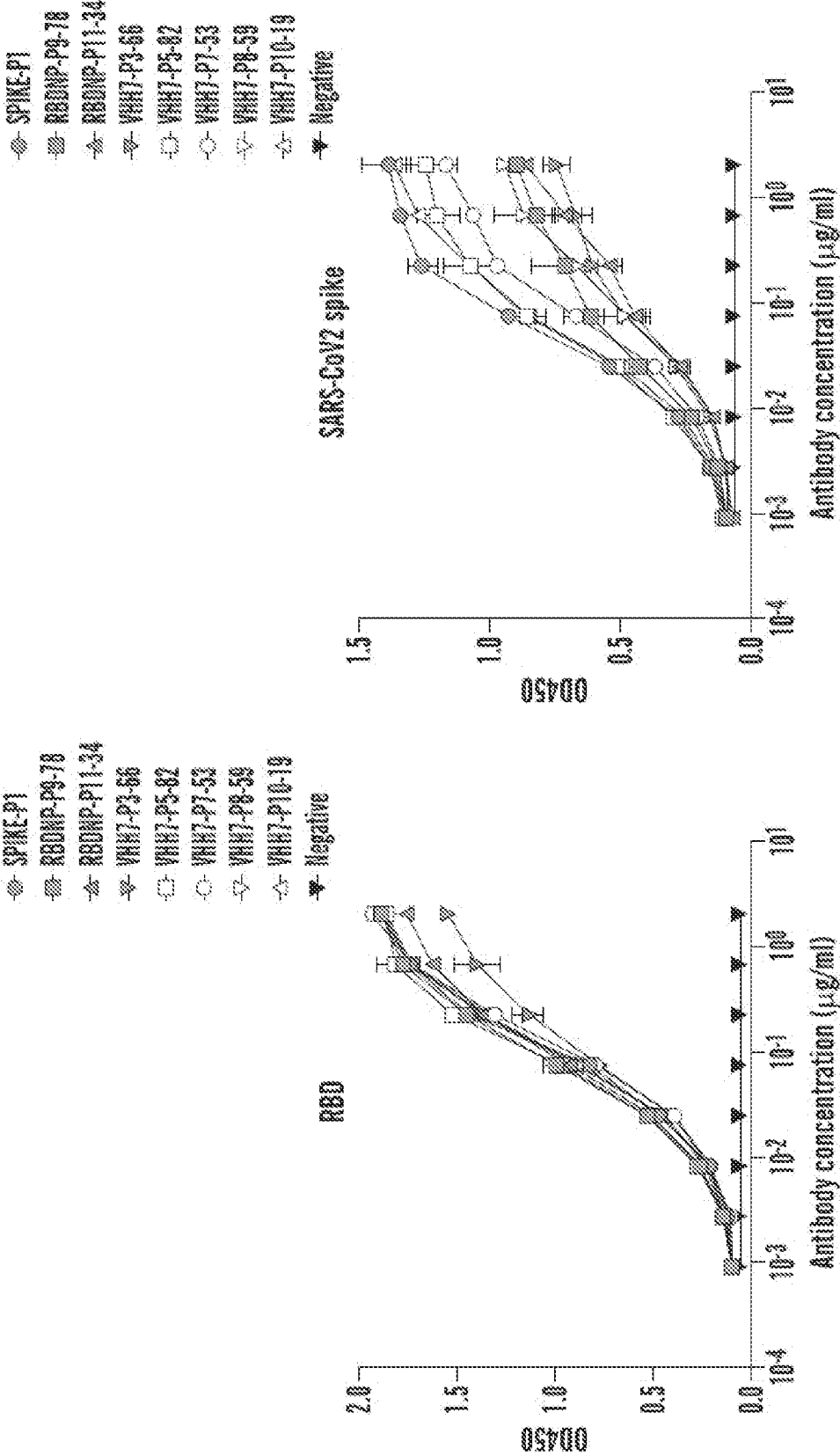


FIG. 15E

Fig. 16

The VH1-2/Vk1-33 antibodies cloned from VH1-2 ONLY mouse model

VH1-2 HC sequence

Antibodies selected from each B cell lineage for expression are boxed

VH1-2	QVQLVQSGAEVKKPGASVKVSCKASGYTFGYYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYC-----	96
SPIKE-P1-37-1-3	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	124
SPIKE-P1-37-6-2	QVQLVQSGAEVKKPGASVKVSCKASGYTFSDSYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	124
SPIKE-P1-37-7-3	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	124
SPIKE-P1-37-7-7	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	124
SPIKE-P1-37-9-4	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	124
BDNP-P6-83-HC78	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	119
BDNP-P11-HC-9	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-23	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-31	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-34	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-47	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-60	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
BDNP-P11-HC-78	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
VH7-2HC-37	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	120
VH7-3HC-66	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	120
VH7-5HC-29	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
VH7-5HC-75	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
VH7-5HC-82	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118
VH7-7HC-53	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	114
VH7-7HC-76	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	114
VH7-7HC-79	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	114
VH7-7HC-85	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	114
VH7-8HC-5	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	119
VH7-8HC-7	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	119
VH7-8HC-59	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	119
VH7-8HC-62	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	119
VH7-10HC-19	QVQLVQSGAEVKKPGASVKVSCKASGYTFDGYTHHWVRQAPGQGLEWMGWINPNSGGTINVAQKFGQGVMTIRDTISISTAYMELSLRSDDTAVYYCQRVYQWFFDWMQDQDTITVSS	118

Fig. 18

Pseudovirus Neutralization - IC50 (ug/ml)

SP1-77 antibody

	D614G or WA-1	B.1.1.7	B.1.351 (Beta)	P.1 (gamma)	B.1.429	B.1.526 E484K	B.1.617.2 (delta)	B.1.1.529 (omicron)
Facility 1	0.019	0.027	0.018	0.016	0.039	0.013	0.082	0.004
Facility 2	0.047	0.077	0.013	0.070	0.066	0.016	0.556	0.039

VHH7-5-82 antibody

	D614G or WA-1	B.1.1.7	B.1.351	P.1	B.1.429	B.1.526 E484K	B.1.617.2	B.1.1.529
Facility 1	0.039	-	>10	>10	>10	>10	>10	>10
Facility 2	0.082	0.081	>10	>10	>10	>10	>10	>10

VHH7-7-53 antibody

	D614G or WA-1	B.1.1.7	B.1.351	P.1	B.1.429	B.1.526 E484K	B.1.617.2	B.1.1.529
Facility 1	0.060	-	>10	>10	0.062	>10	0.088	>10
Facility 2	0.162	0.127	>10	>10	0.126	>10	0.131	>10

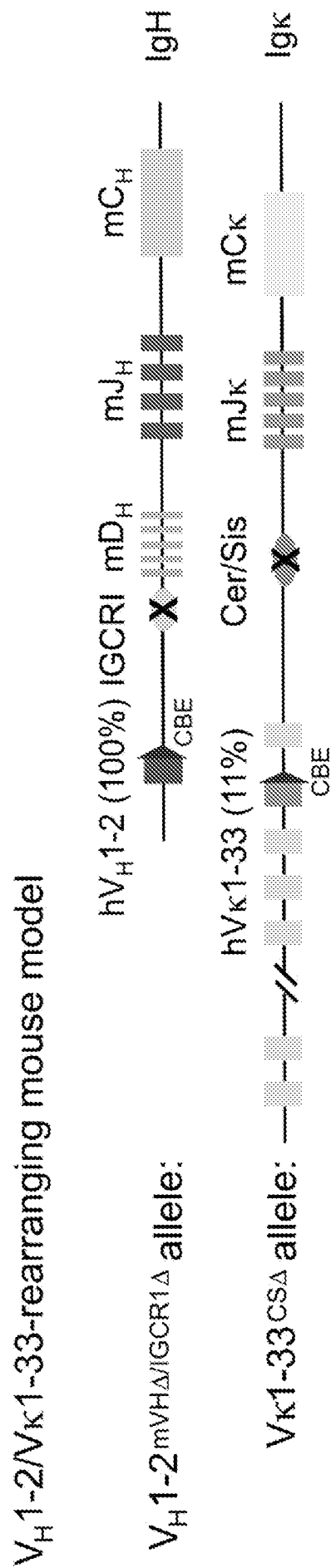


Fig. 19A

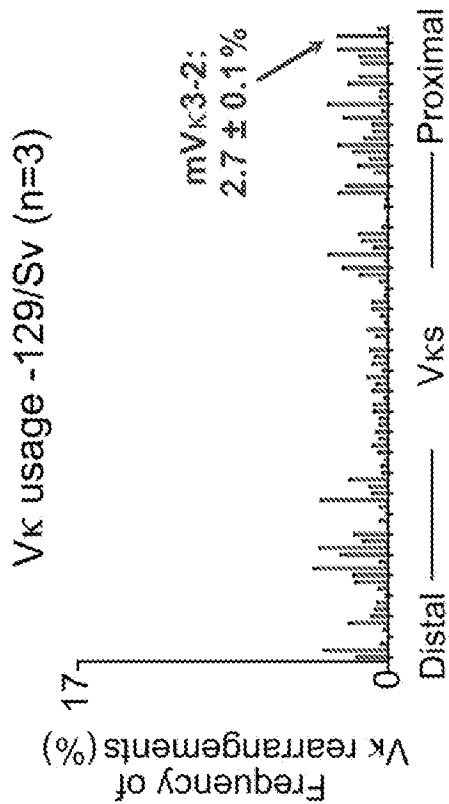


Fig. 19B

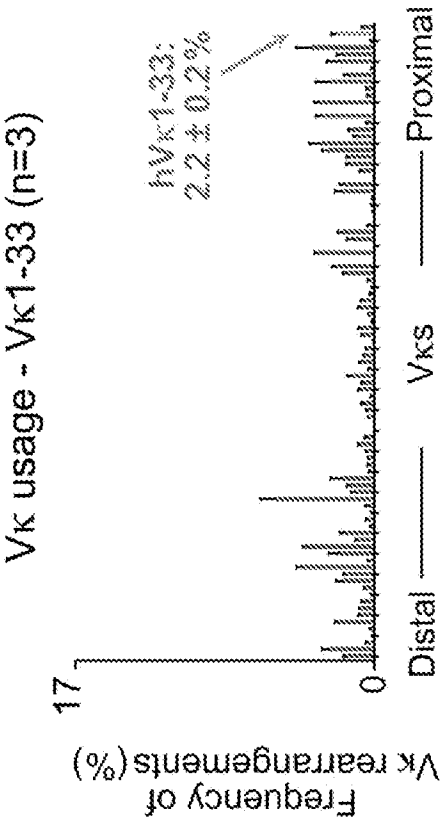


Fig. 19C

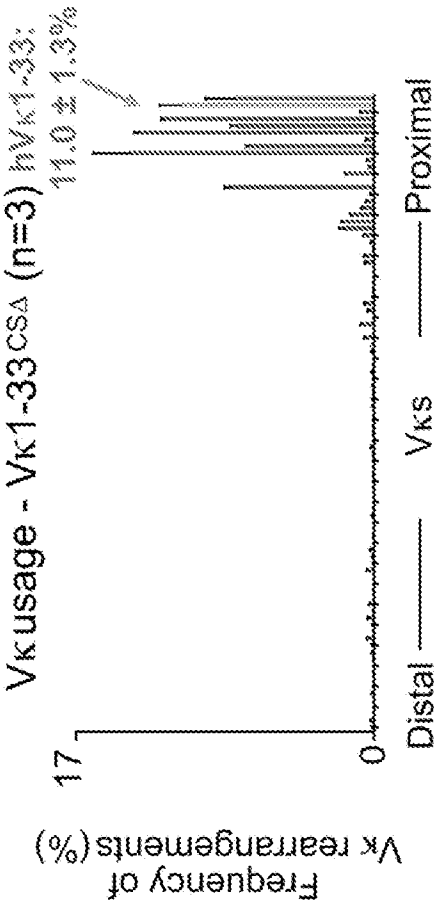


Fig. 19D

Overlapping of
unique Vκ1-33 LC CDR3s

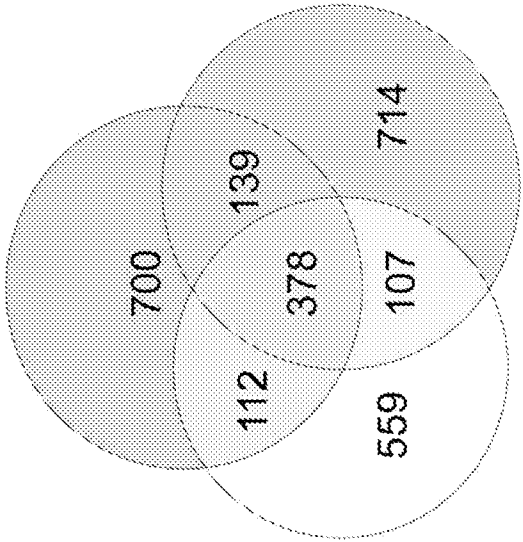


Fig. 19F

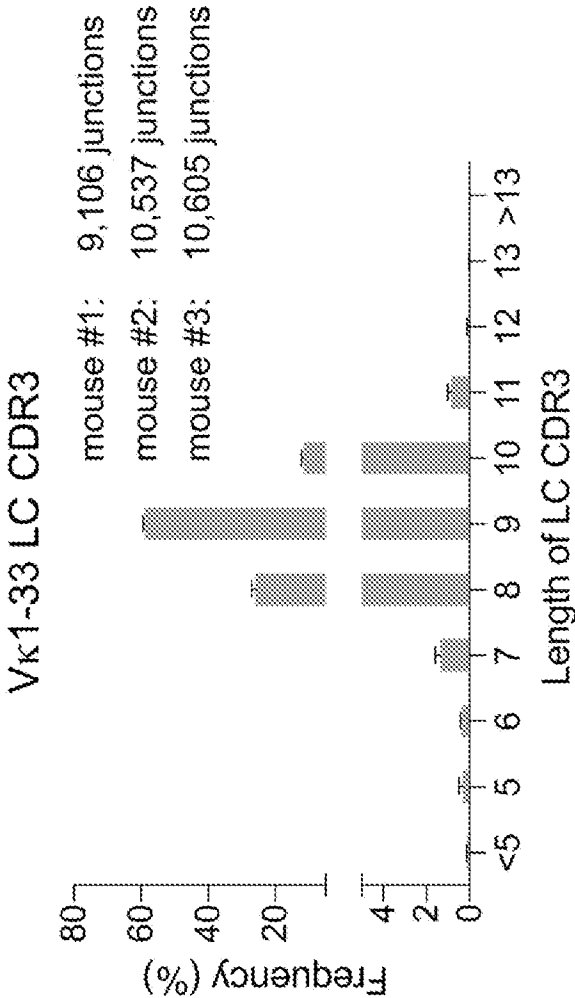


Fig. 19E

Immunogen: 1. 25µg SARS-CoV-2 Spike with Poly I:C
2. 25µg VHH7-RBD with Poly I:C

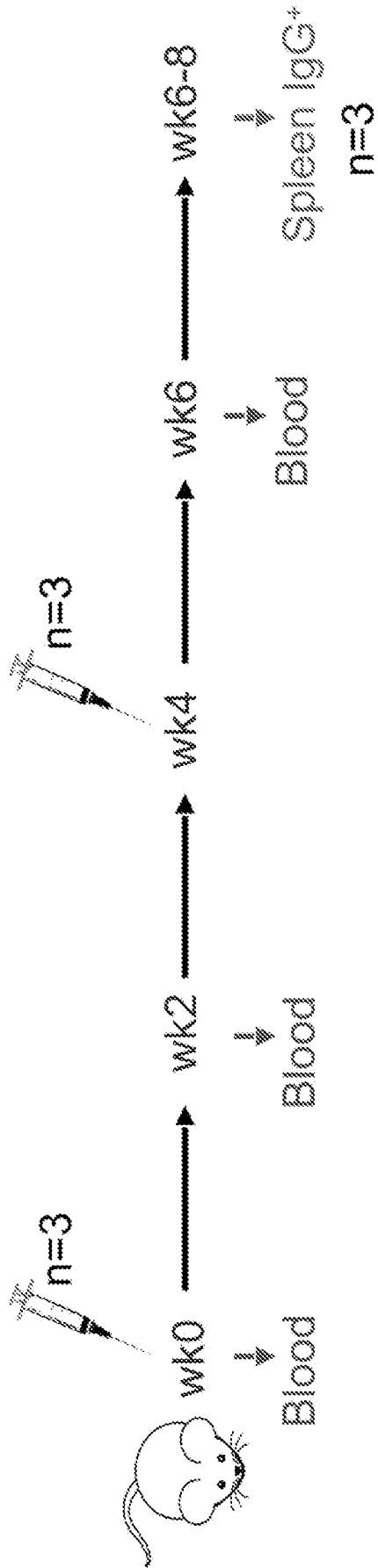


Fig. 20A

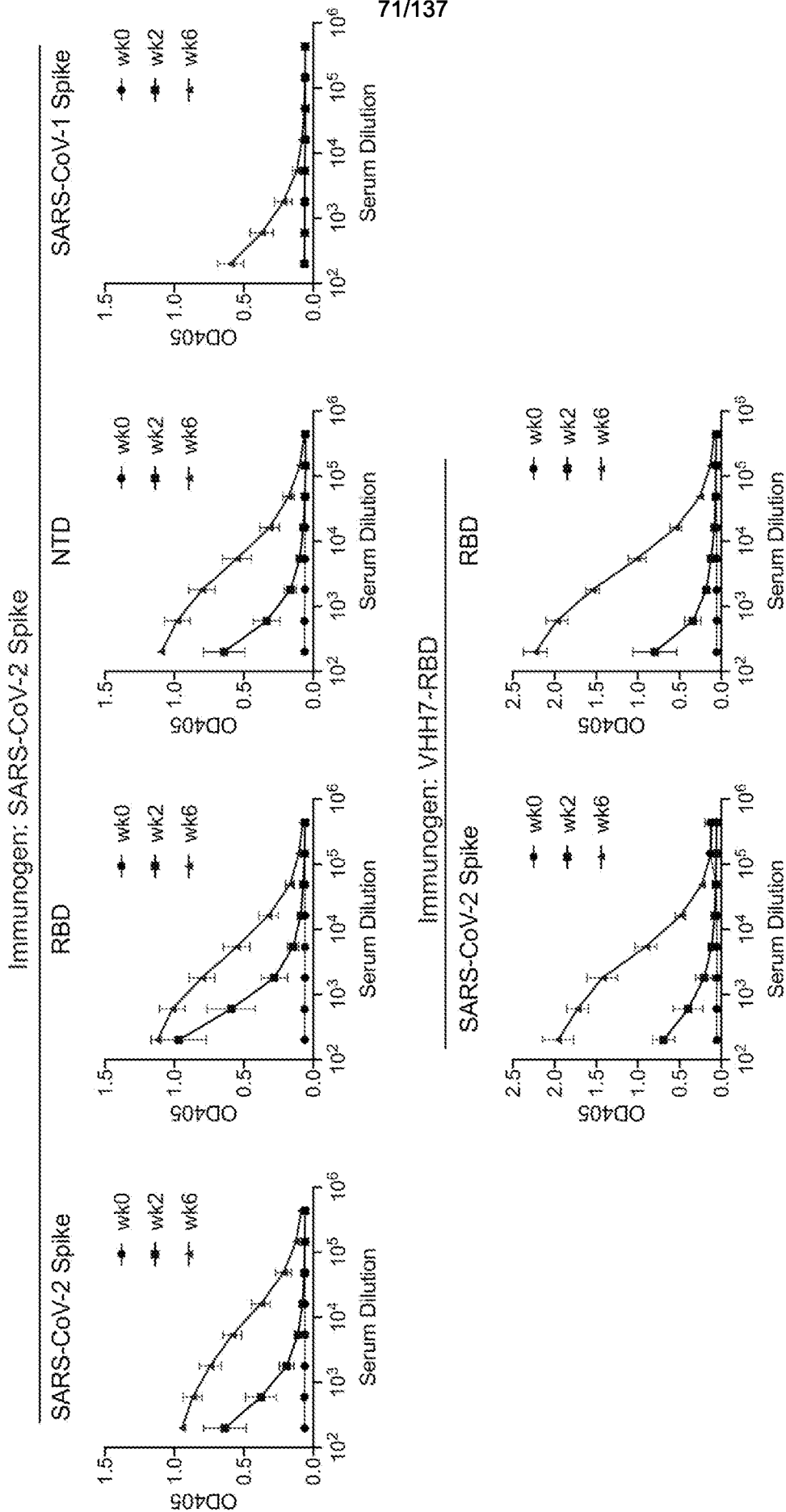


Fig. 20B

B cell lineage	Immunogen	Cells	HC CDR3	length of HC CDR3	LC CDR3	length of LC CDR3
SP1	SARS-CoV-2 spike	5	ARDRVLYGRSFGWYFDV	17 aa	QQYDNLPT	8 aa
SP2	SARS-CoV-2 spike	4	ARERRVIHYSGILYALDY	18 aa	QQYDNLHMFT	9 aa
SP3	SARS-CoV-2 spike	4	AITGRRPWFPF	11 aa	QQYDYLPT	9 aa
SP4	SARS-CoV-2 spike	6	ARGERRSWFTY	11 aa	QQYDNLPT	9 aa
VHH7-3	VHH7-RBD	2	ARDLGDYDAWFAY	13 aa	QQYDNLRWT	9 aa
VHH7-5	VHH7-RBD	3	ARDRVGQRFPPY	11 aa	QQYDNLWT	8 aa
VHH7-7	VHH7-RBD	4	ARRNCDY	7 aa	QQYDNLPT	9 aa
VHH7-8	VHH7-RBD	4	ARGKGTSWFAY	12 aa	QQYDNLCT	8 aa
VHH7-10	VHH7-RBD	1	ARDPLTGIFDY	11 aa	QQYDNLPYMYT	11 aa

Fig. 20C

Pseudotyped Virus Neutralization of Monoclonal Antibodies (IC₅₀: ng/ml)

	Alpha	Beta	Gamma	Epsilon	Iota_K484E	Iota	Delta	Omicron					Neutralization titer IC ₅₀ (ng/ml)	
	G614	B.1.1.7	B.1.351	P.1	B.1.429	B.1.526_K484E	B.1.526	B.1.617.2	BA.1	BA.2	BA.3	BA.4/BA.5	BA.2.12.1	
SP1-77	20	28	16	36	15	19	11	76	6.5	33	7	16	8	<10
VHH7-5-82	38	20	>10,000	>10,000	>10,000	26	>10,000	>10,000	>10,000	-	-	-	-	10-100
VHH7-7-53	68	43	>10,000	>10,000	62	43	>10,000	96	>10,000	-	-	-	-	100-1,000
														1,000-10,000
														>10,000

Pseudotyped Virus Neutralization of Monoclonal Antibodies (IC₈₀: ng/ml)

	Alpha	Beta	Gamma	Epsilon	Iota_K484E	Iota	Delta	Omicron				Neutralization titer IC ₅₀ (ng/ml)		
	G614	B.1.1.7	B.1.351	P.1	B.1.429	B.1.526_K484E	B.1.526	B.1.617.2	BA.1	BA.2	BA.3	BA.4/BA.5	BA.2.12.1	
SP1-77	47	166	35	213	81	37	29	327	26	82	28	56	37	<10
VHH7-5-82	201	109	>10,000	>10,000	>10,000	128	>10,000	>10,000	>10,000	-	-	-	-	10-100
VHH7-7-53	423	363	>10,000	>10,000	315	264	>10,000	792	>10,000	-	-	-	-	100-1,000
														1,000-10,000
														>10,000

