Cell Reports, Volume *36*

Supplemental information

Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals

Elaine C. Chen, Pavlo Gilchuk, Seth J. Zost, Naveenchandra Suryadevara, Emma S. Winkler, Carly R. Cabel, Elad Binshtein, Rita E. Chen, Rachel E. Sutton, Jessica Rodriguez, Samuel Day, Luke Myers, Andrew Trivette, Jazmean K. Williams, Edgar Davidson, Shuaizhi Li, Benjamin J. Doranz, Samuel K. Campos, Robert H. Carnahan, Curtis A. Thorne, Michael S. Diamond, and James E. Crowe Jr.

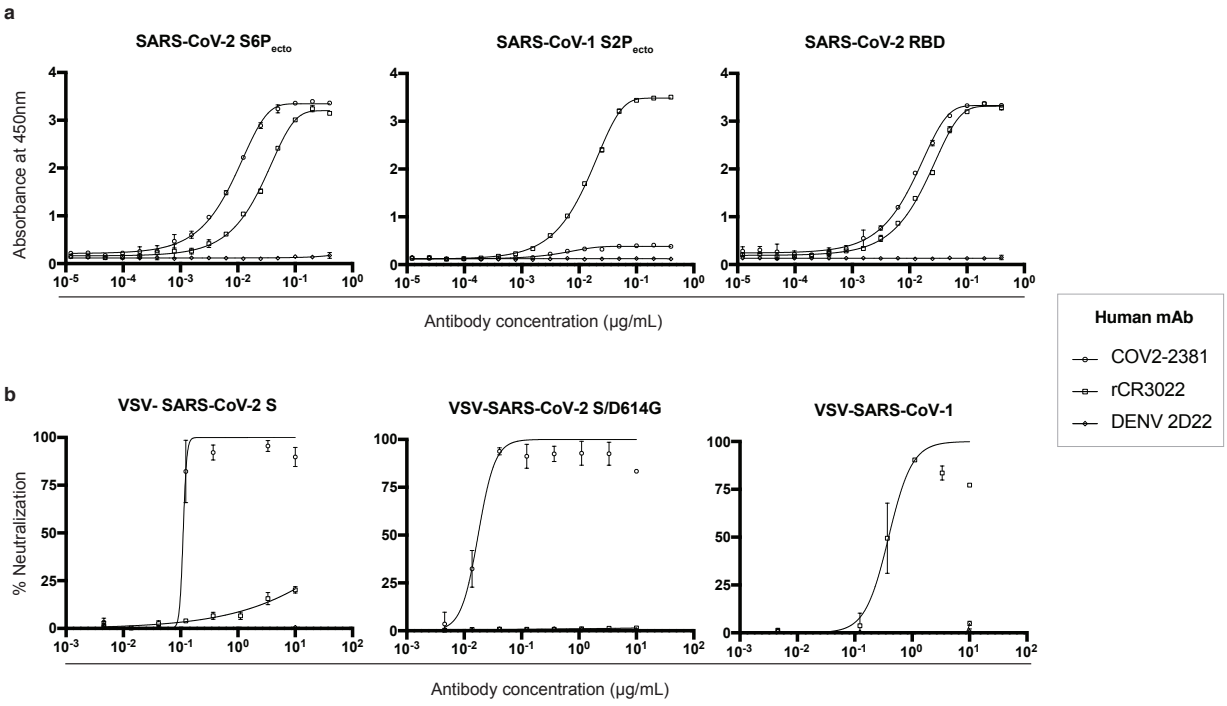

Figure S1. Controls for ELISA and neutralization assays, Related to Figure 2.

a. Positive or negative controls used for testing antibody binding in ELISA to SARS-CoV2-S6P$_{ecto}$, SARS-CoV-1 S2P$_{ecto}$, or SARS-CoV-2 RBD proteins. The positive control antibody COV2-2381 binds to SARS-CoV-2 S2P$_{ecto}$ and RBD but not to SARS-CoV-1 S2P$_{ecto}$, and the positive control antibody rCR3022 also binds to SARS-CoV-1 S2P$_{ecto}$.

b. Positive or negative controls used for replication-competent chimeric VSV neutralization assays. COV2-2381 was used as a positive control for SARS-CoV-2 WT and D614G, whereas rCR3022 was used as a positive control for SARS-CoV-1.

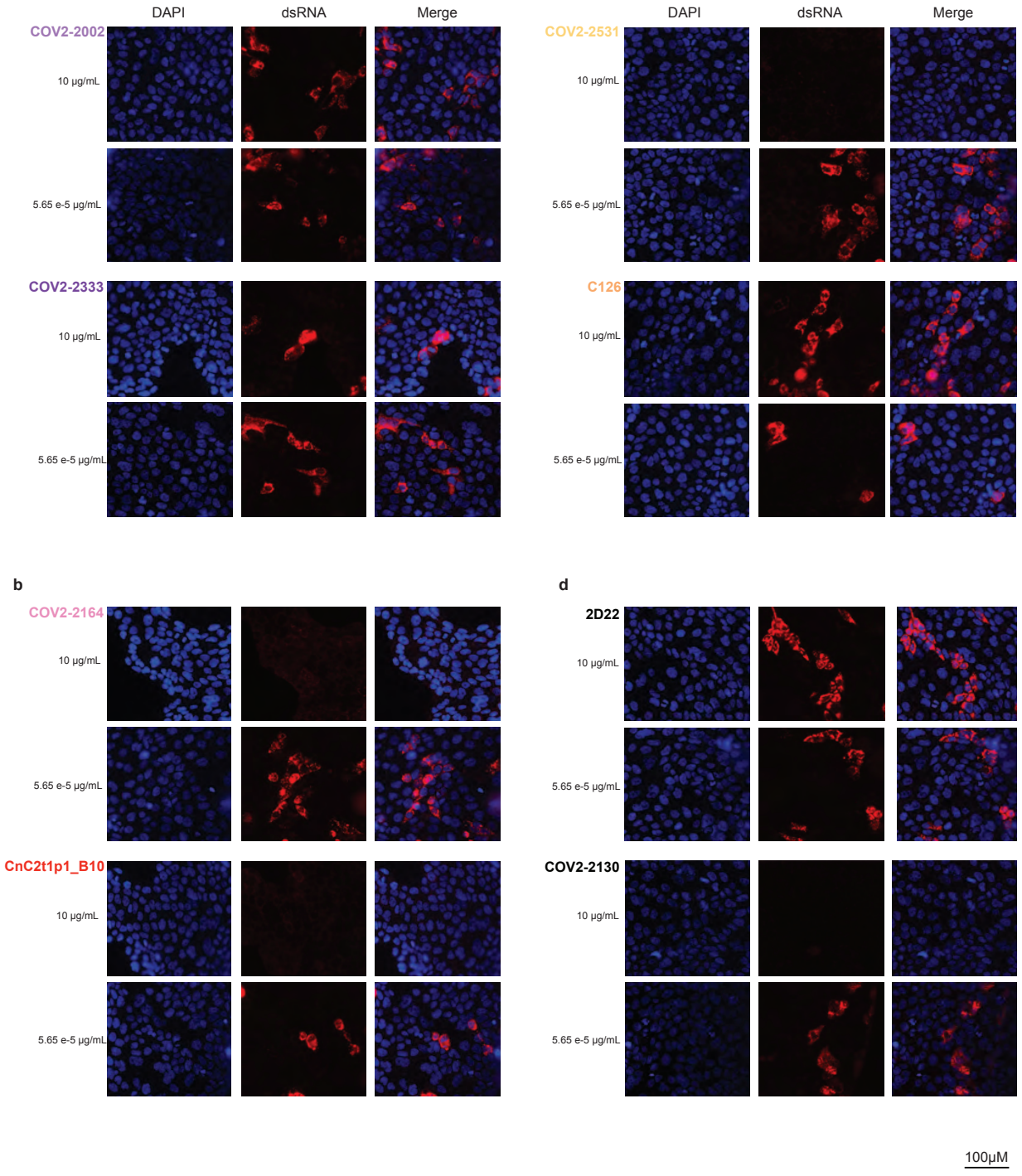

Figure S2. Staining of dsRNA intensity, Related to Figure 2. Staining of dsRNA intensity split into DAPI stain, dsRNA stain, and the two merged for each antibody group.