Fig. 21A

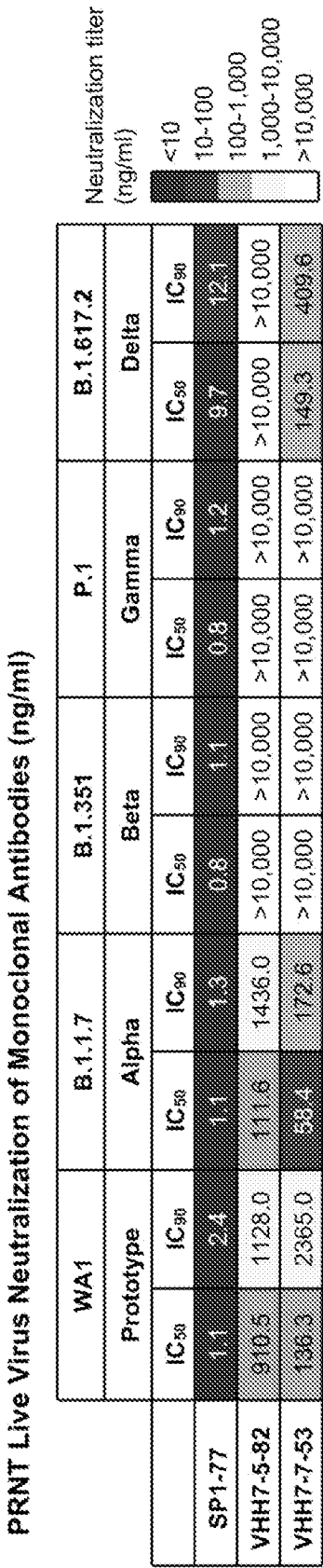


Fig. 21B

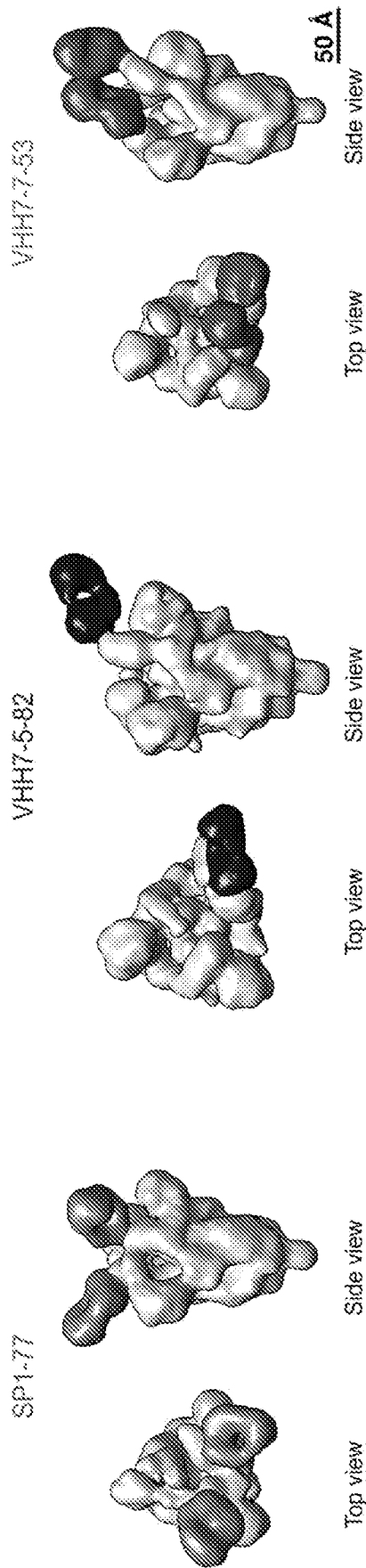


Fig. 21C

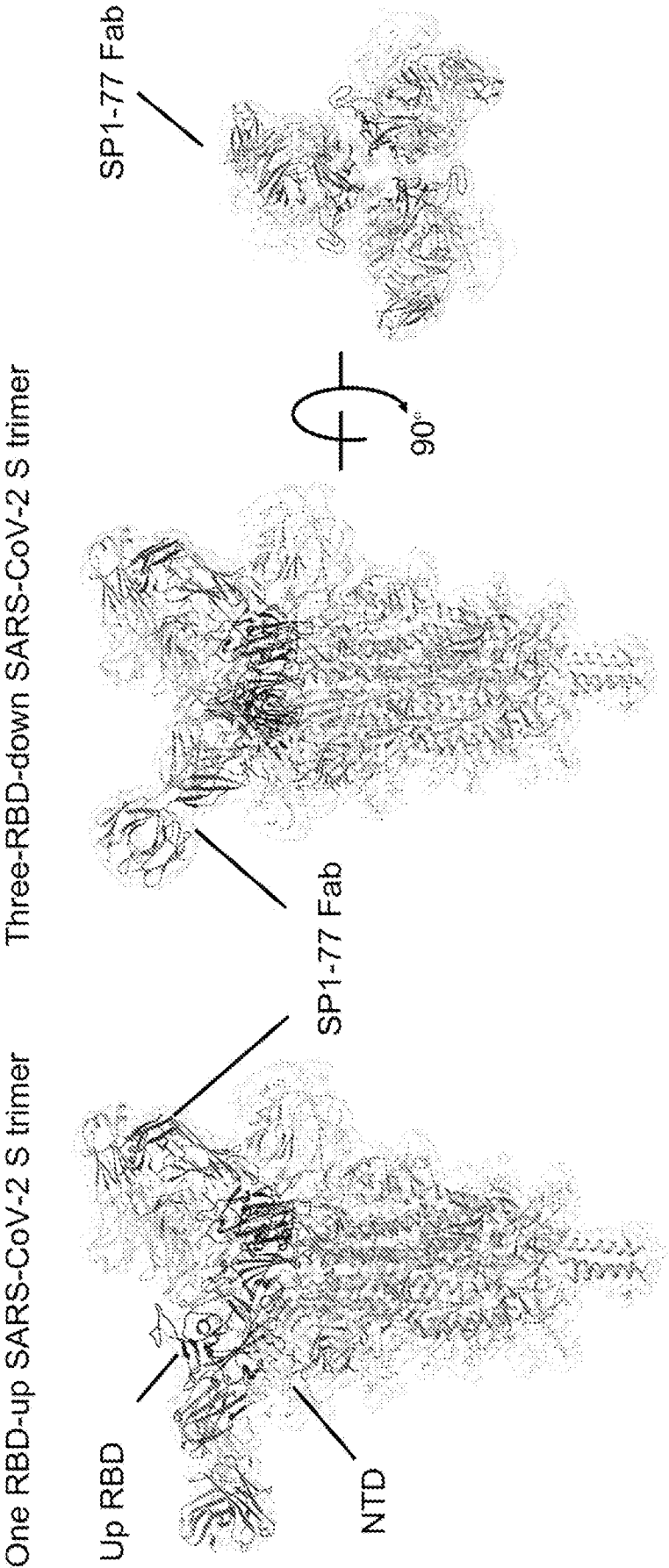


Fig. 22A

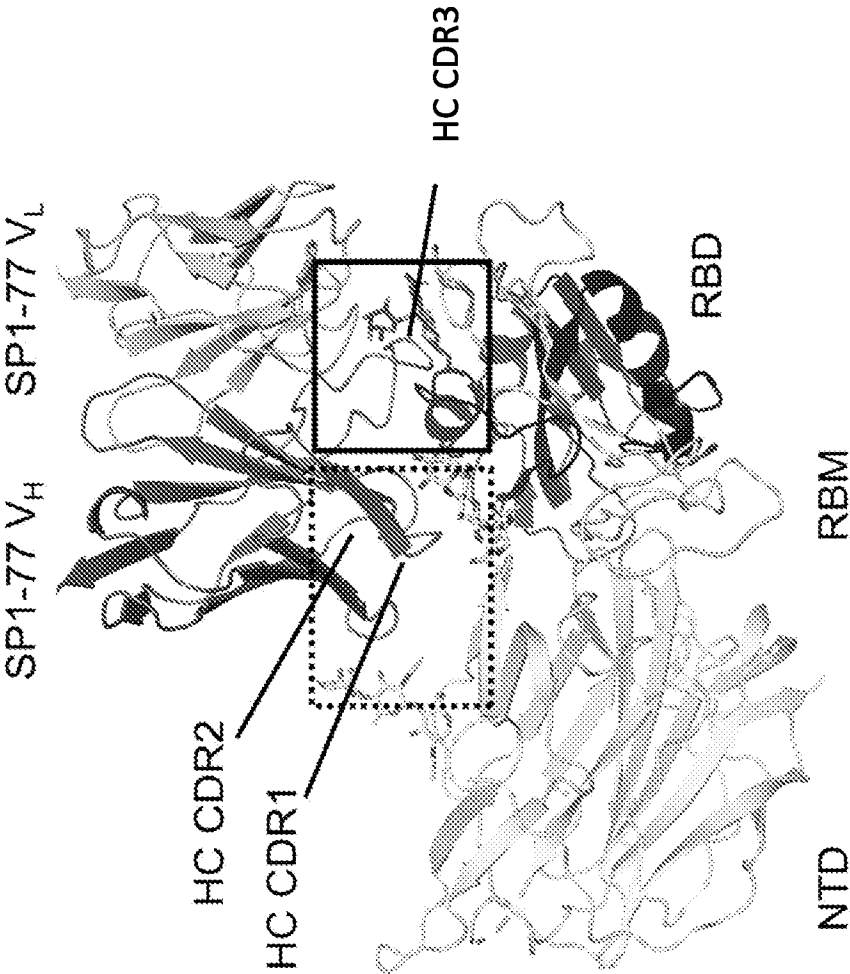


Fig. 22B

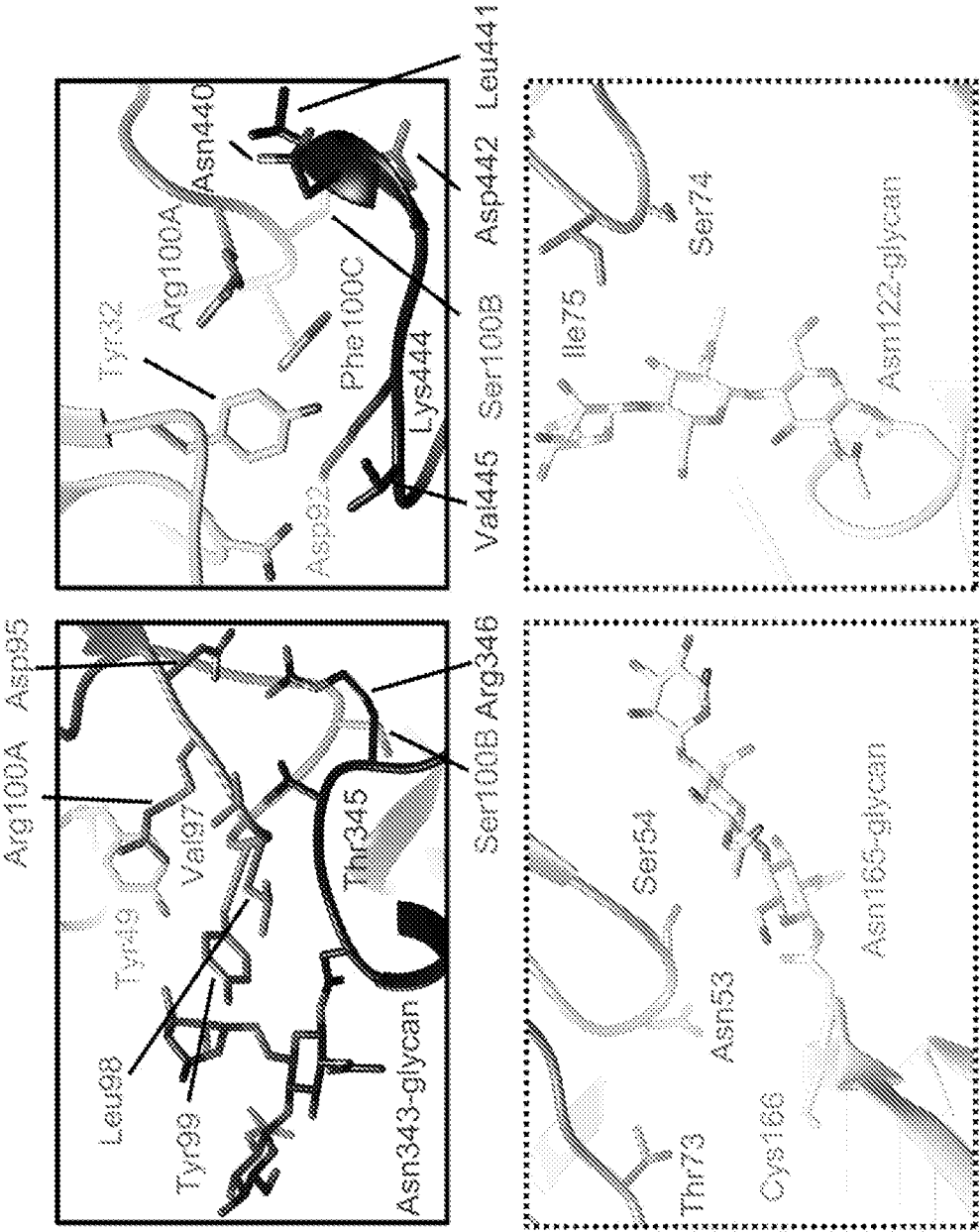


Fig. 22C

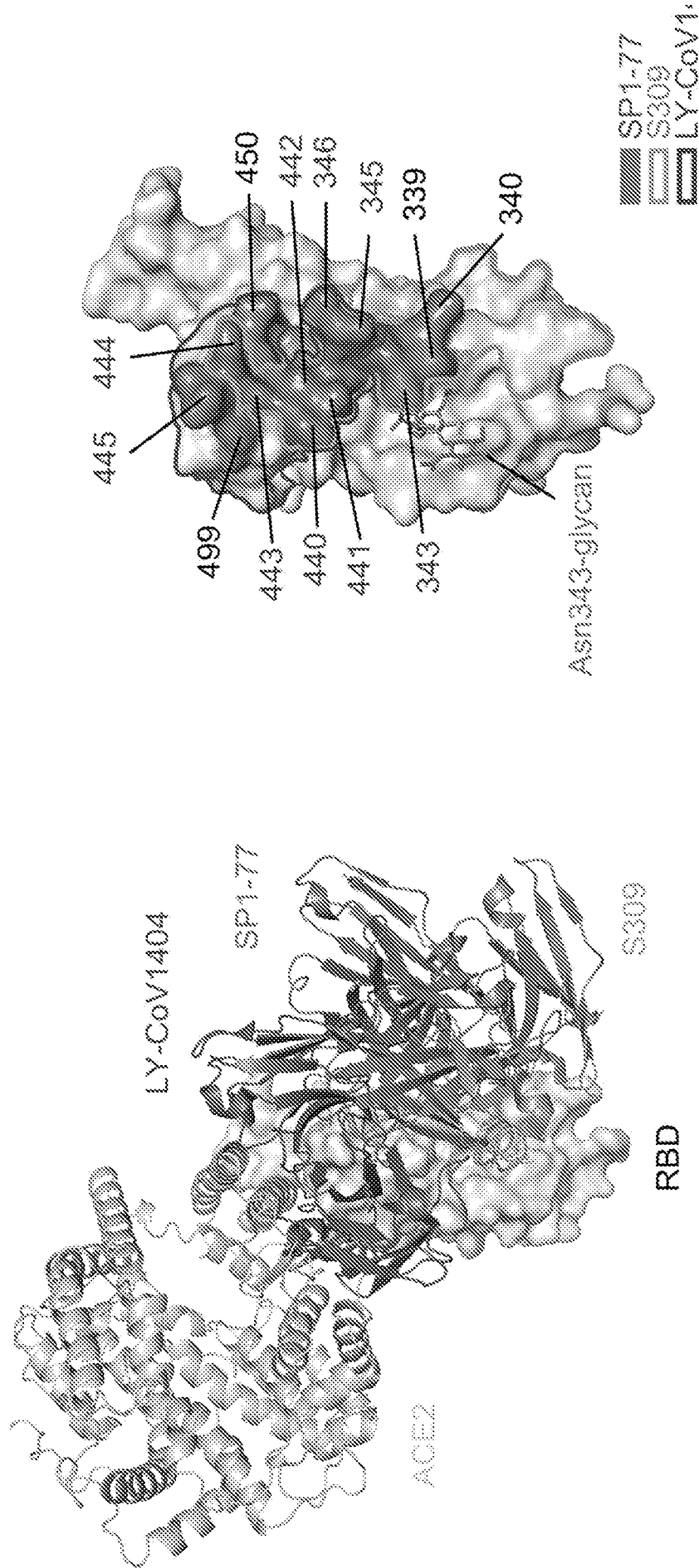


Fig. 22D

Fig. 22E

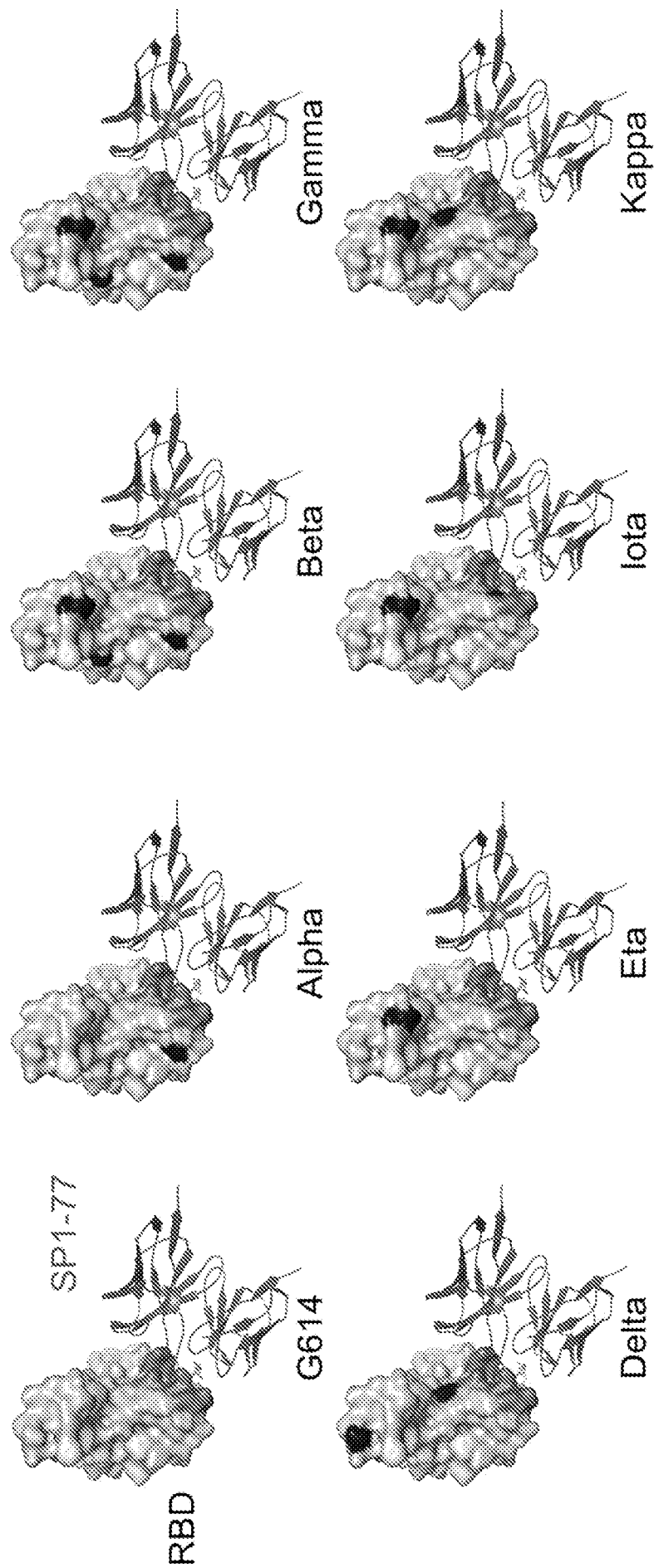


Fig. 23A

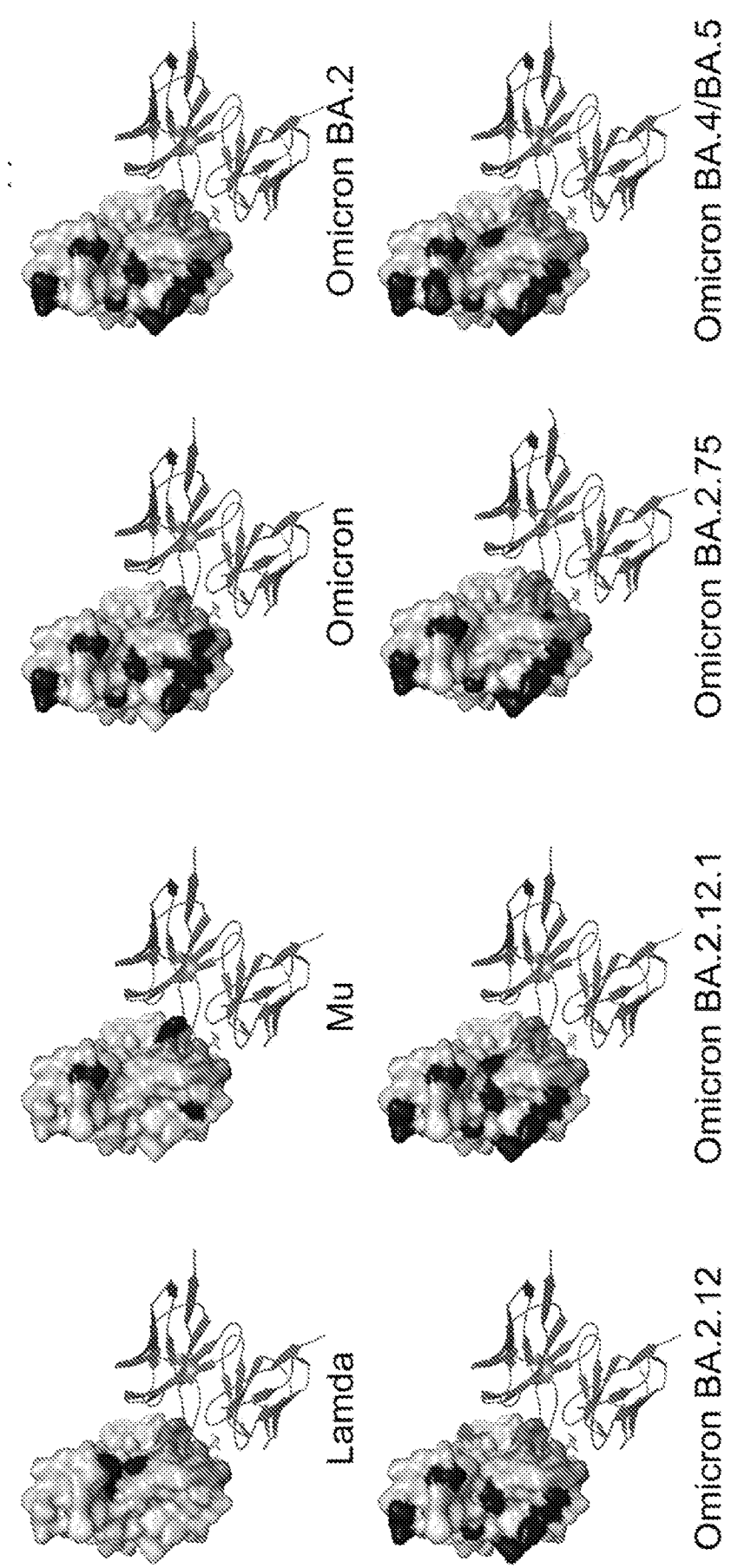


Fig. 23B

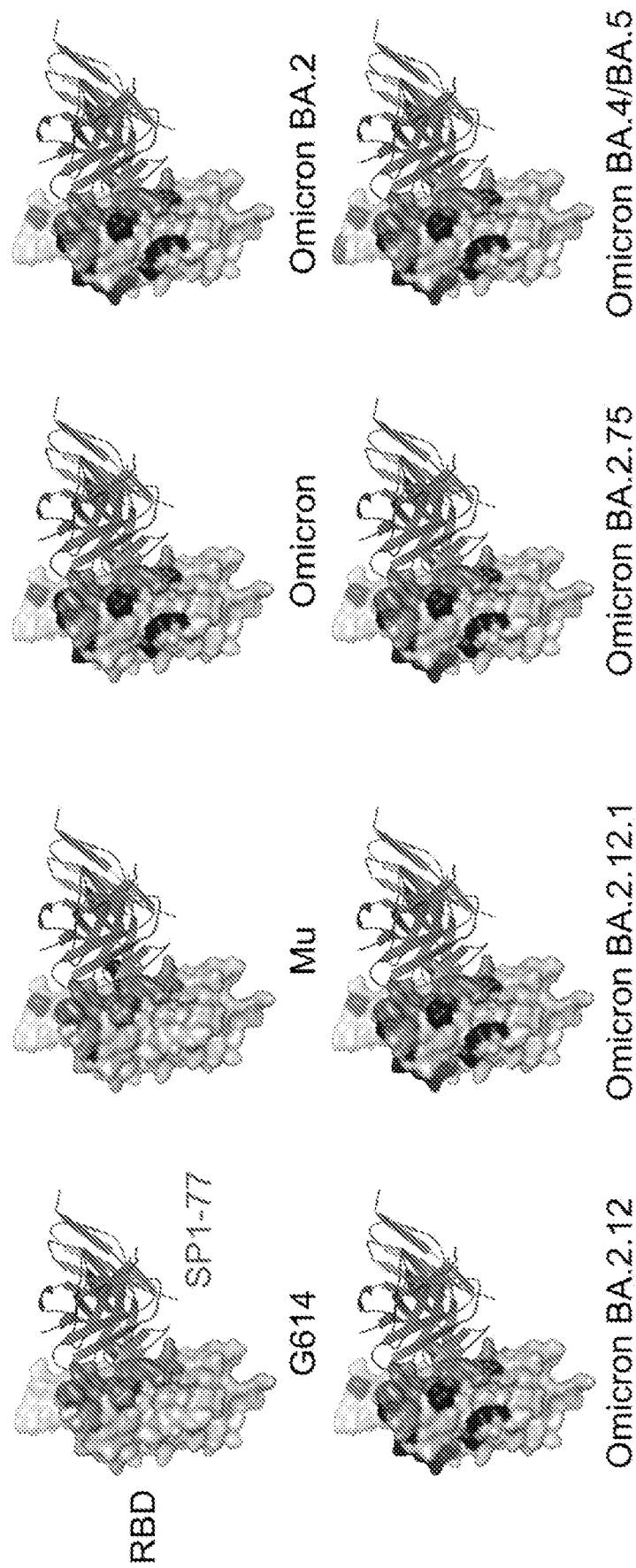


Fig. 23C

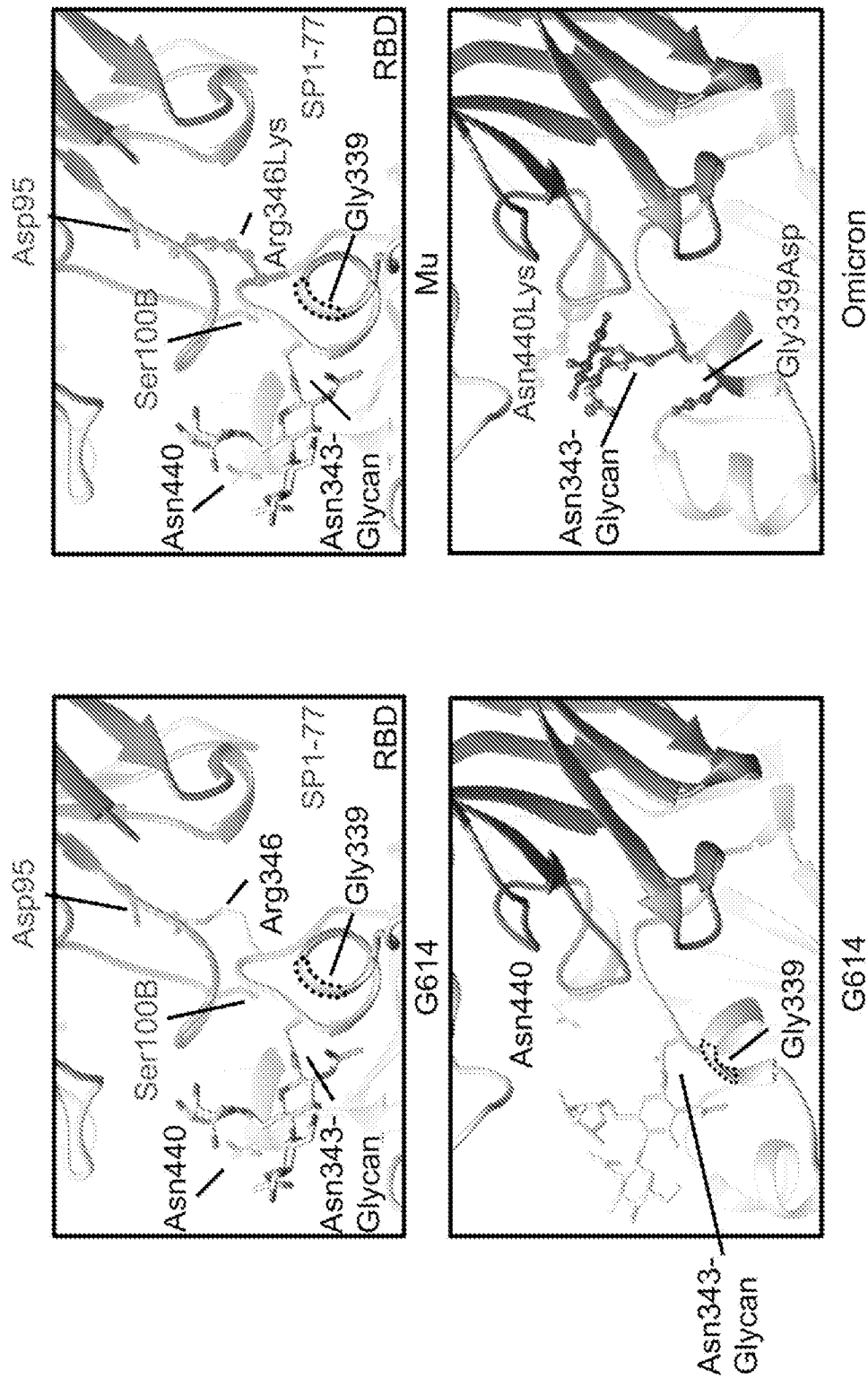


Fig. 23D

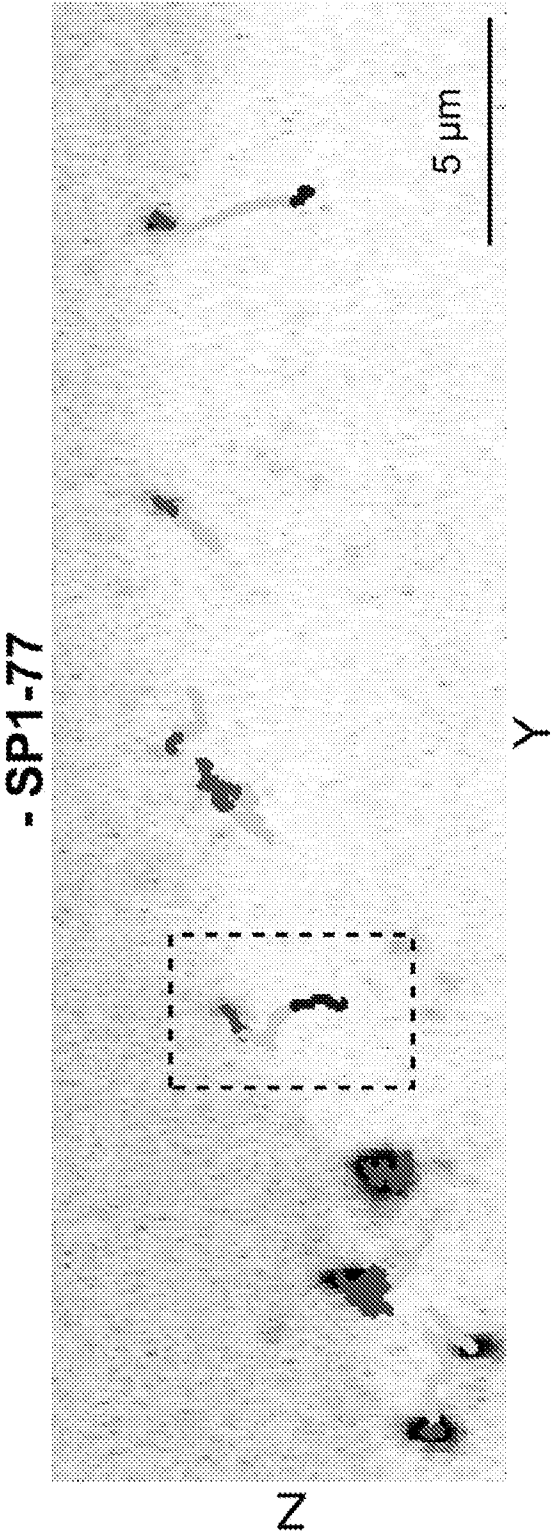


Fig. 24A

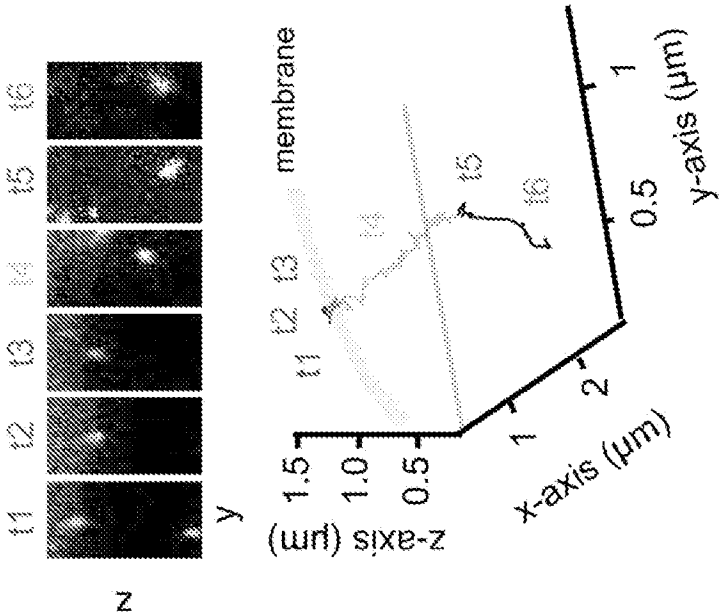


Fig. 24B

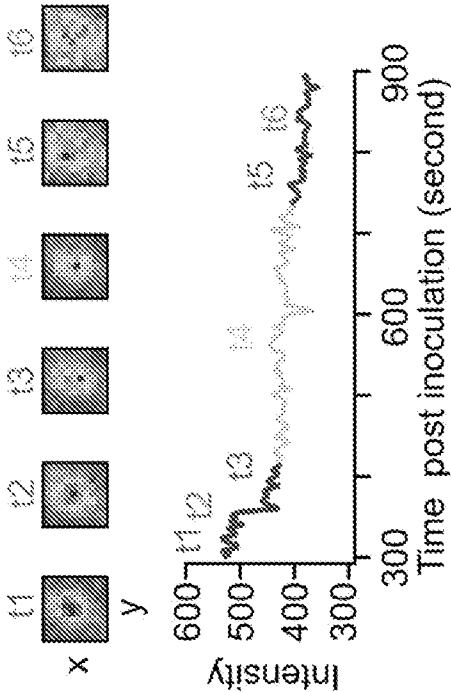


Fig. 24C

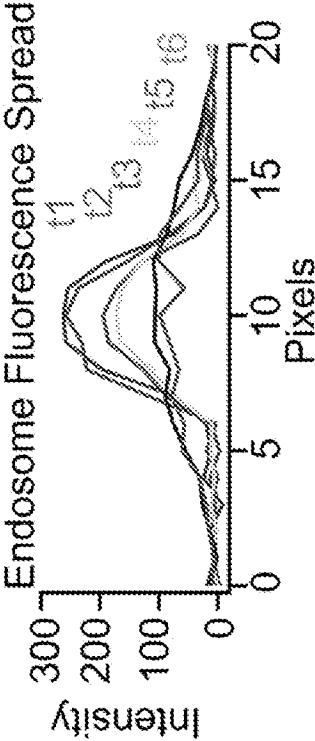


Fig. 24D

+ SP1-77

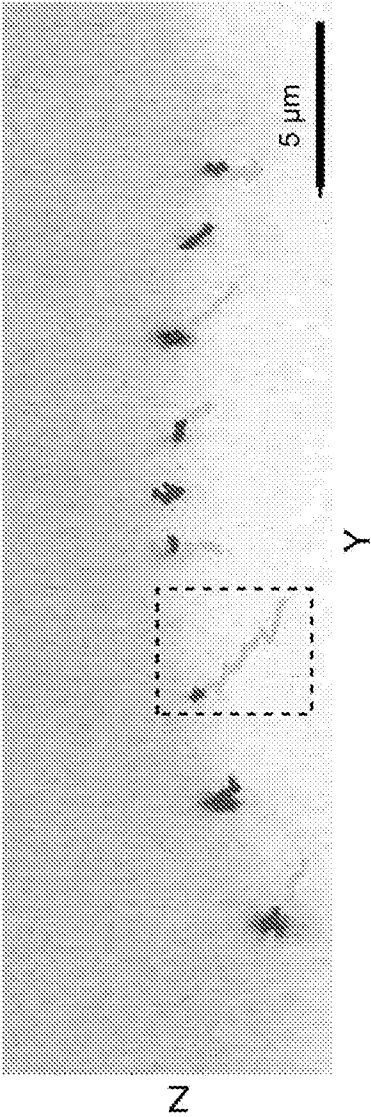


Fig. 24E

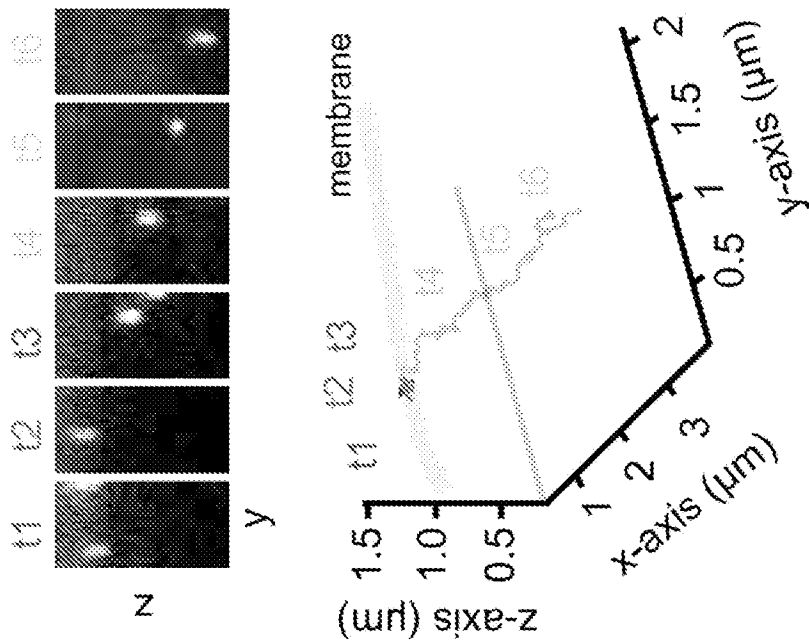


Fig. 24F

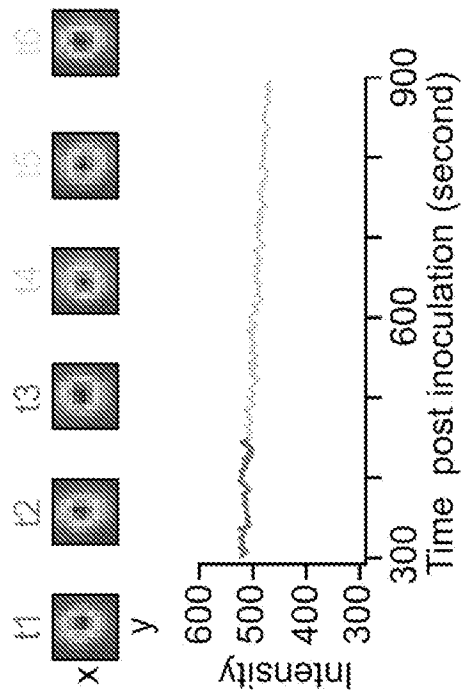


Fig. 24G

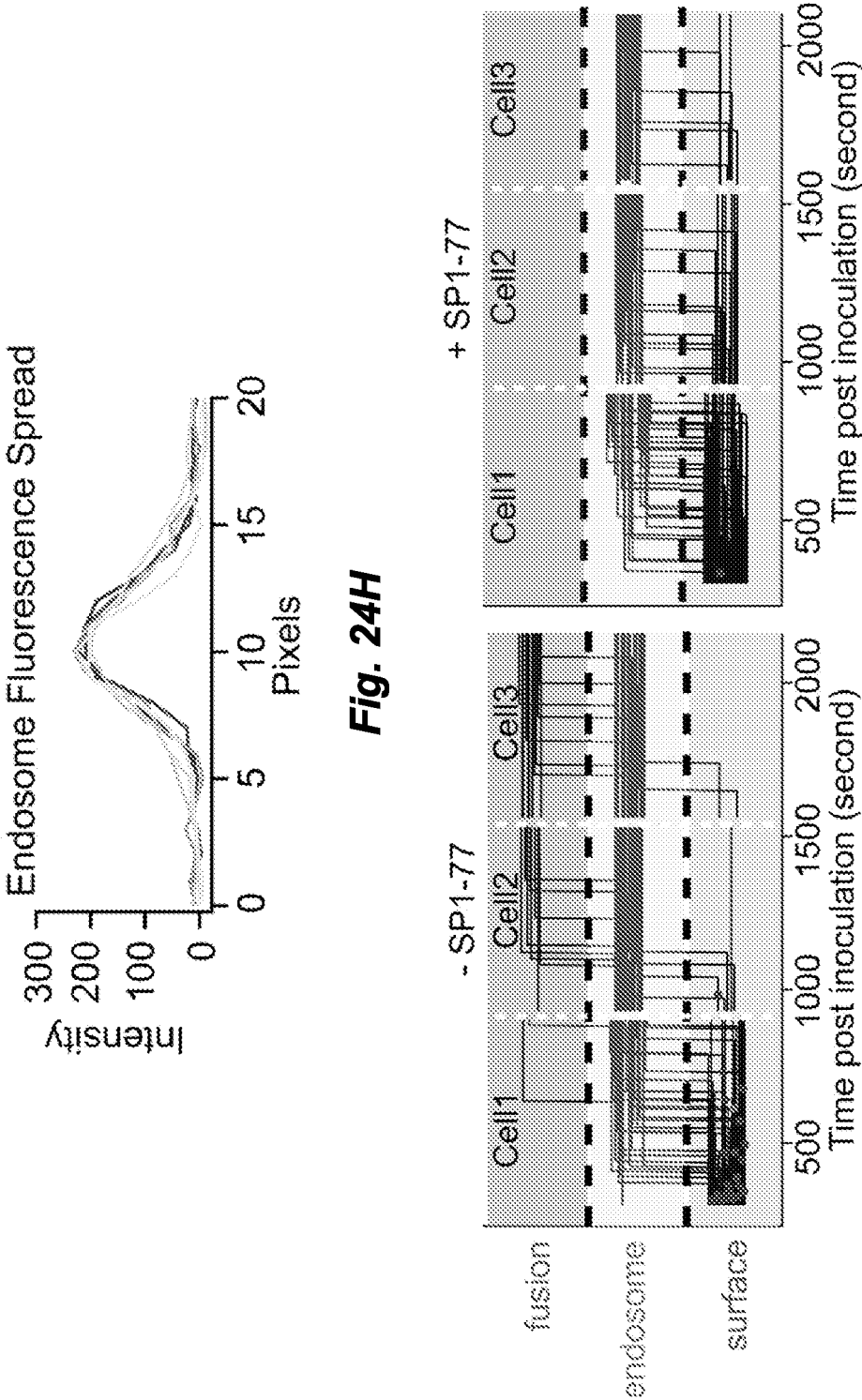


Fig. 24H

Fig. 24I

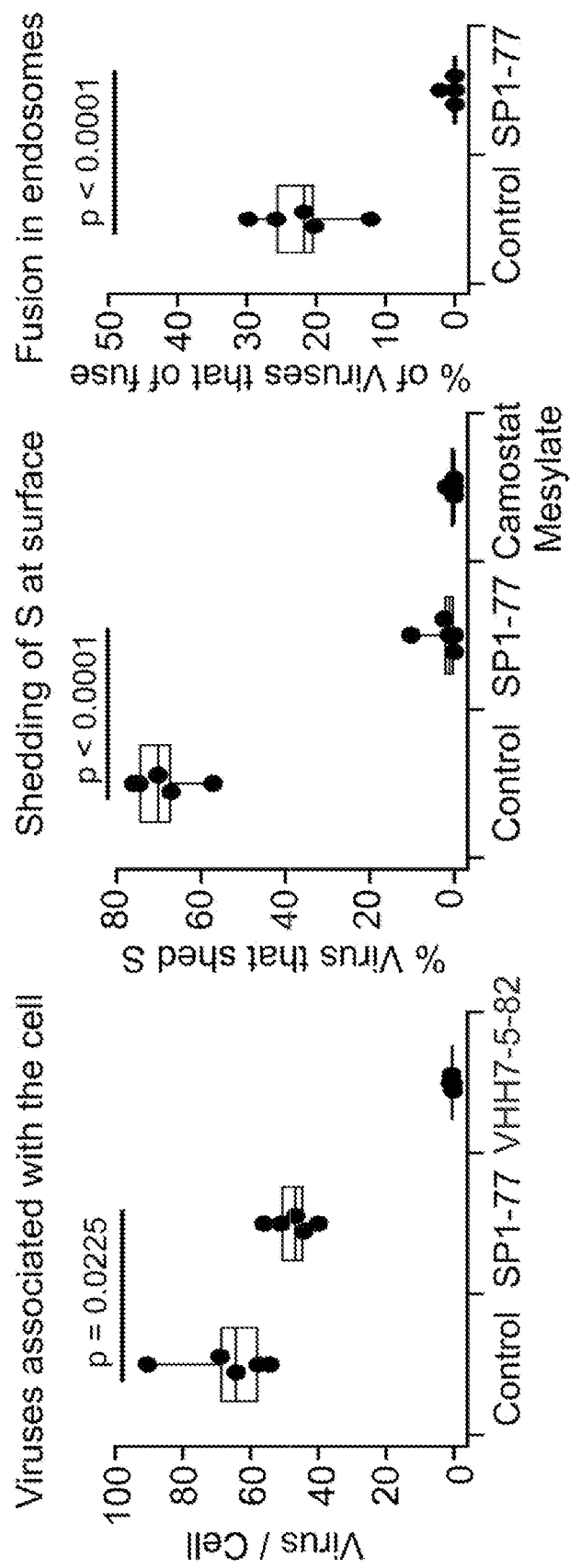


Fig. 24J

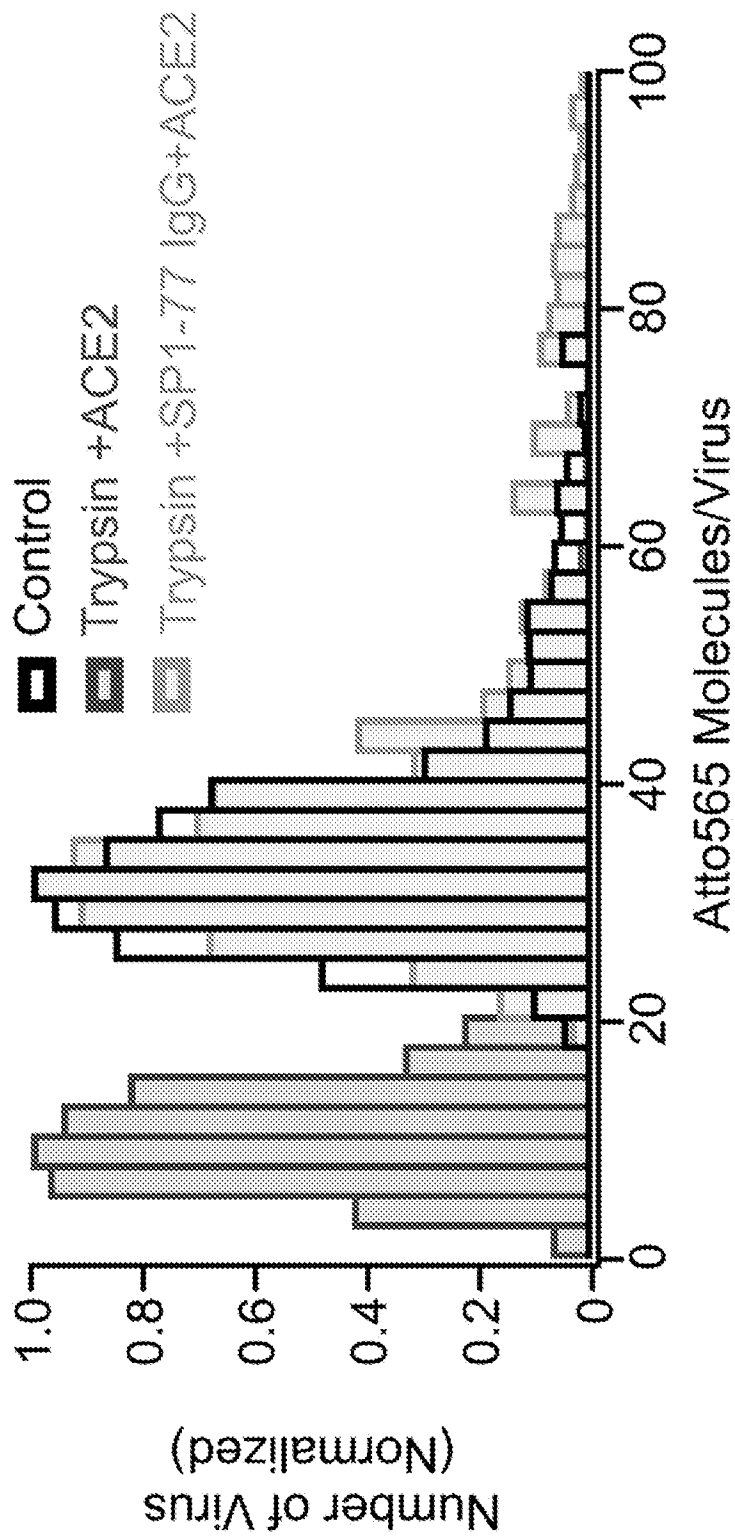


Fig. 25A

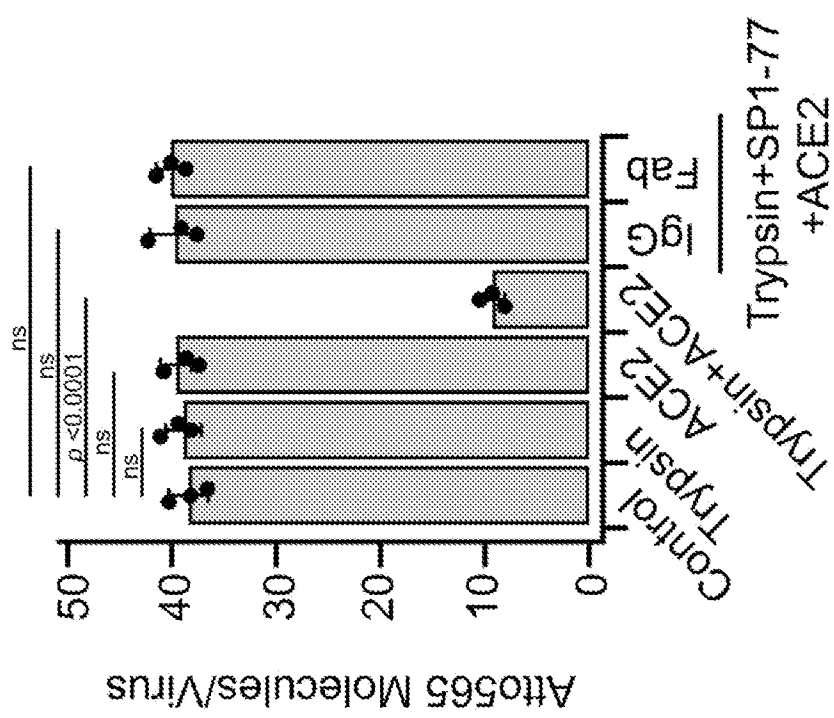


Fig. 25B

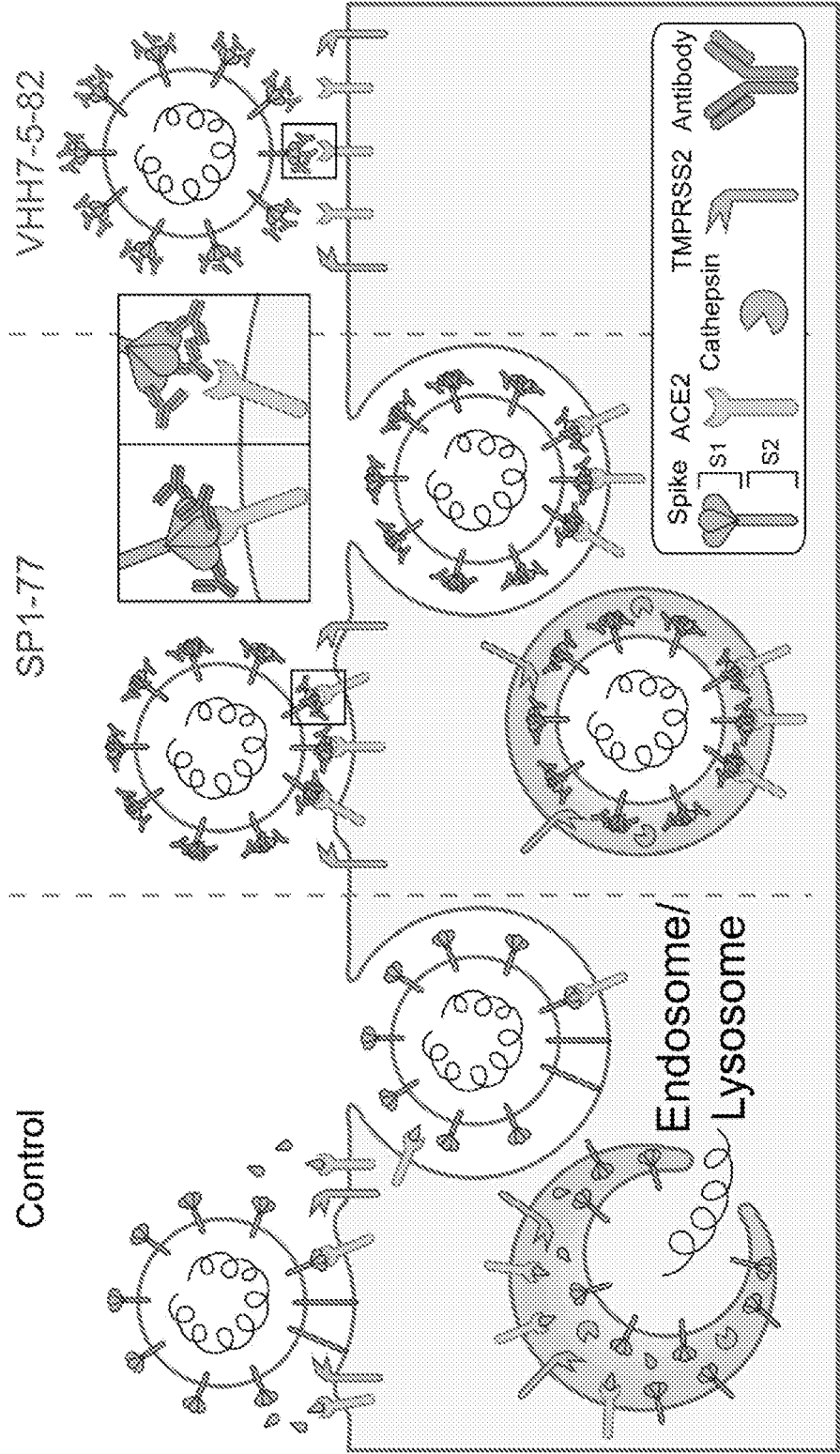


Fig. 25C

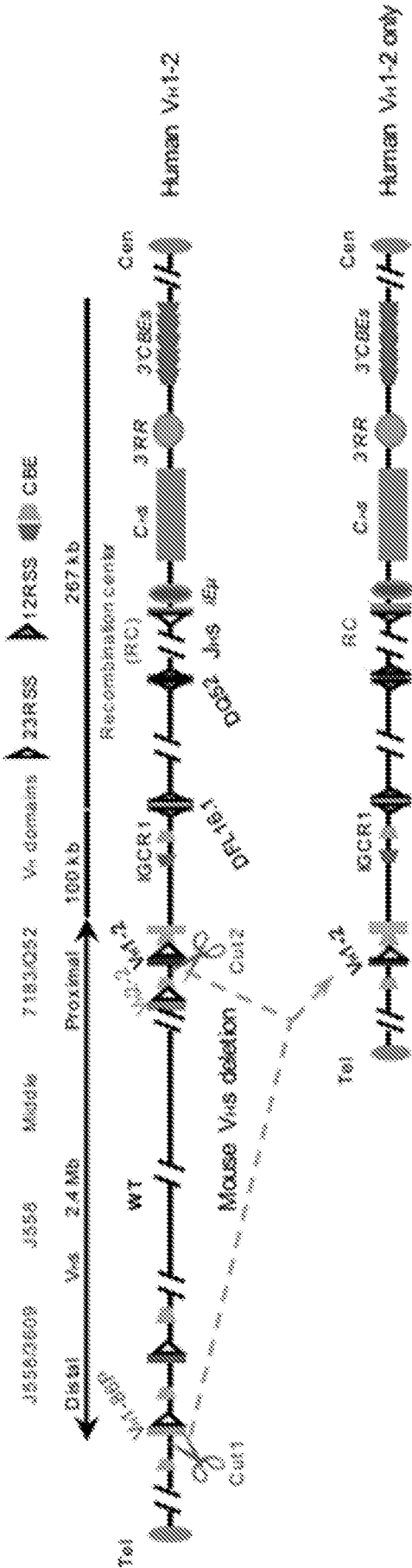


Fig. 26A

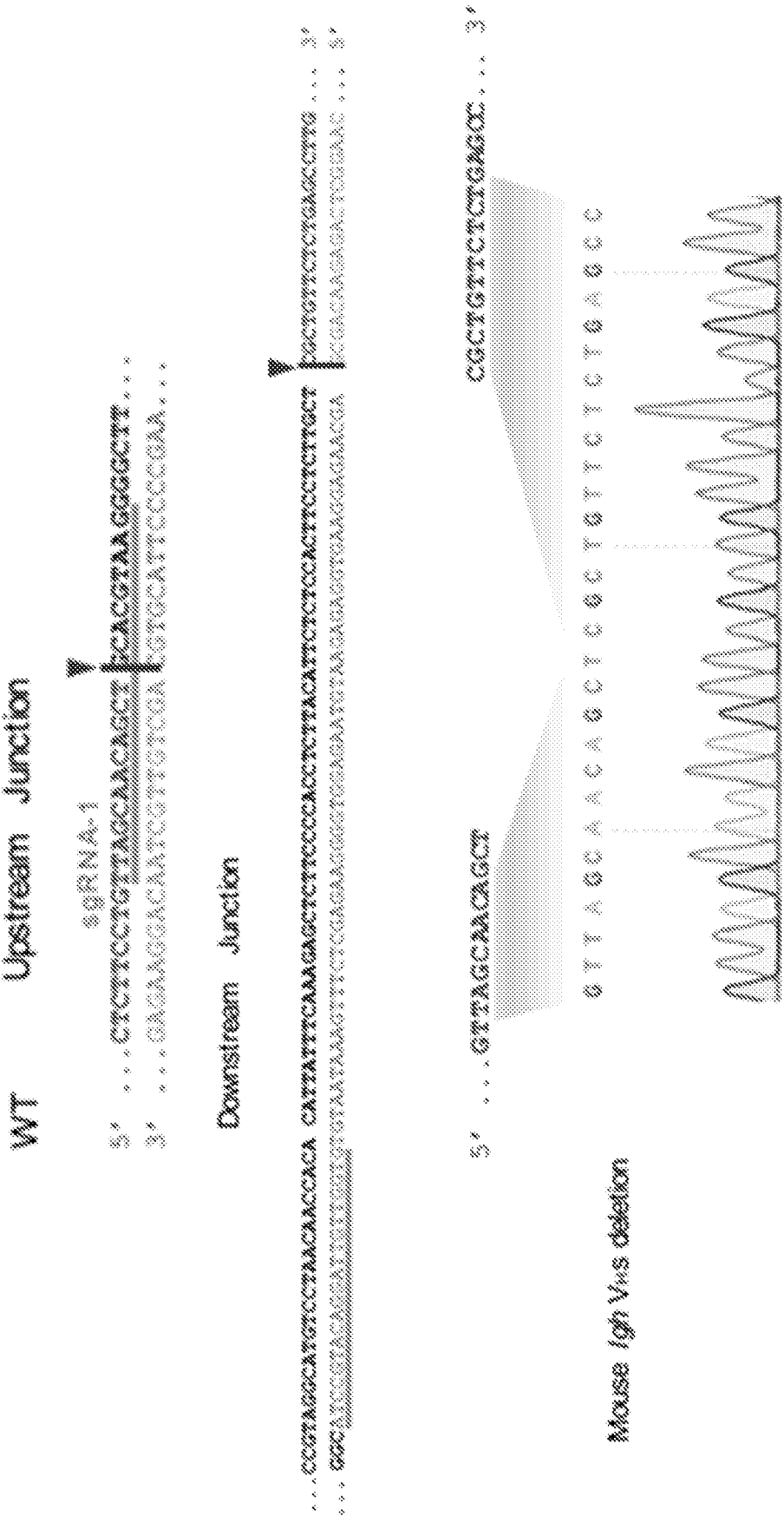


Fig. 26B

hJH1	-----AEYFQHWGQGTLLVTVSS	hJK1	WTFGGGTRKVEIK