a. Staining for Group 1 antibodies.

b. Staining for Group 2 antibodies.

c. Staining for Group 3 antibodies.

d. Staining for control antibodies. 2D22[47] is used as a negative control antibody. COV2-2130[43] is used as a positive control antibody.

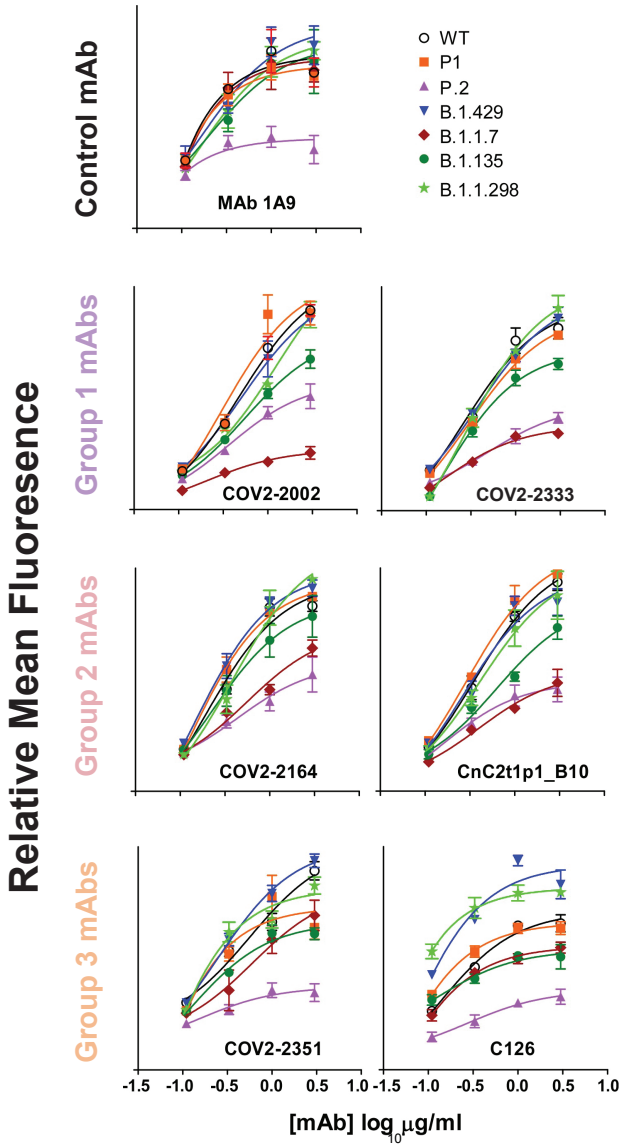

Figure S3. Antibody binding to cell surface displayed variant S protein, Related to Figure 2. All public clonotypes were tested against all variants listed, starting at 1 μg/mL. 1A9 from Genetex, an anti-S2 non-conformational antibody, was used as a control to test for spike protein expression.