hJH2	-----YWYFDLWGRGCTLLVTVSS	mJK1	WTFGGGTRKLEIK
mJH1	-----YWYFDVWGRGCTLLVTVSS	hJK2	YTFGGGTRKLEIK
hJH3~1	-----DAFDVWCGQGTMTVTSS	mJK2	YTFGGGTRKLEIK
hJH3~2	-----DAFDLWCGQGTMTVTSS	hJK3	YTFGGGTRKVEIK
hJH4~1	-----YFDYWGCGCTLLVTVSS	mJK4	YTFGGGTRKLEIK
hJH4~2	-----YFDYWGCGCTLLVTVSS	hJK4	LTFGGGTRKVEIK
hJH4~3	-----YFDYWGCGCTLLVTVSS	mJK5	LTFGGGTRKLEIK
mJH2	-----YFDYWGCGCTLLVTVSS	hJK5	ITFGGGTRNLEIK
hJH5~1	-----NWFDSWCGQGTLLVTVSS		
hJH5~2	-----NWFDPWCGQGTLLVTVSS		
mJH3	-----NFAIHWCGQGTLLVTVSA		
hJH6~1	YYYYYGMDVWCGQGTLLVTVSS		
hJH6~2	YYYYYGMDVWCGQGTLLVTVSS		
hJH6~3	YYYYYGMDVWCGKGTLLVTVSS		
hJH6~4	YYYYYGMDVWCGKGTLLVTVSS		
mJH4	-----YYANDVWCGGCTLLVTVSS		

Fig. 26C

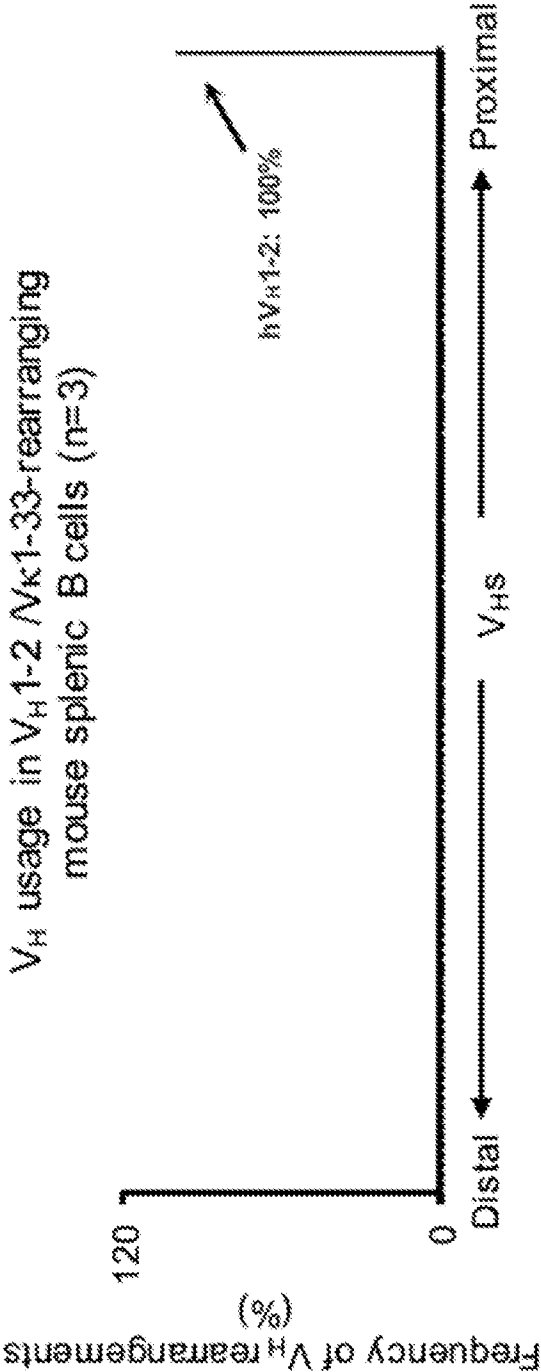


Fig. 27A

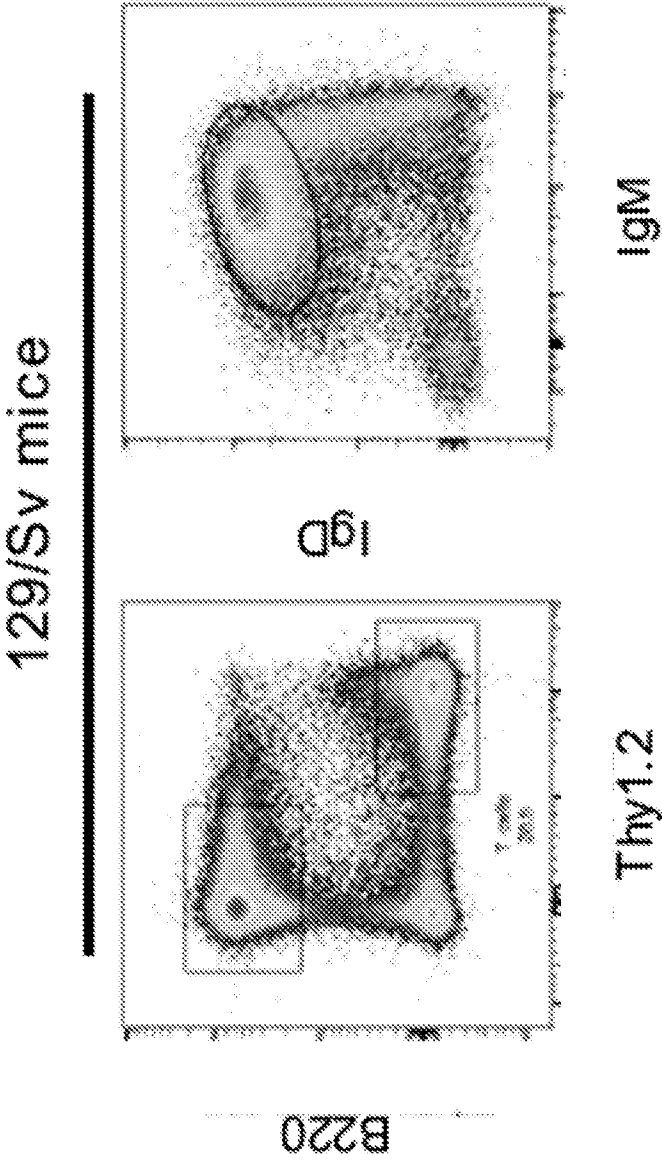


Fig. 27B

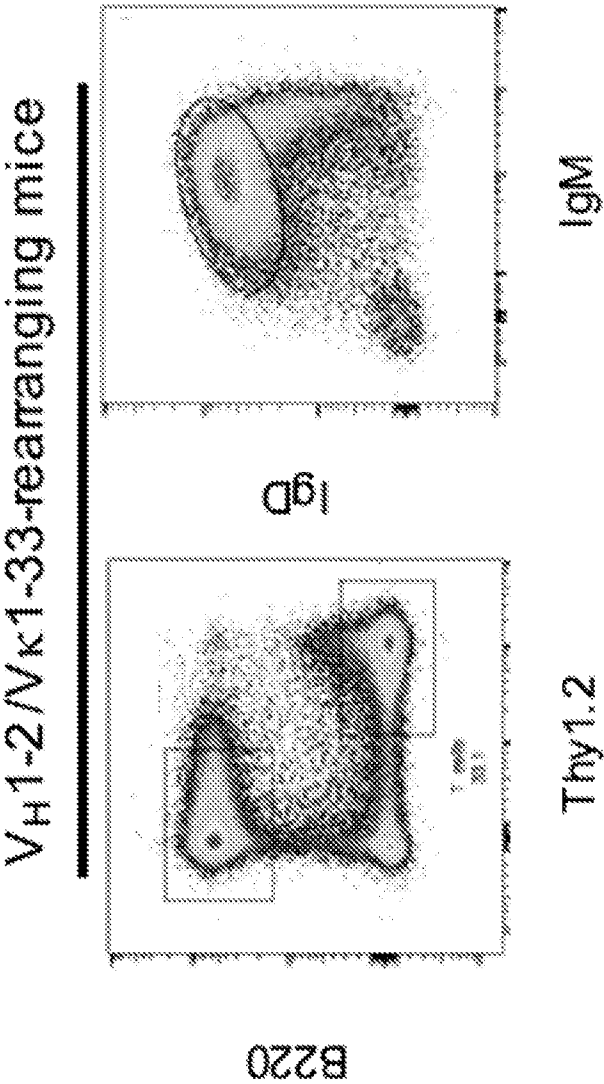


Fig. 27C

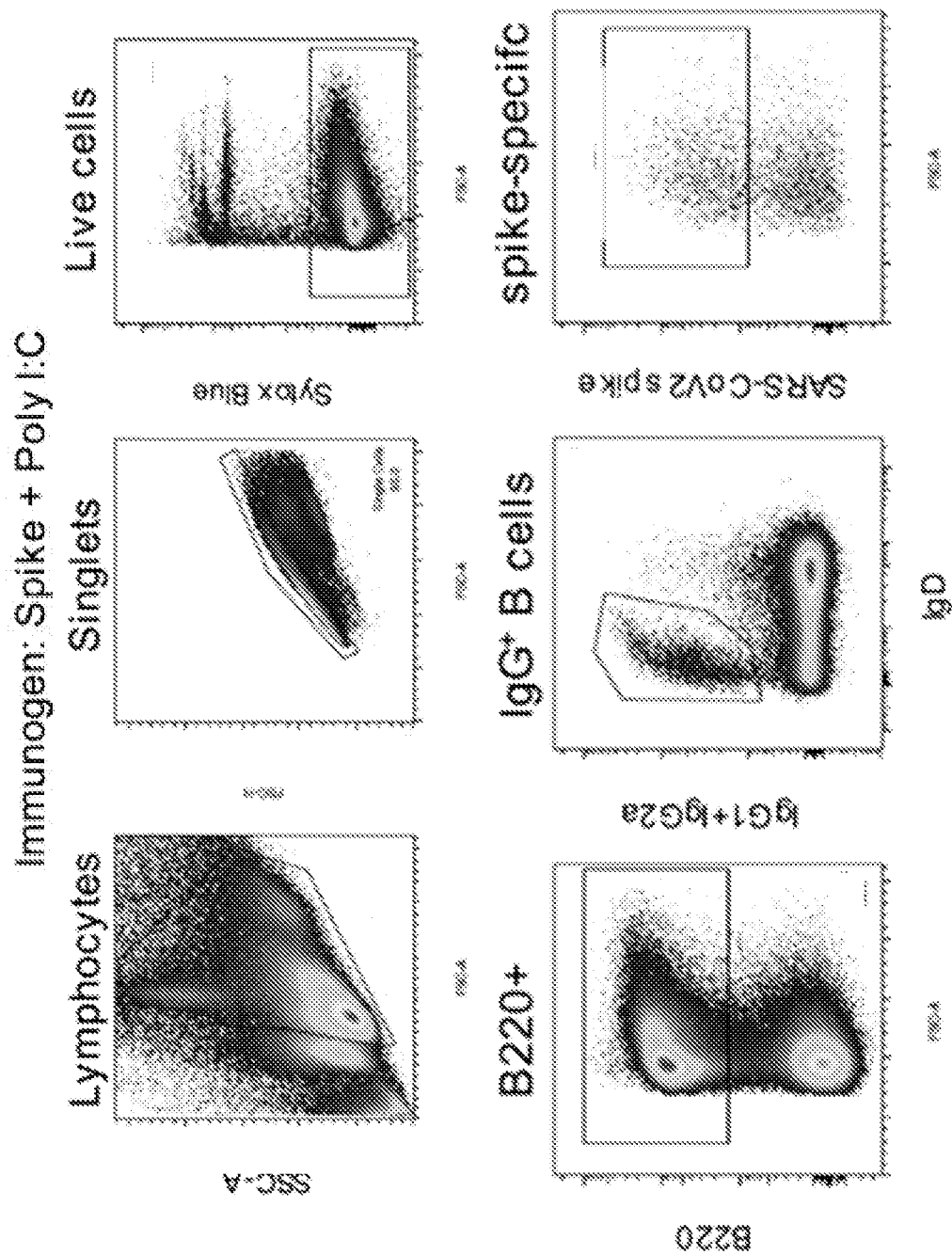


Fig. 28A

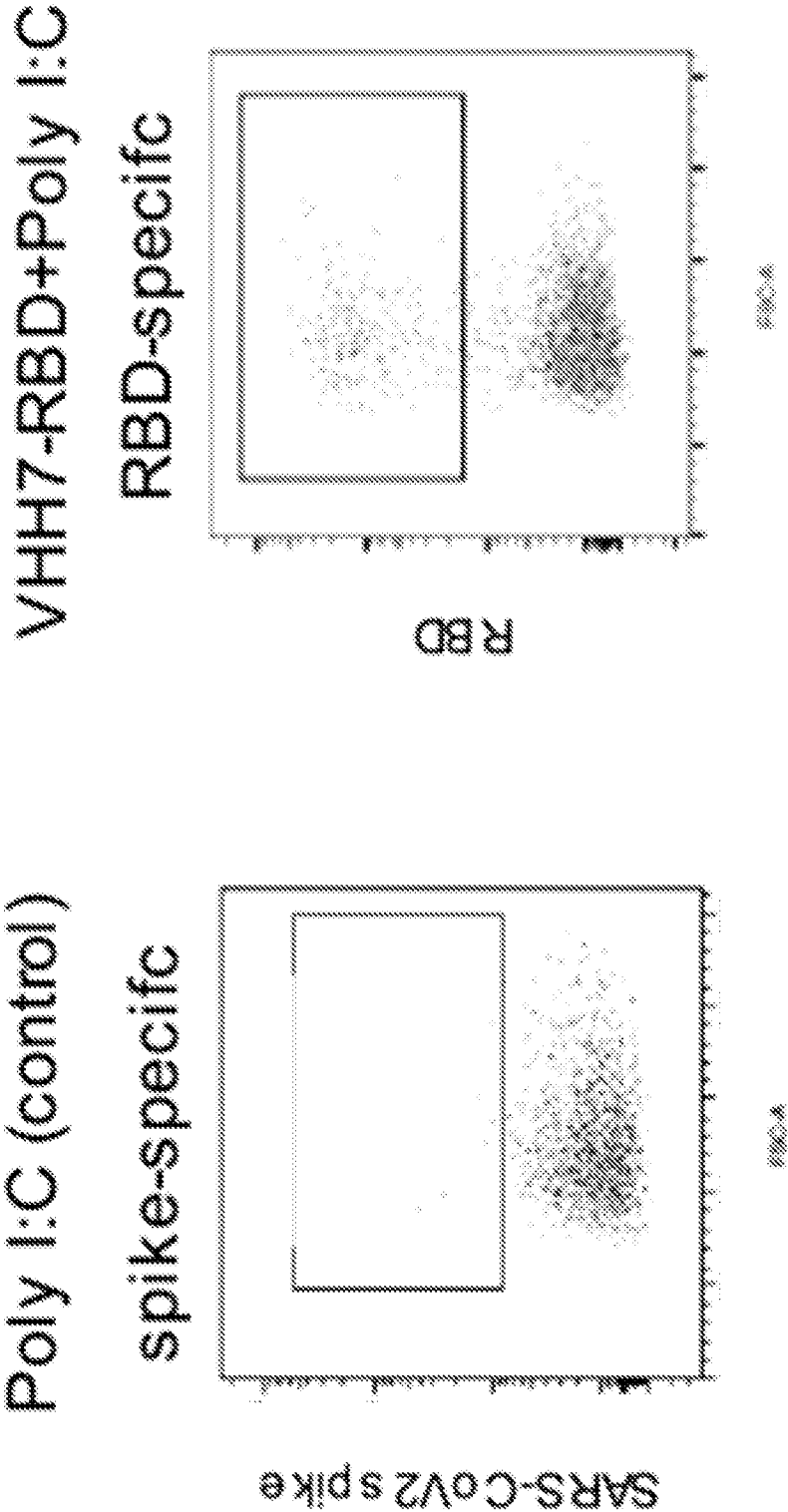


Fig. 28B

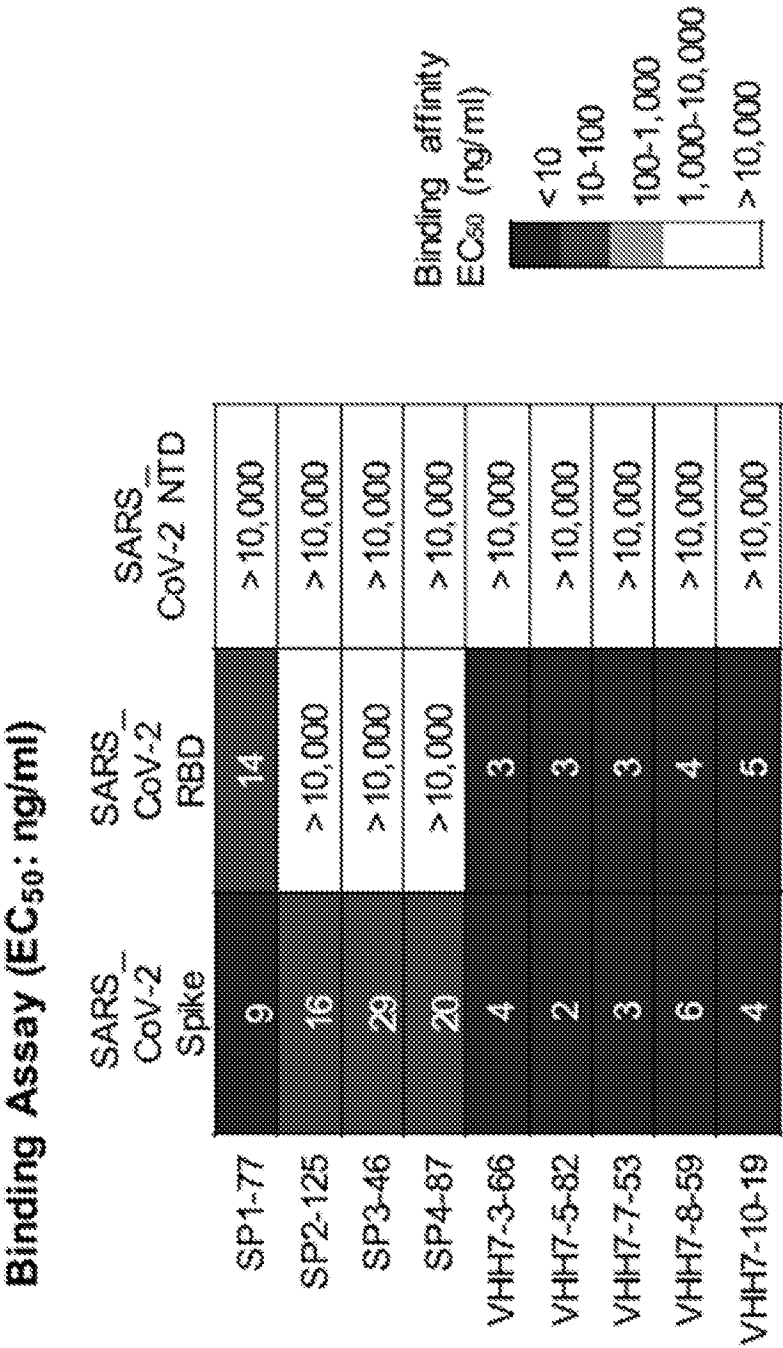


Fig. 28C

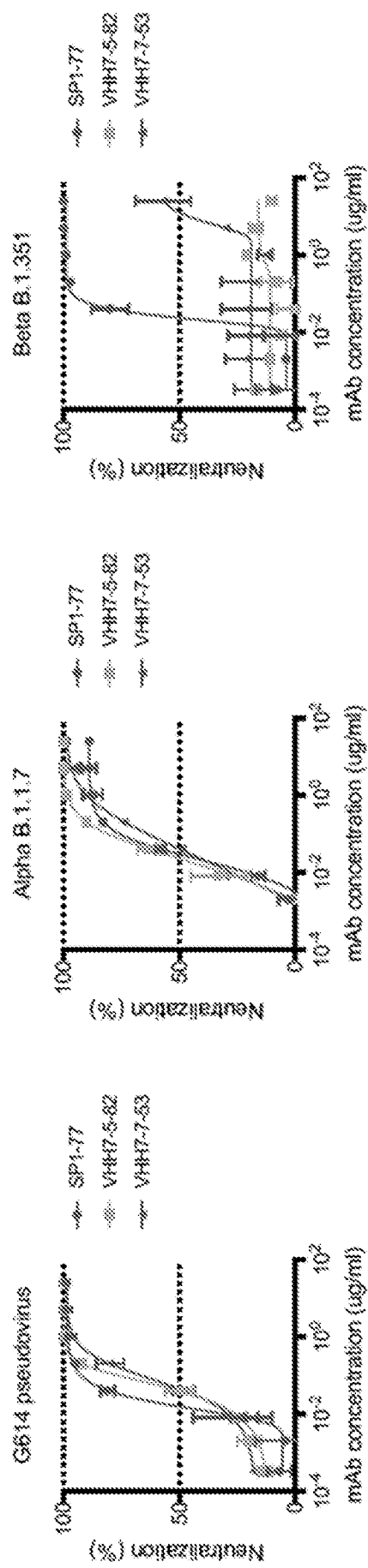


Fig. 29A

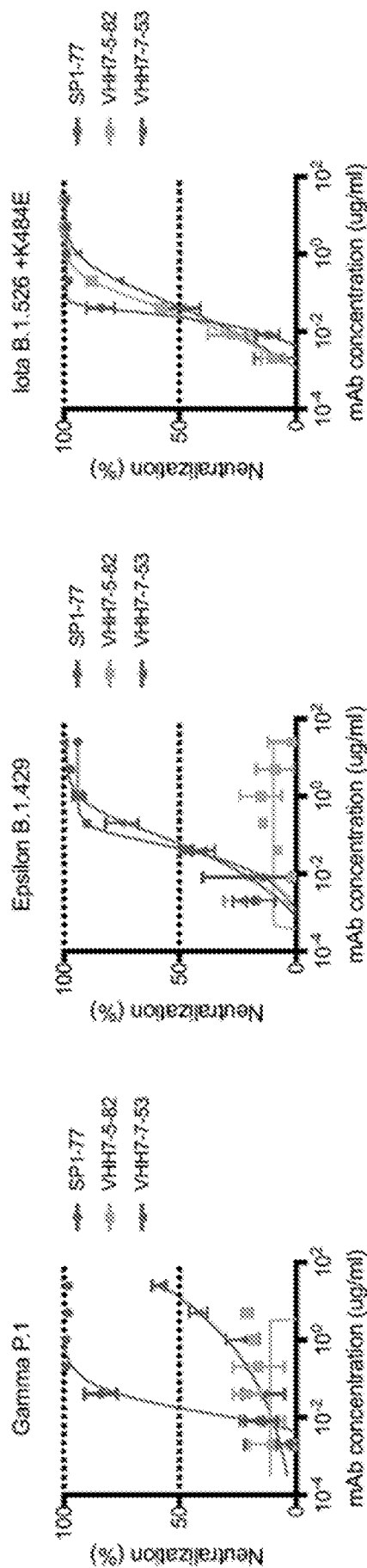


Fig. 29B

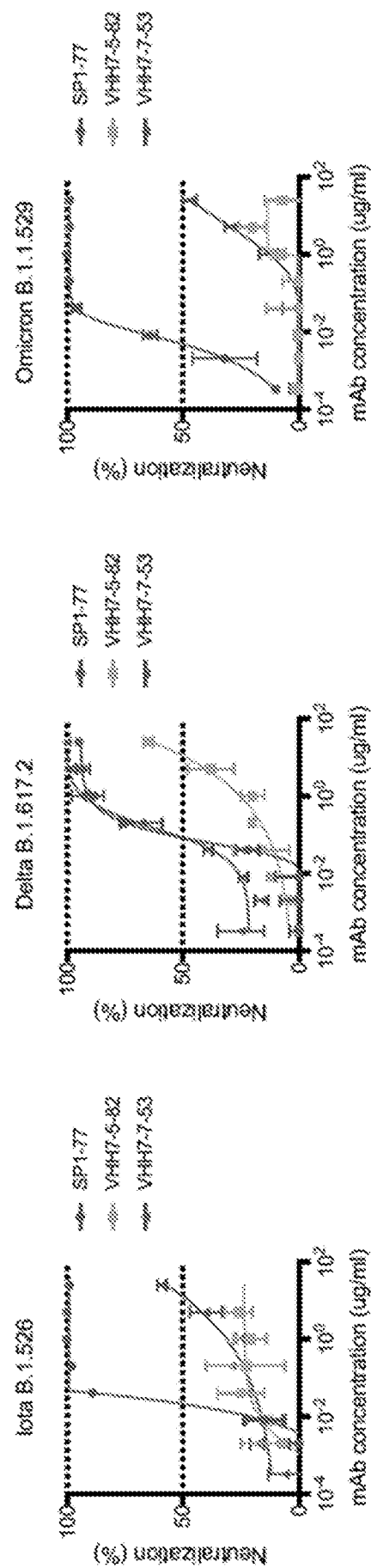


Fig. 29C

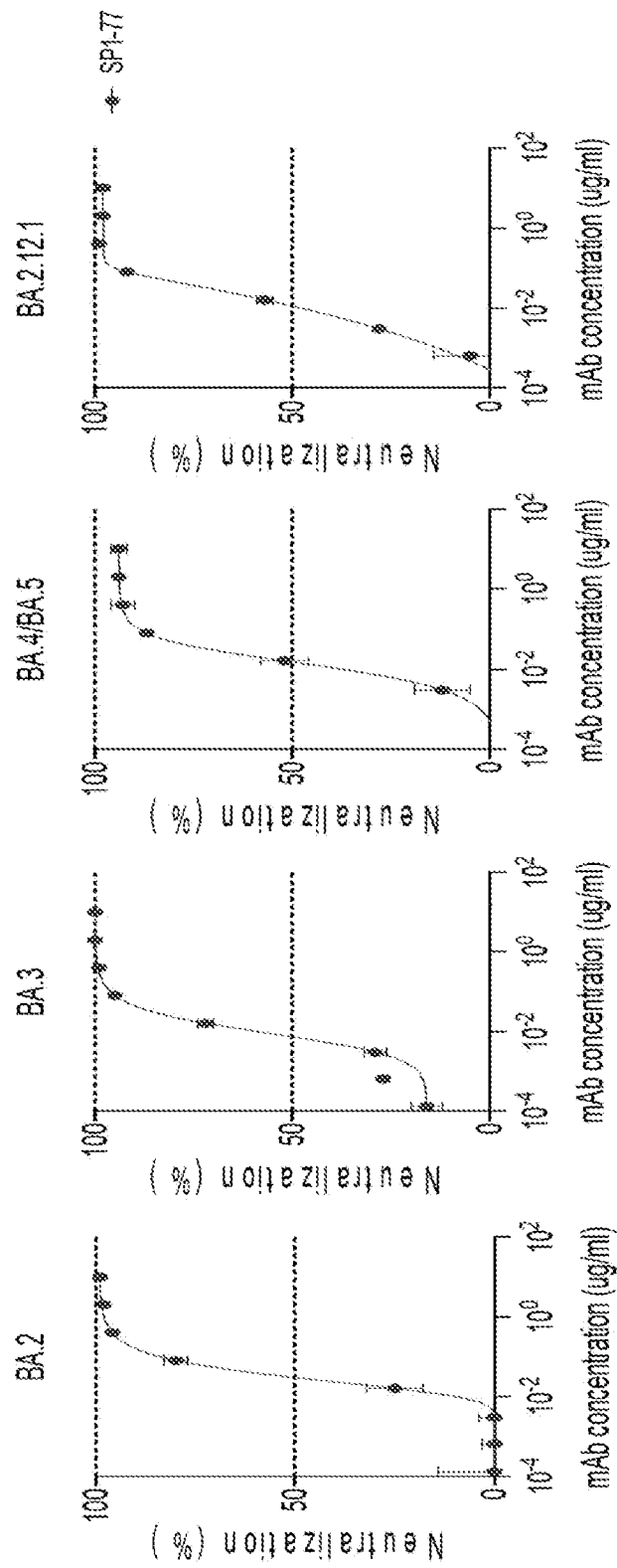


Fig. 29D

Pseudotyped Virus Neutralization of Monoclonal Antibodies – 293T/ACE2/TMPRSS2 (IC₅₀: ng/ml)

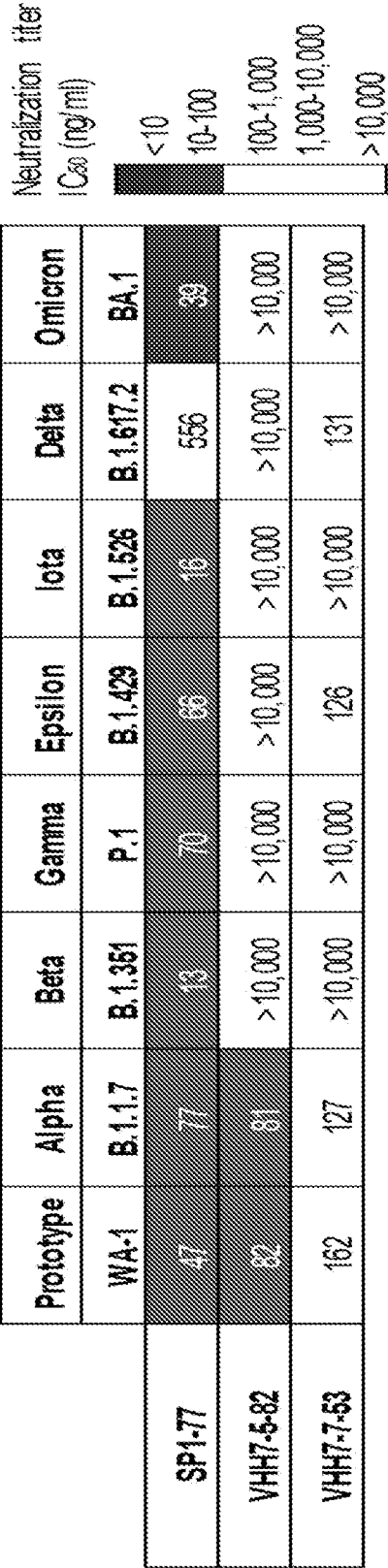


Fig. 29E

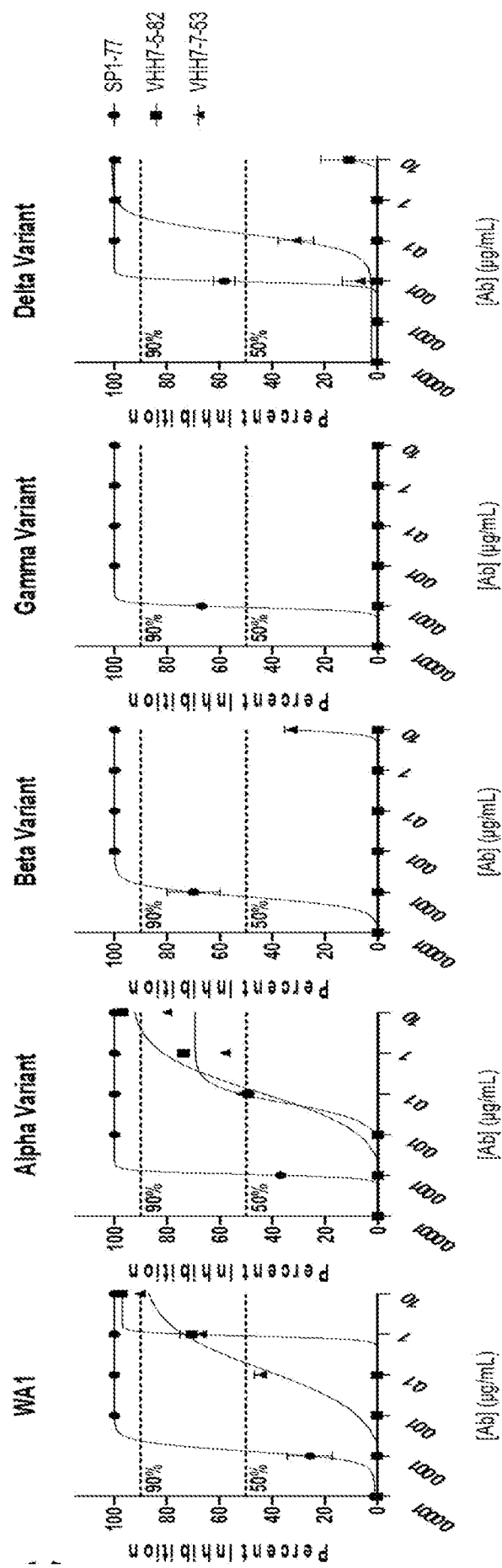


Fig. 29F

Pseudotyped Virus Neutralization of Monoclonal Antibodies – 293T/ACE2 (IC₅₀: ng/ml)