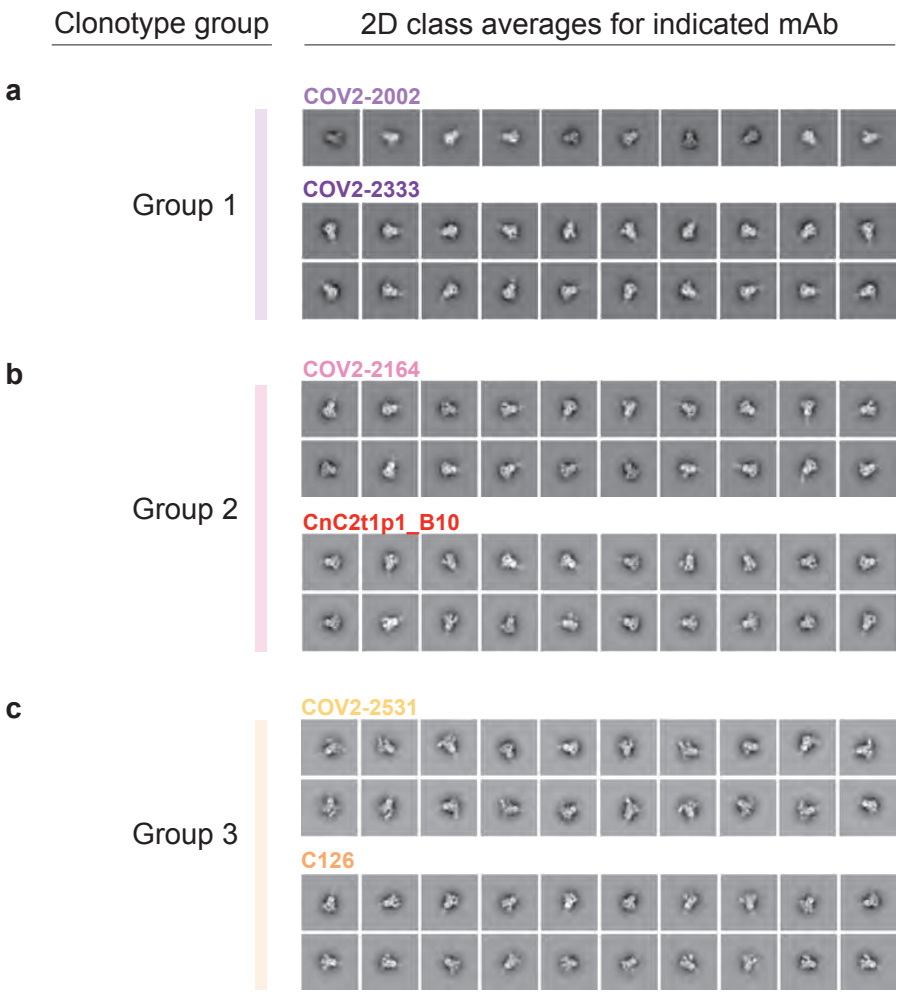

Figure S4. Negative stain electron microscopy complexes of each public clonotype, Related to Figure 3.

a. Negative stain EM of SARS-CoV-2 $S6P_{ecto}$ protein in complex with Fab forms of COV2-2002 or COV2-2333.

b. Negative stain EM of SARS-CoV-2 $S6P_{ecto}$ protein in complex with Fab forms of COV2-2164 or CnC2t1p1_B10.

c. Negative stain EM of SARS-CoV-2 $S6P_{ecto}$ protein in complex with Fab forms of COV2-2531 or C126.