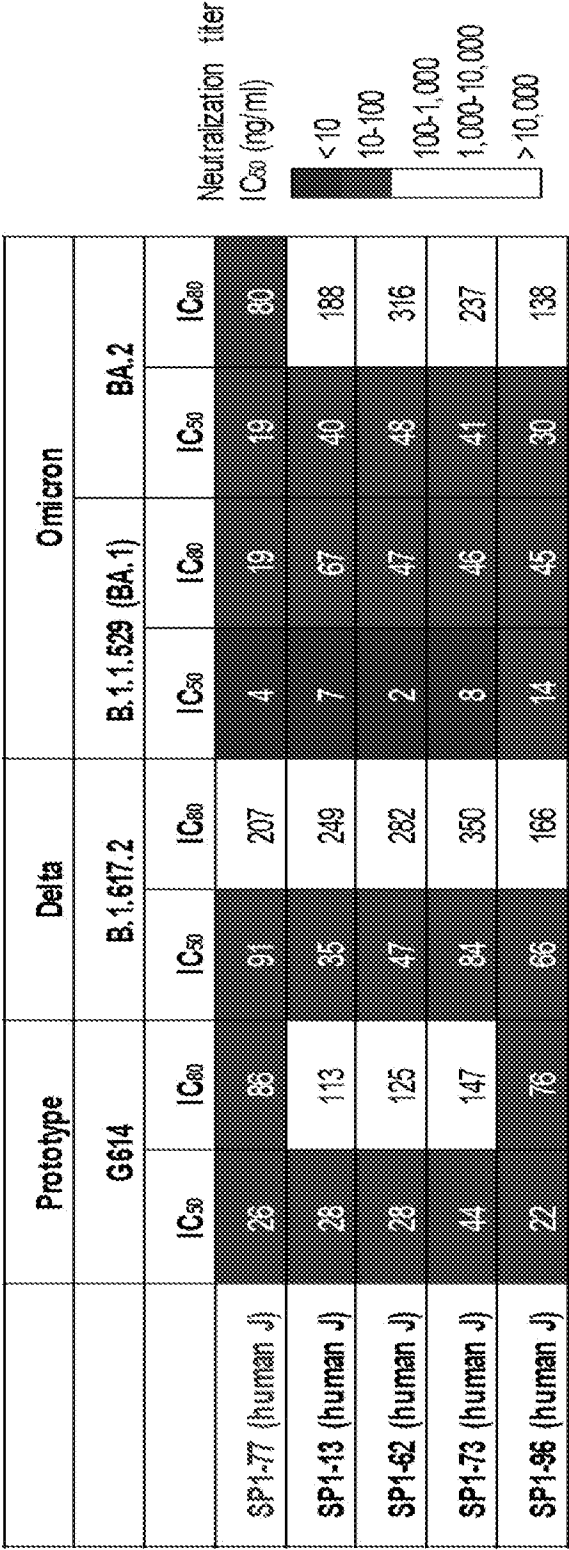


Fig. 29G

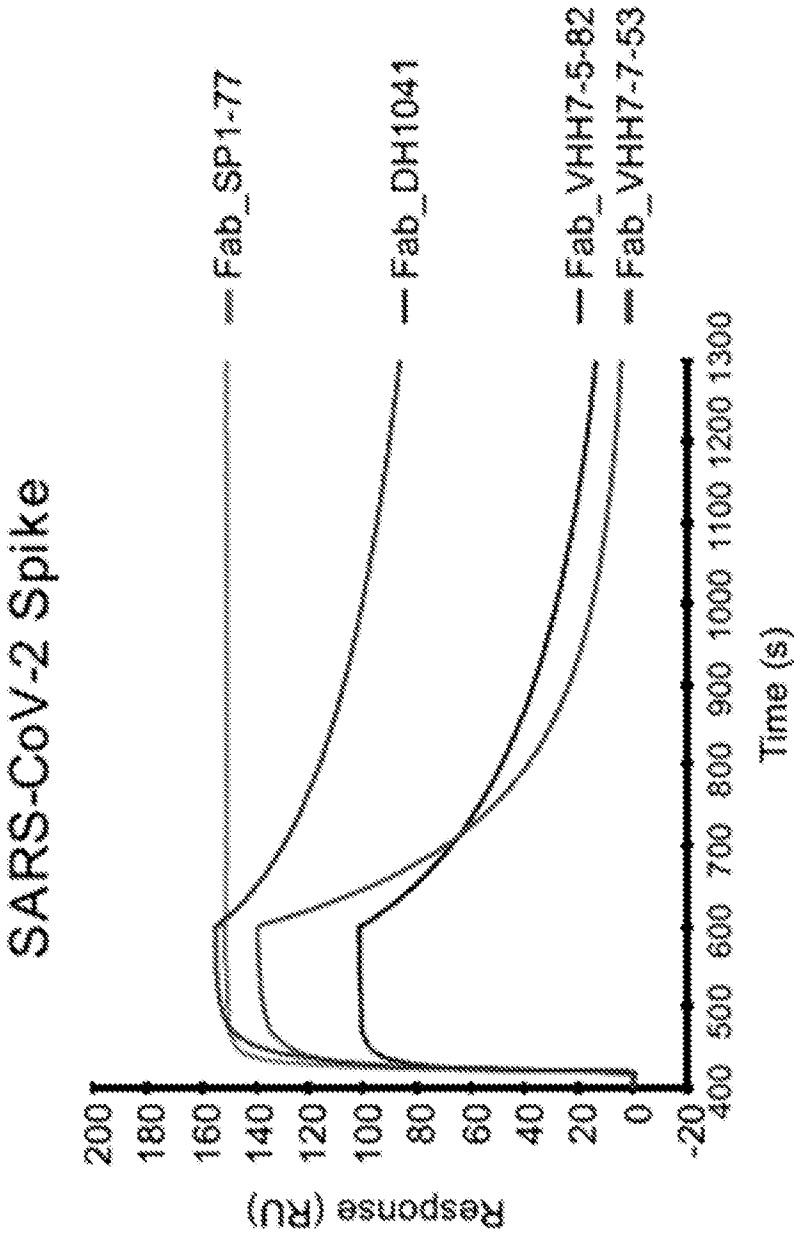


Fig. 30A

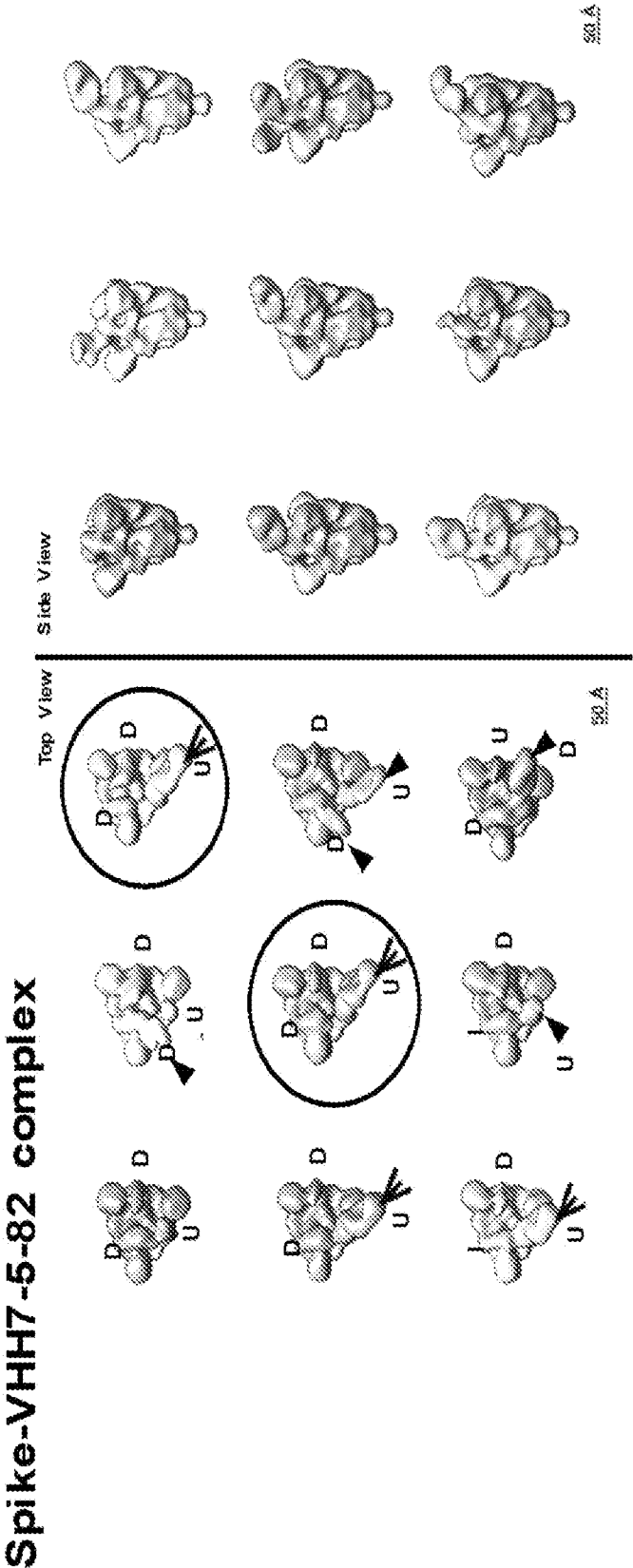
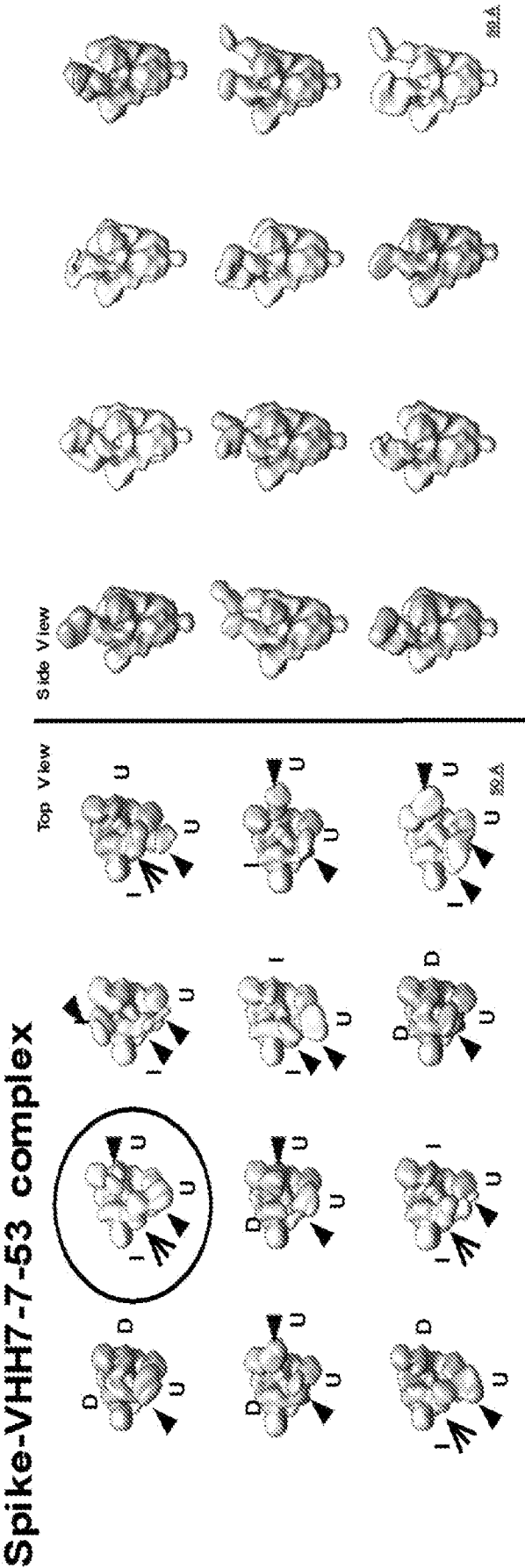


Fig. 30B



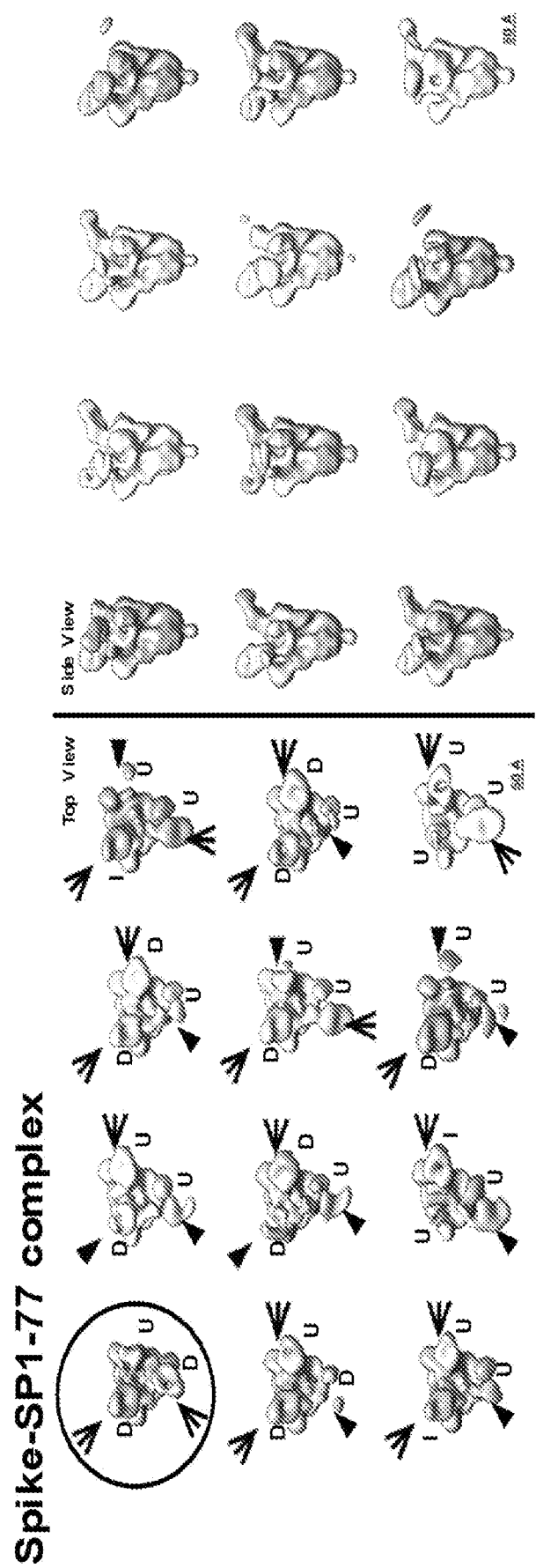


Fig. 30D

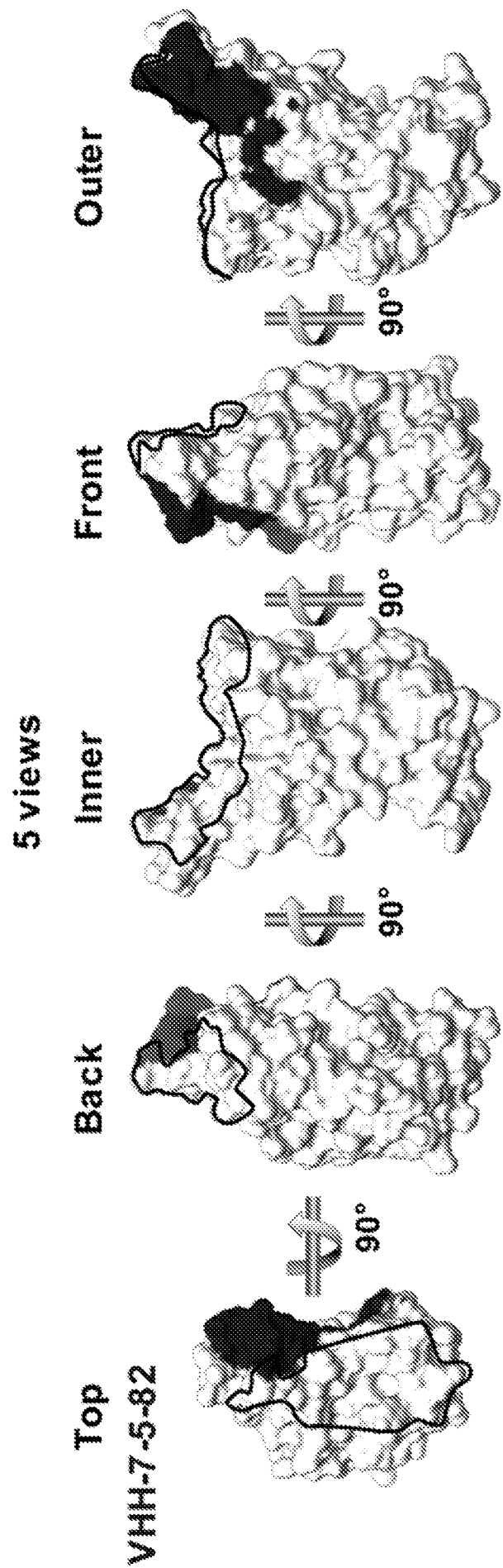


Fig. 31A

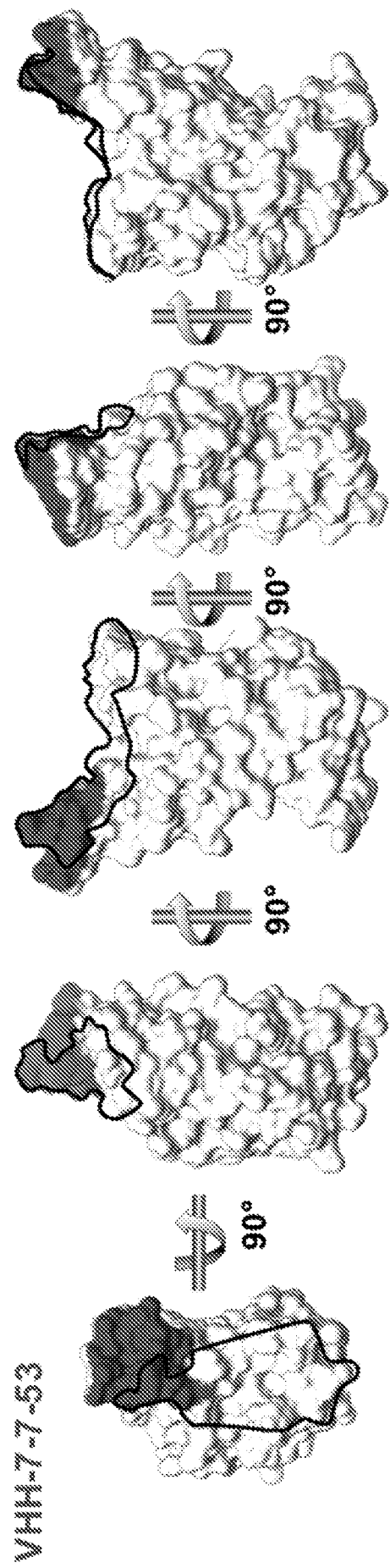


Fig. 31B

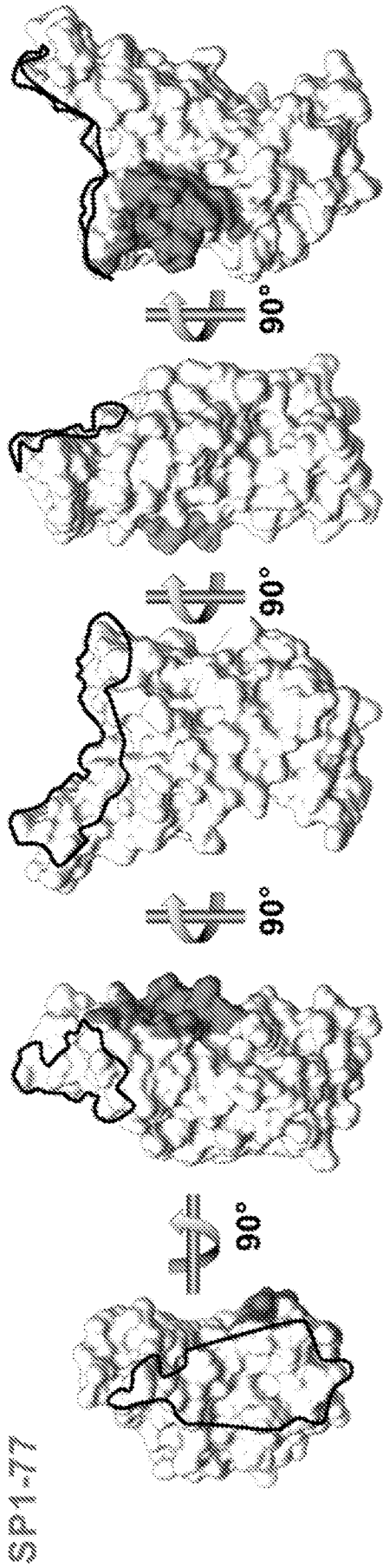


Fig. 31C

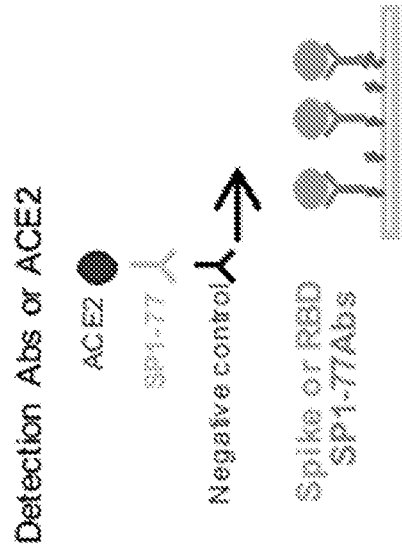


Fig. 31D

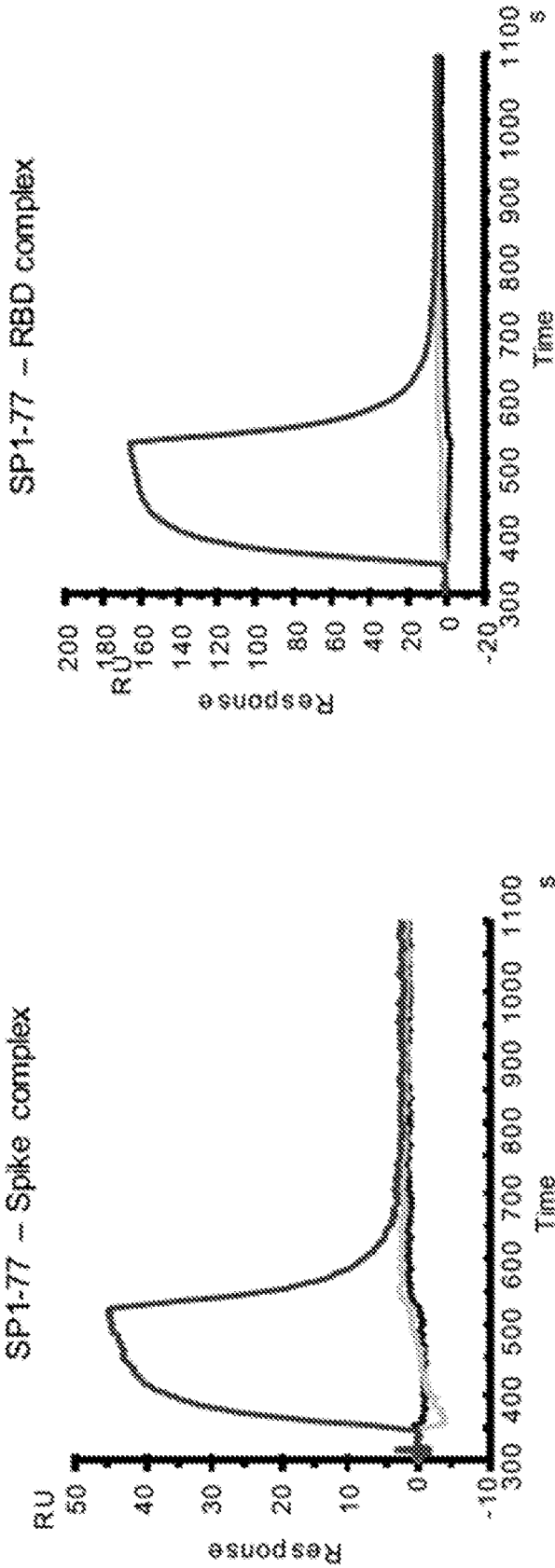


Fig. 31E

Fig. 31F

Name	Heavy V Gene	Light V Gene	CDRH3	CDRL3
SP1-77	IGHV1-2	IGKV1-33	ARDRLVLYGRSEGWYEDV	QQYDNLPT
VHH7-5-82	IGHV1-2	IGKV1-33	ARDRVGOREPY	QQYDNLWT
VHH7-7-53	IGHV1-2	IGKV1-33	ARRNCDY	QQYDNLPT
Fab2-43	IGHV1-2	IGLV2-14	ARGLGVGCSGGNCYLDYYIMDV	SSYTSSSTWV
Fab2-15	IGHV1-2	IGLV2-14	ARGSRCSCGNCYGWAYDAFDI	SSYTSSSTFV
S2-M11	IGHV1-2	IGKV3-20	ARAAPEYDFWSGYSYEDY	QQYGSSAWT
H4	IGHV1-2	IGKV2-40	ARVPYCSSTSCHRDWYEDL	MORIEFPLT
COVOX-316	IGHV1-2	IGLV2-8	ARDMAFSMVRGSEFY	SSYAGSNHWV
C121	IGHV1-2	IGLV2-23	ARAPLEPTIGVLAGDYYYYGMDV	CSYAGSSILV
Fab2-4	IGHV1-2	IGLV2-8	ARDRSWAVVYYIMDV	SSYAGSNNLV
CV05-163	IGHV1-2	IGKV3-11	AREVMVRGALPPYGMVDV	QQRSNWPPVT
REGN10989	IGHV1-2	IGLV2-14	ARGSRYDWNQNNWFDP	SSFTTSTTV

Fig. 32A

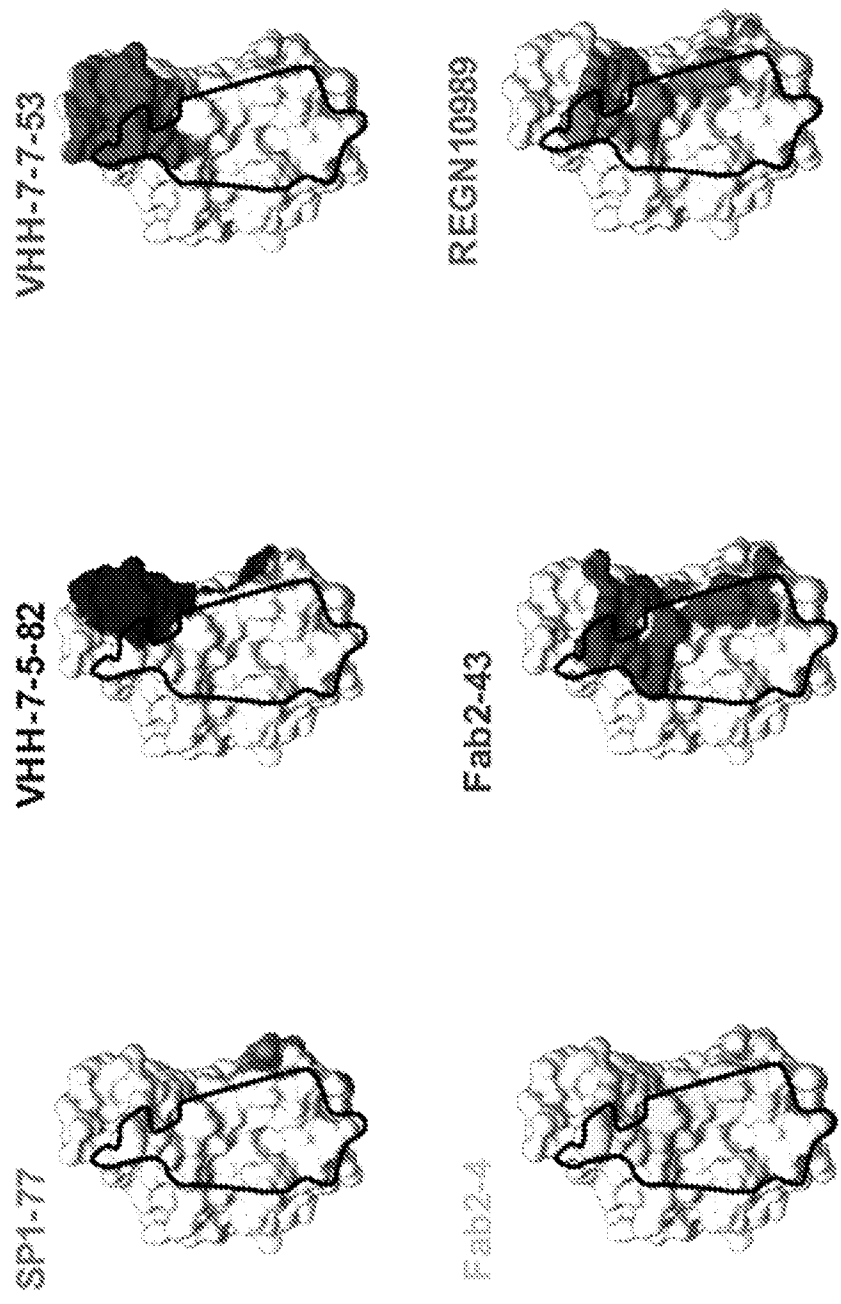


Fig. 32B

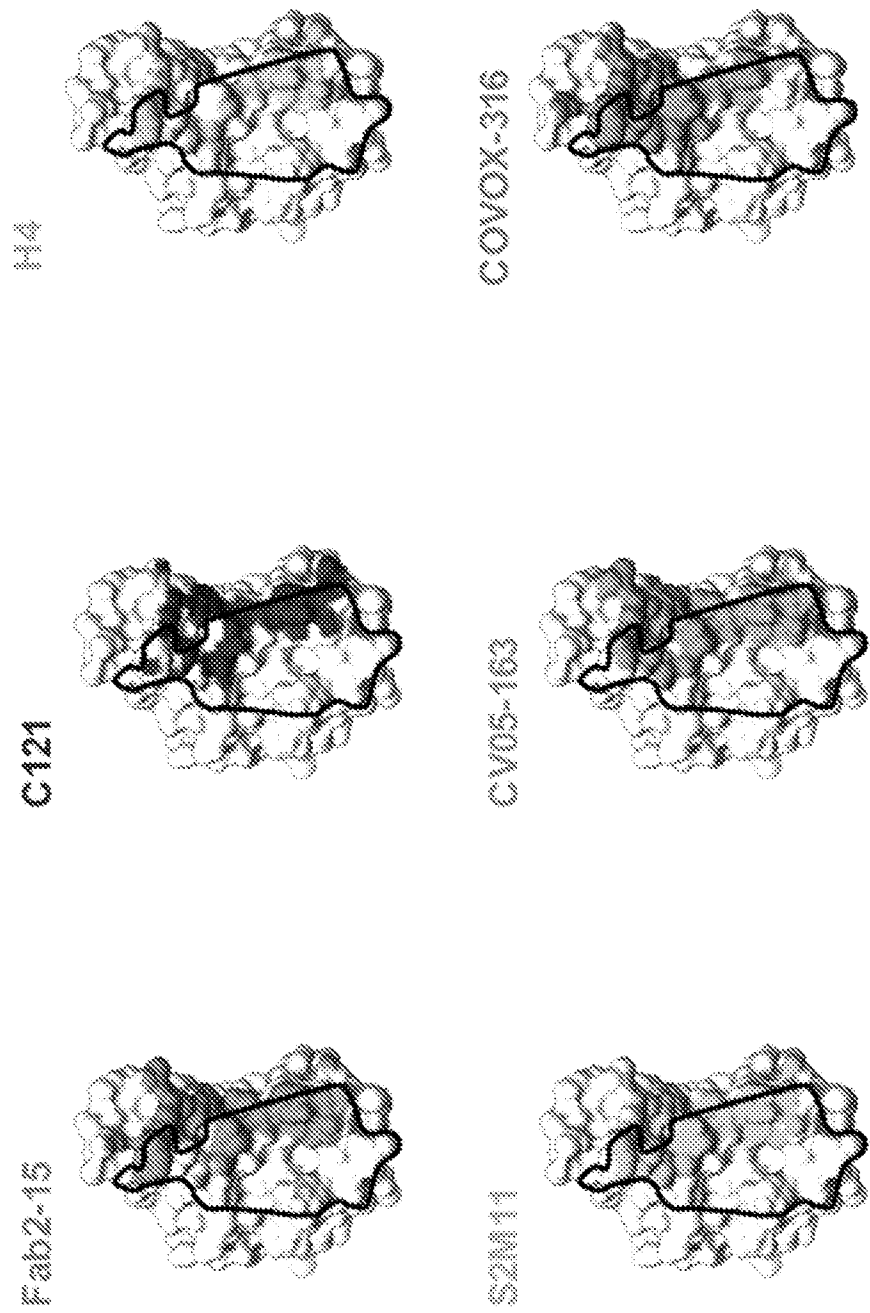


Fig. 32C

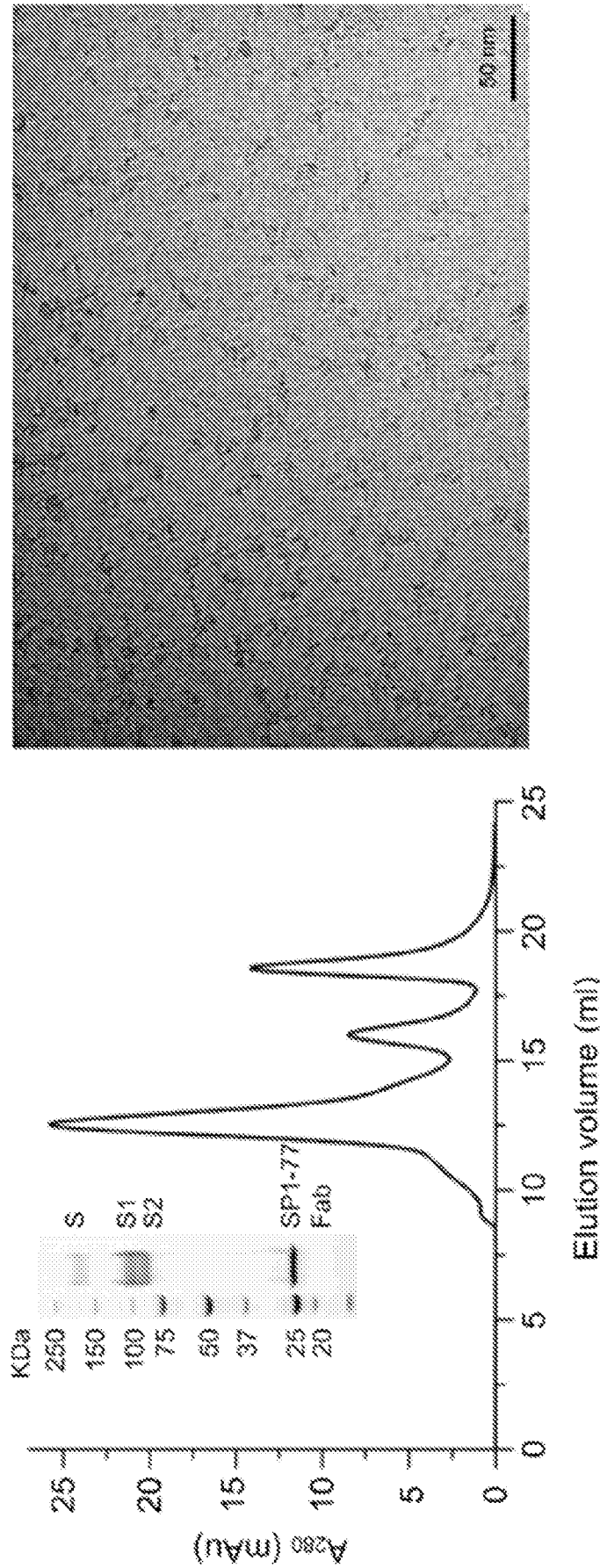


Fig. 33A

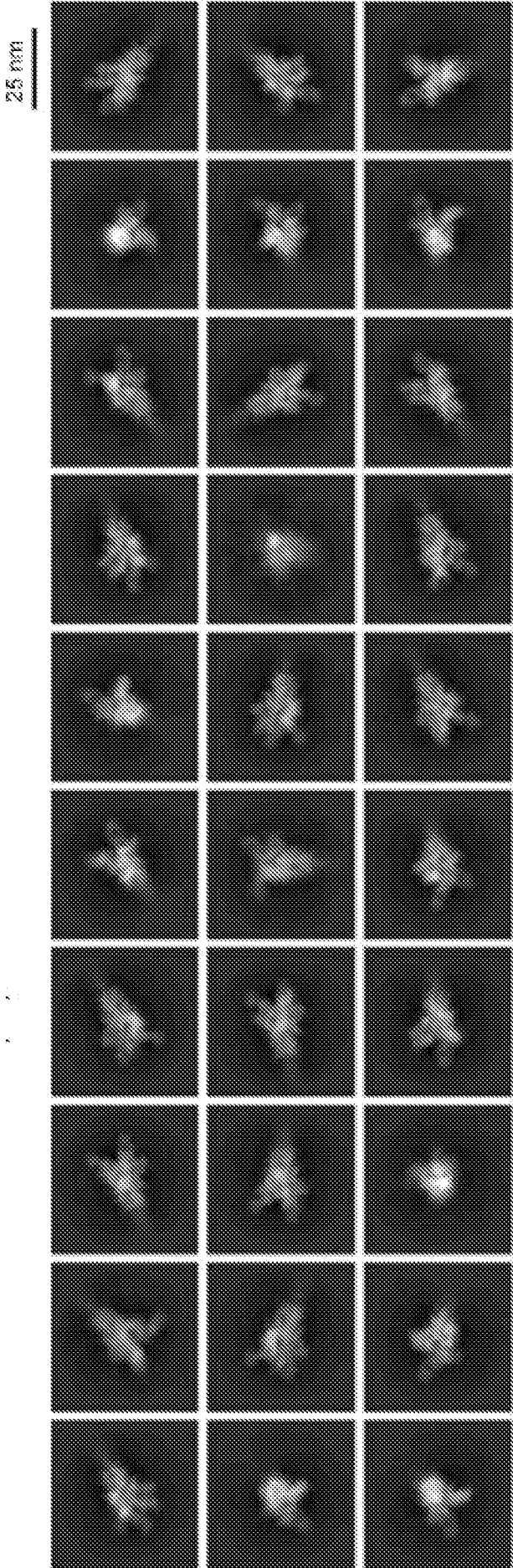
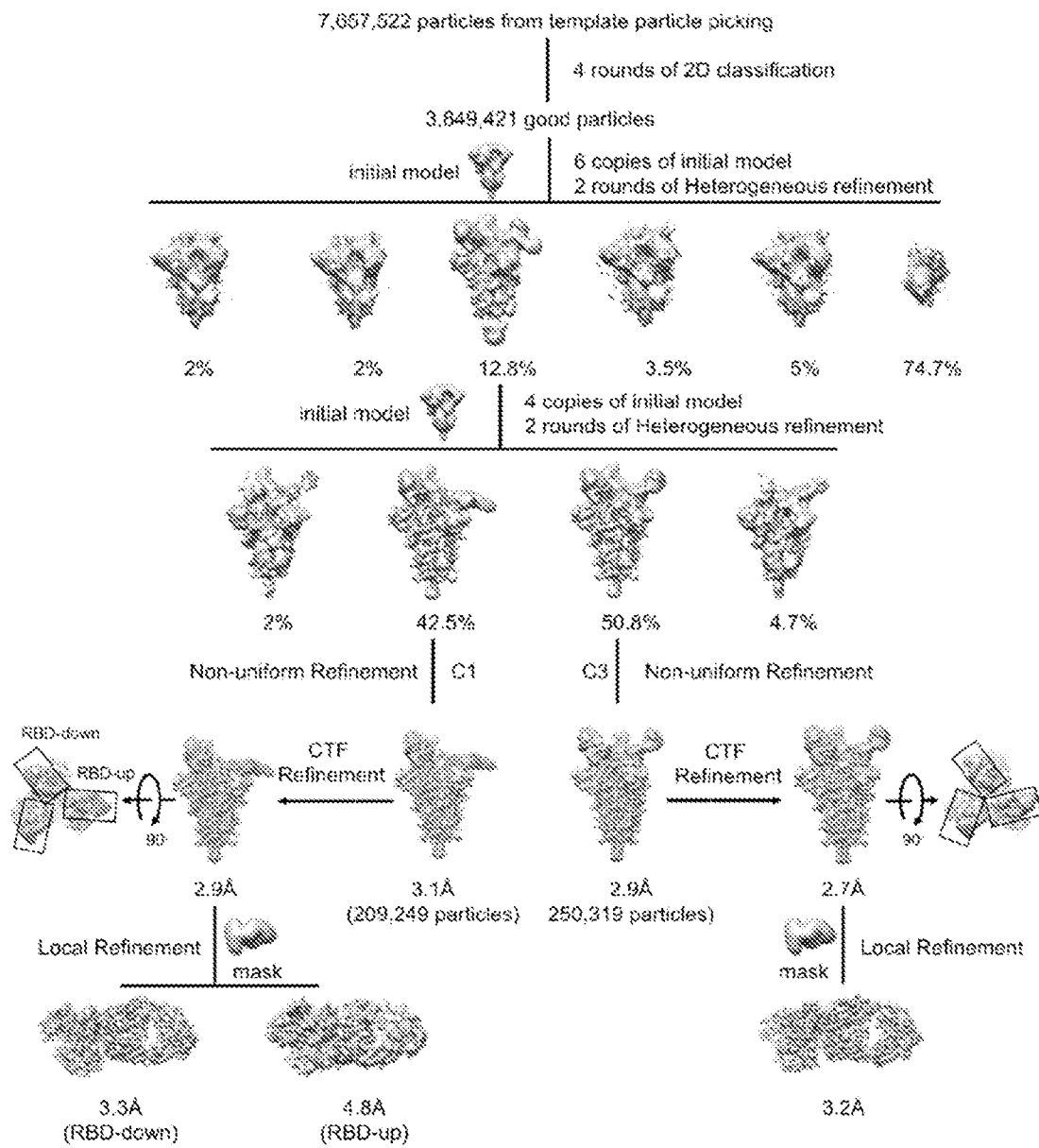


Fig. 33B

**Fig. 33C**

C3 symmetry, RBD-down G614 with SP1-77 Fab
250,319 particles
GSFSC Resolution 2.68Å

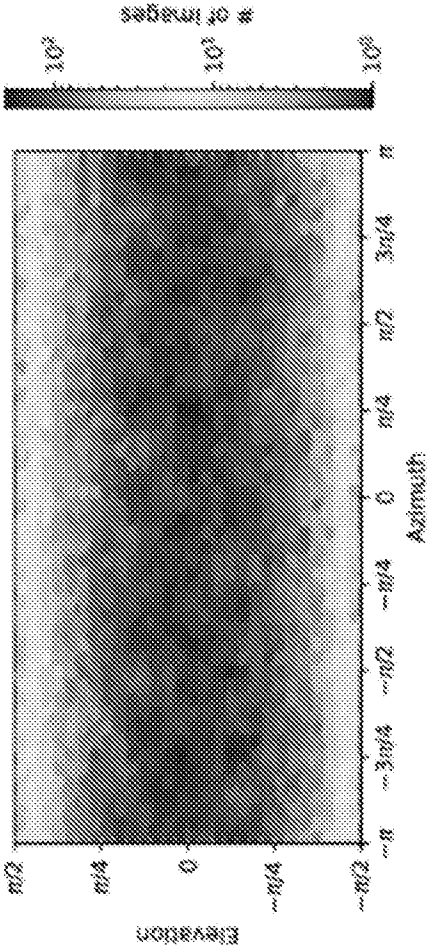
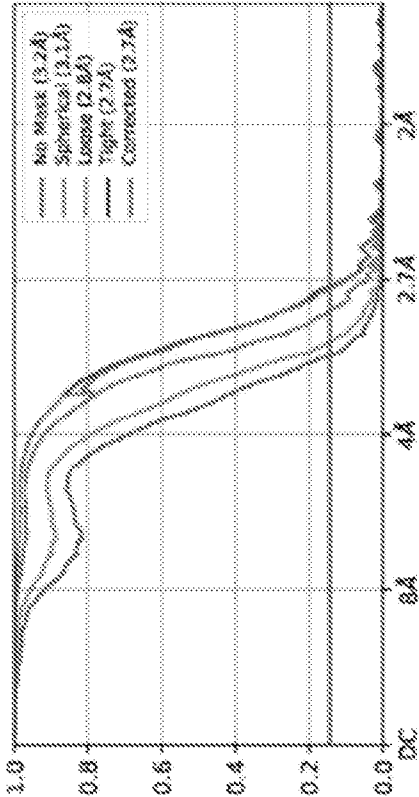


Fig. 34A

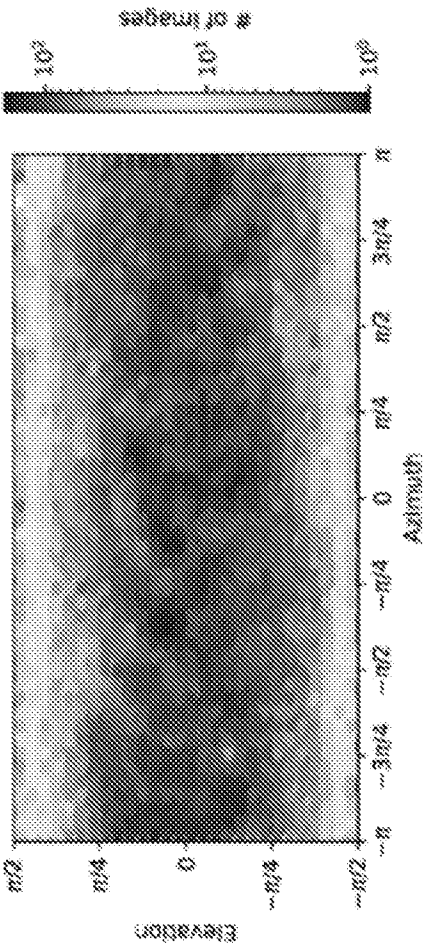
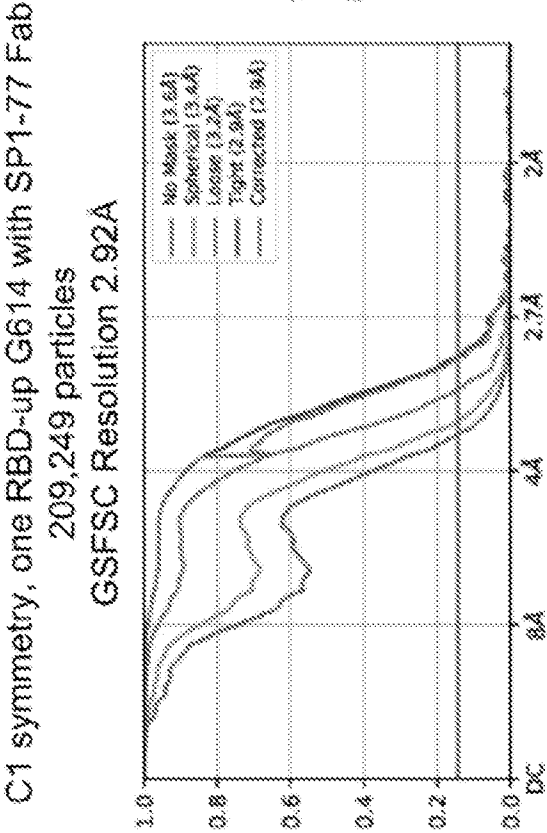


Fig. 34B

C1 symmetry, RBD-down with SP1-77 Fab local refine
250,319 particles
GSFSC Resolution 3.23Å

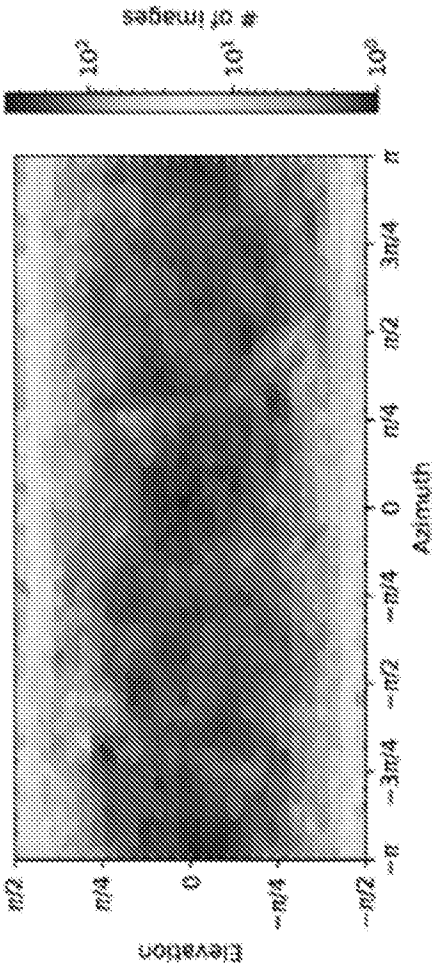
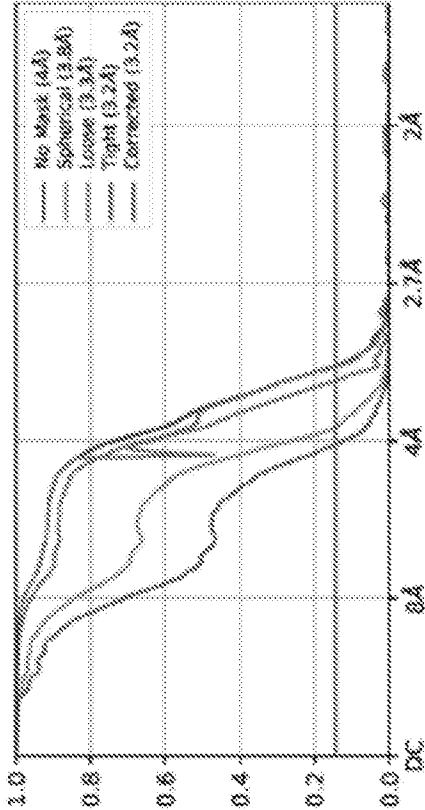


Fig. 34C

C1 symmetry, RBD-down with SP1-77 Fab local refine
209,249 particles
GSFSC Resolution 3.31Å

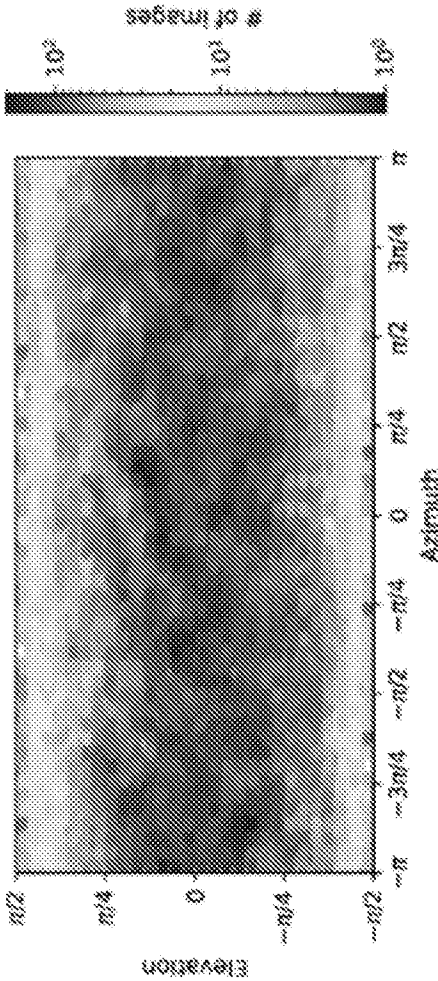
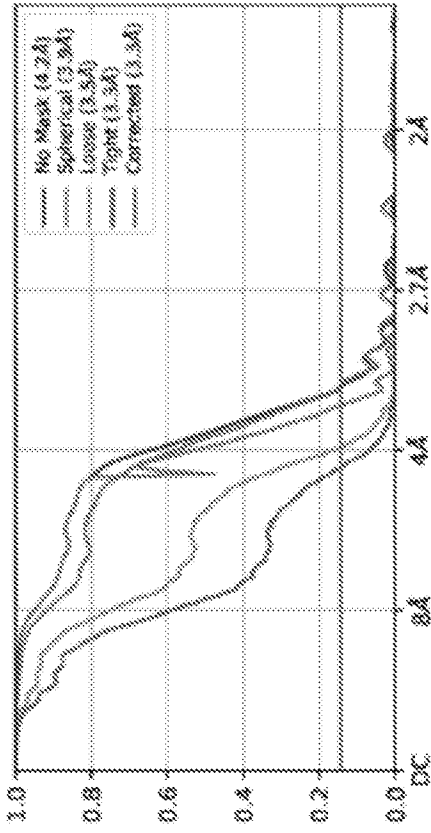


Fig. 34D

C1 symmetry, RBD-up with SP1-77 Fab local refine

209,249 particles

GSFSC Resolution 4.84Å

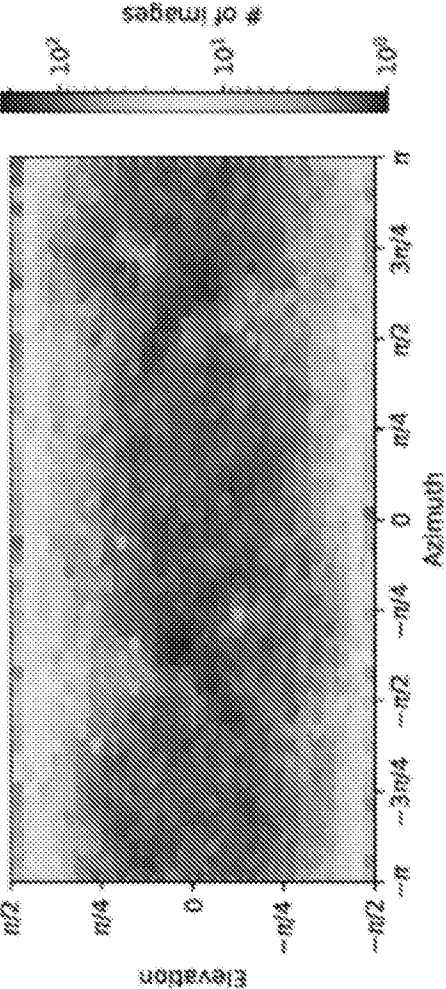
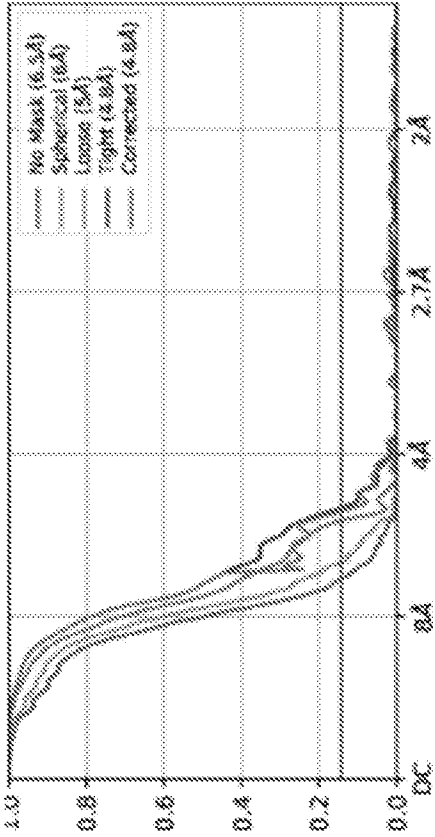


Fig. 34E

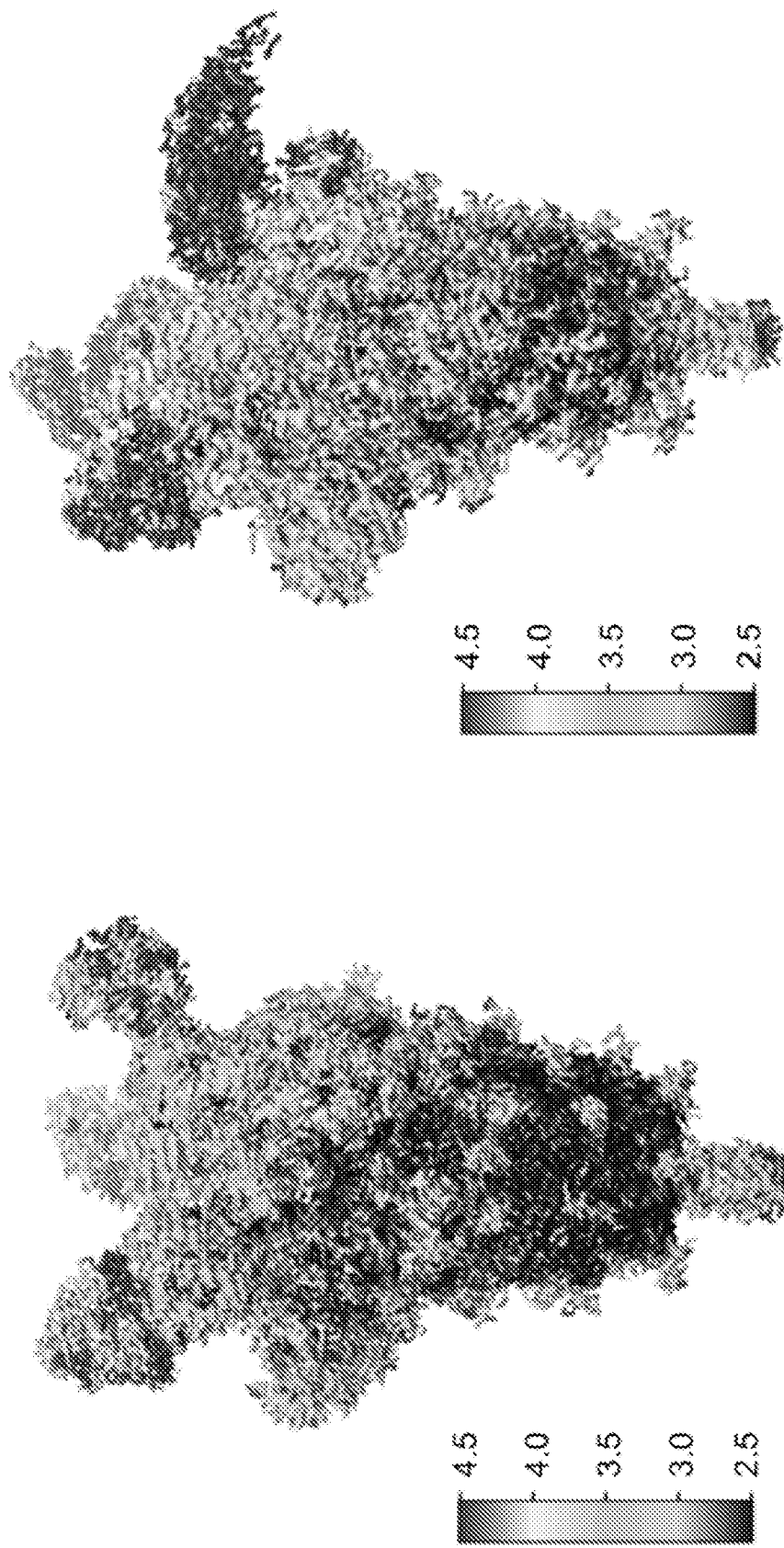


Fig. 35A

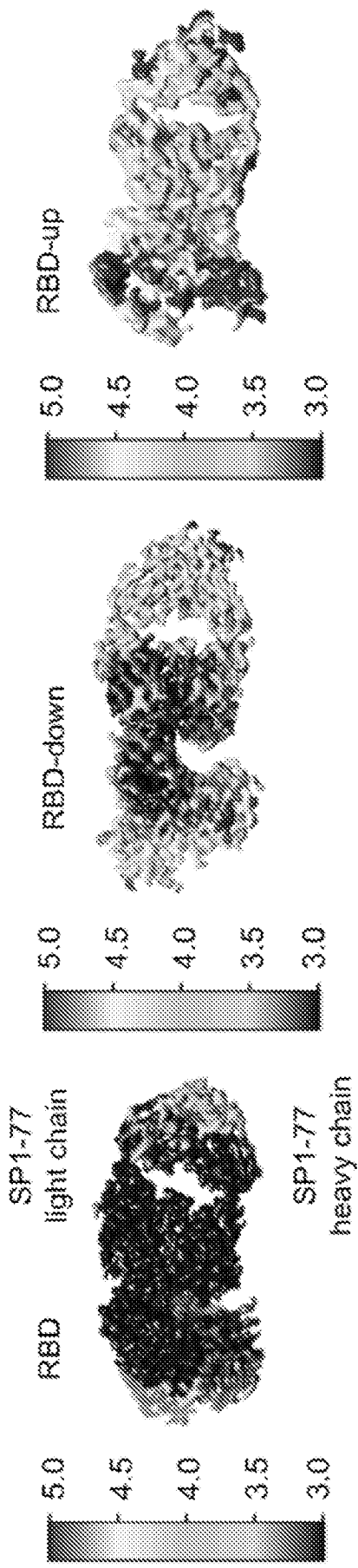


Fig. 35B

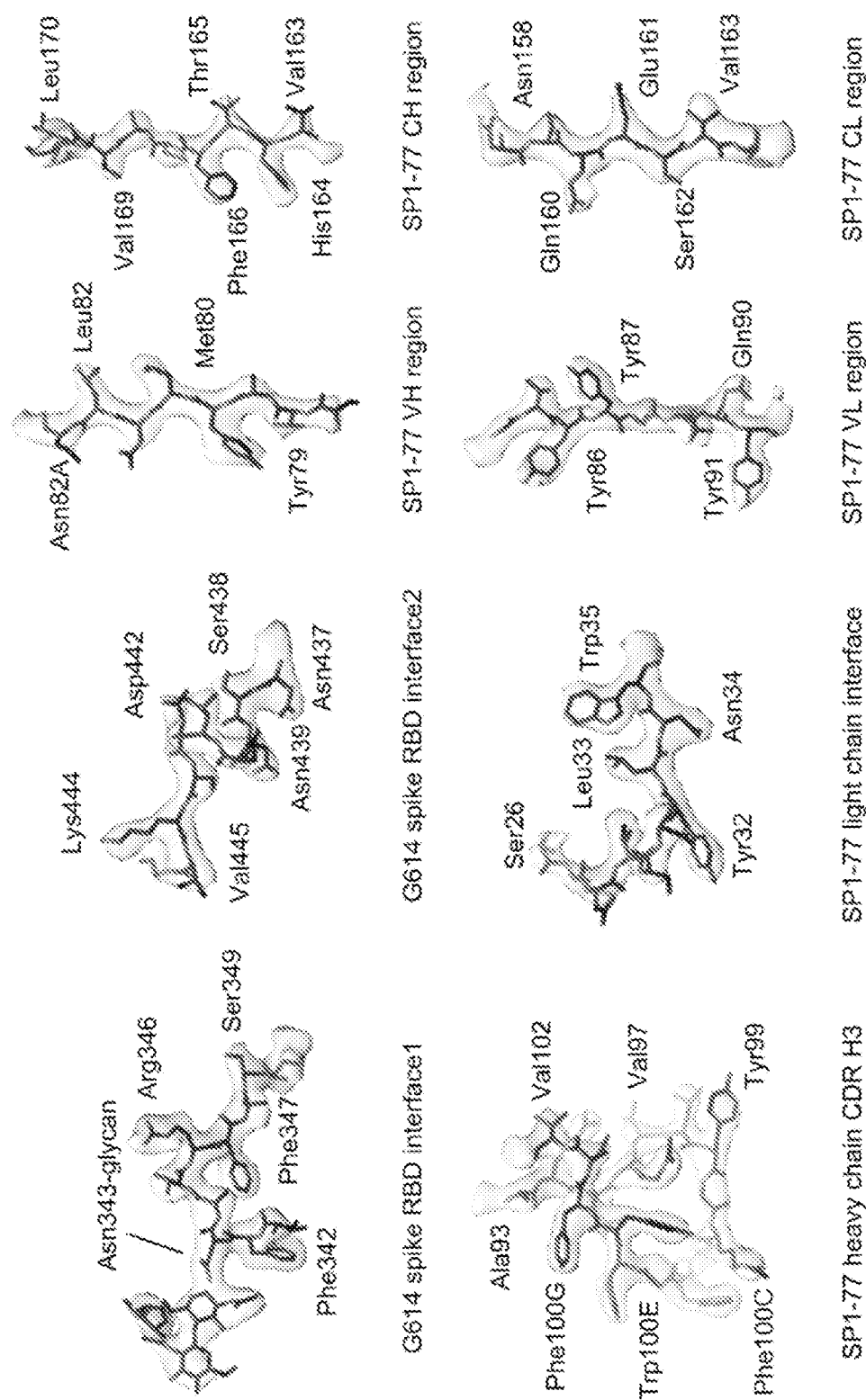


Fig. 35C

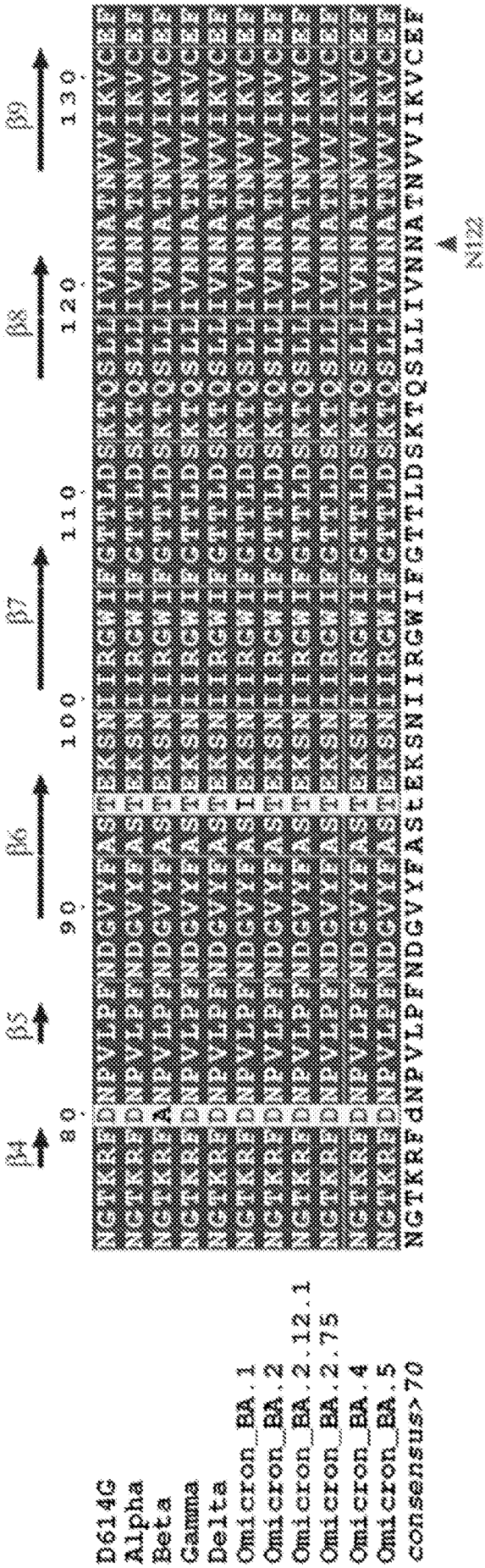


Fig. 36A

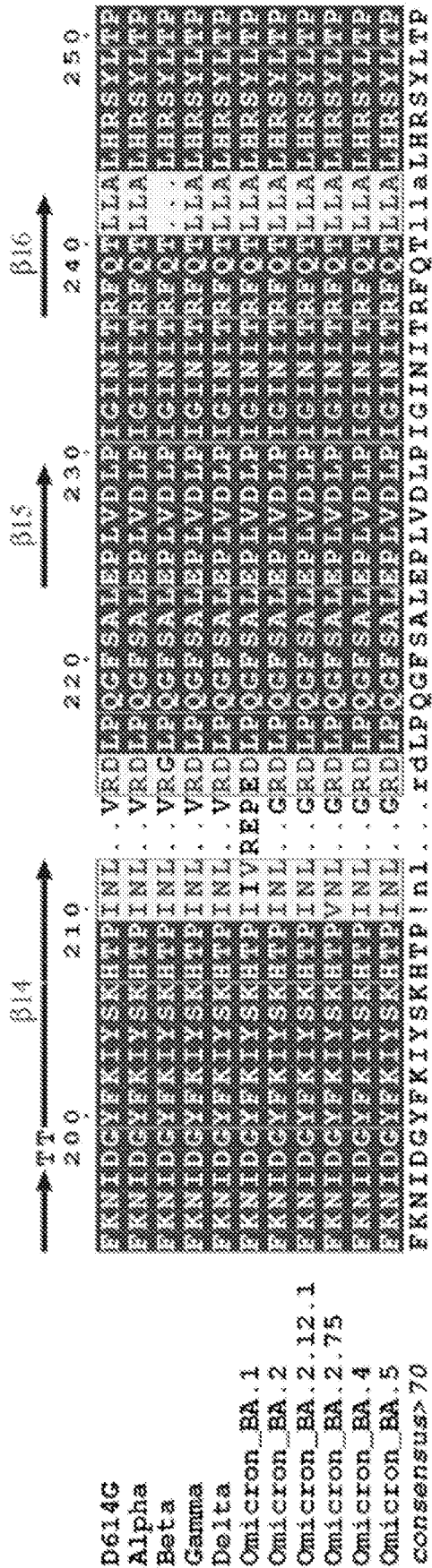


Fig. 36C

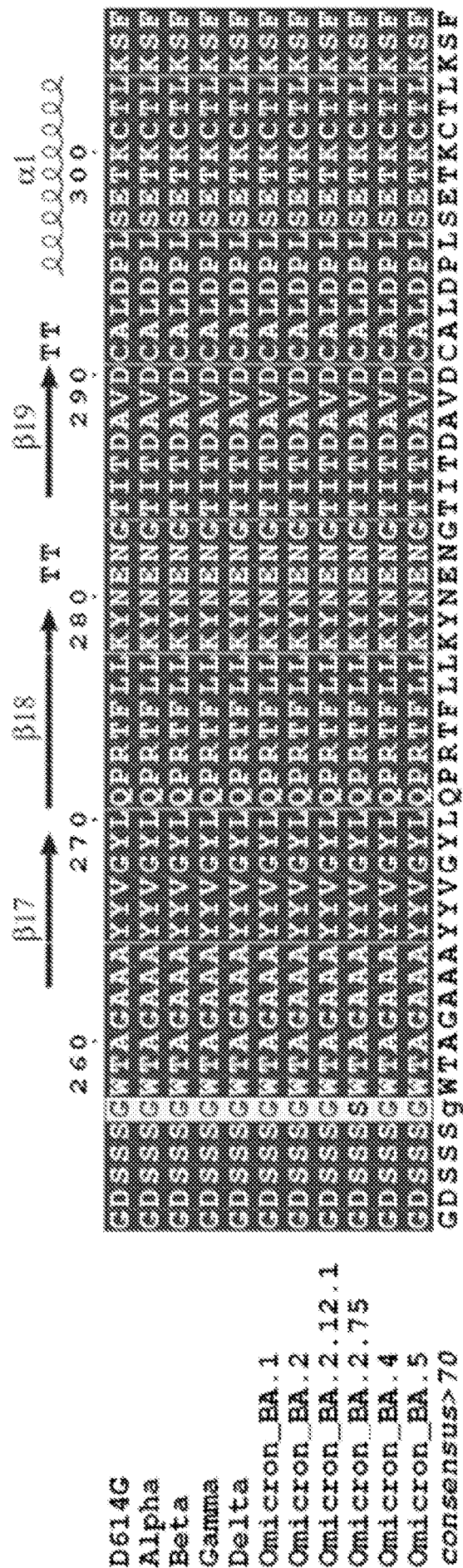


Fig. 36D

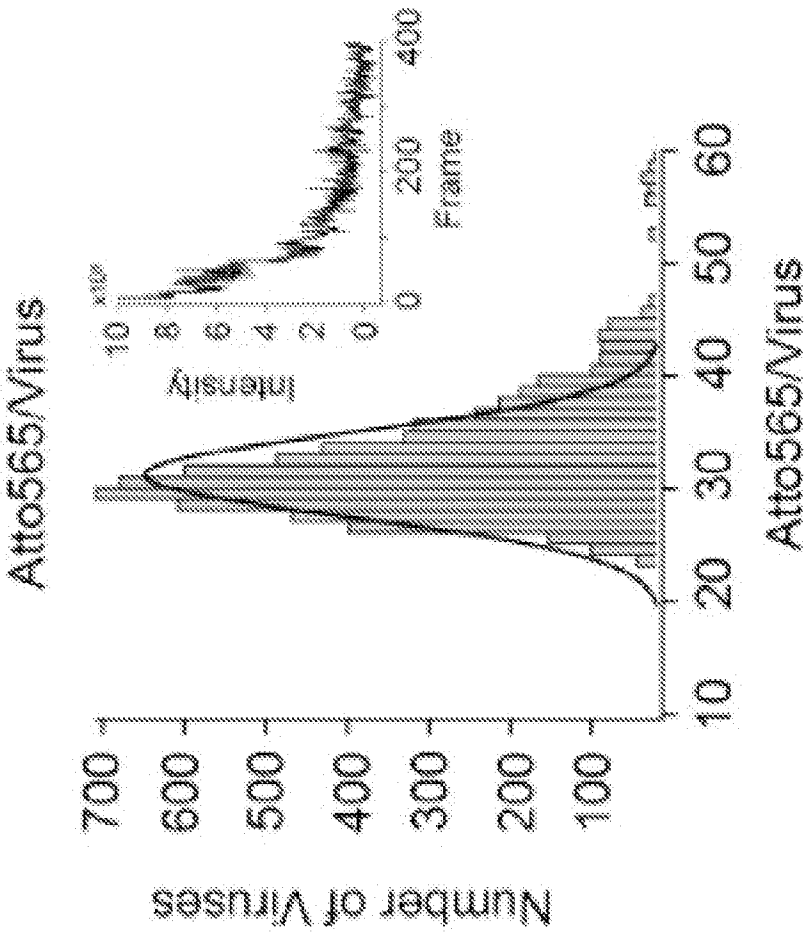


Fig. 37B

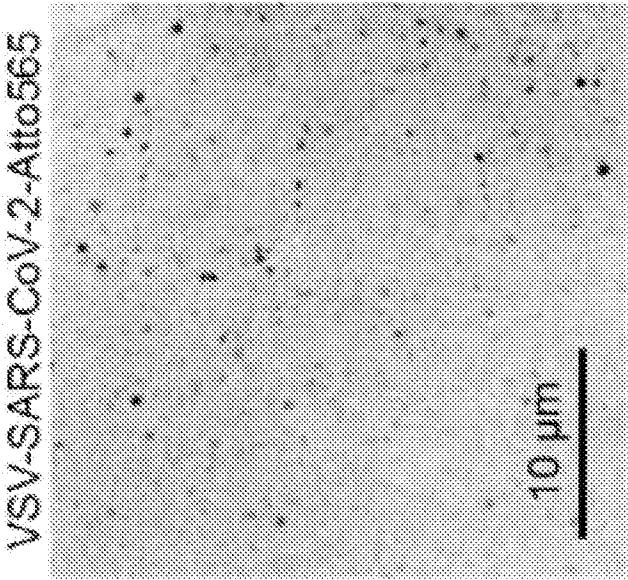


Fig. 37A

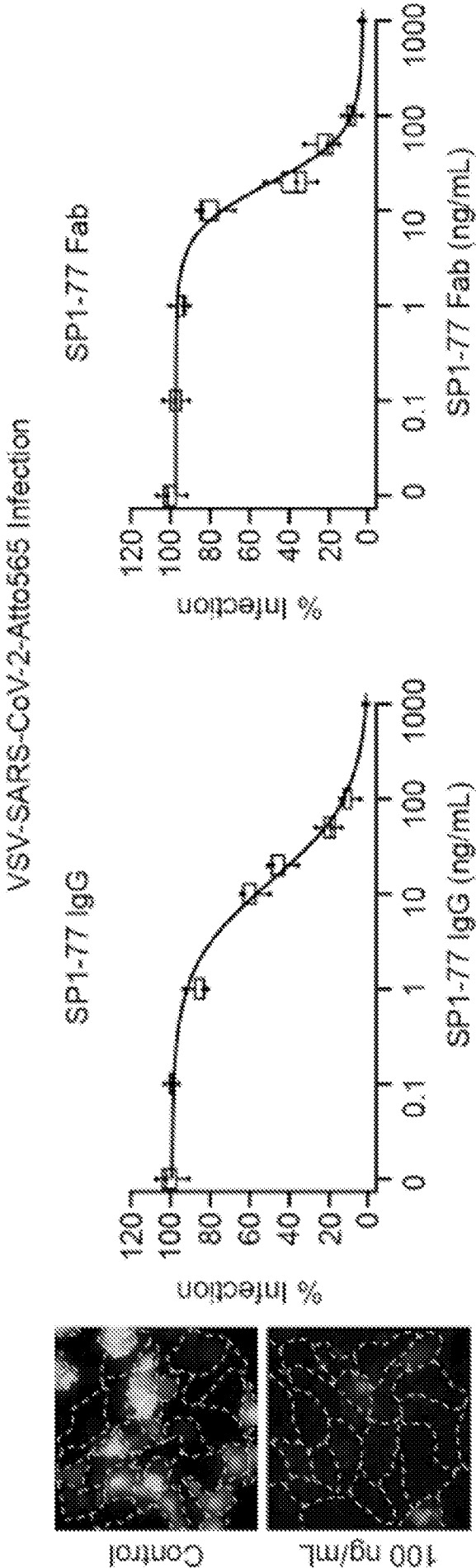


Fig. 37C