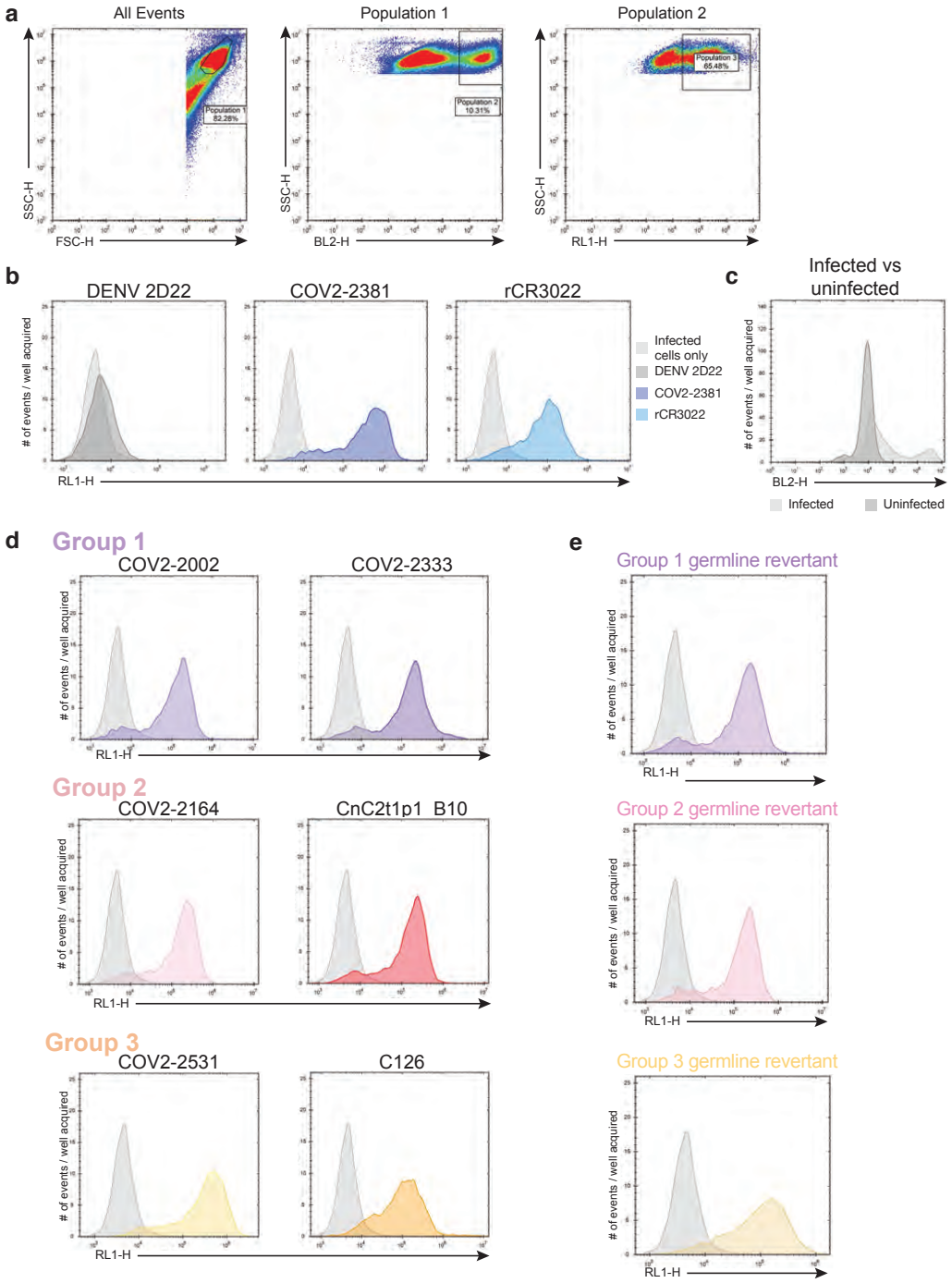

Figure S5. Control reagents for detection of antibody binding to membrane-anchored S protein in cell-surface antigen-display assays, Related to Figures 2 and 3.

a. Gating strategy used for cell-surface antigen-display experiment. The first gate is for all cells, the second gate is for infected cells, and the third gate is for antibody binding to infected cells.

b. Controls used for cell-surface antigen-display antibody binding experiment. Cell-only control in shown in light grey. The unrelated mAb DENV 2D22 was used as an antibody negative control, shown in dark grey. The mAb COV2-2381 shown in dark blue and mAb rCR3022 shown in turquoise were used as positive antibody controls.

c. Histogram of data obtained using infected or uninfected cells. Infected cells are shown in light grey, and uninfected cells are shown in dark grey.

d. Group 1, 2, or 3 antibody binding to infected cells. The antibody concentration used was 10 µg/mL for all antibodies.

e. Group 1, 2, or 3 germline-revertant antibody binding to infected cells. The antibody concentration used was 10 µg/mL for all antibodies.

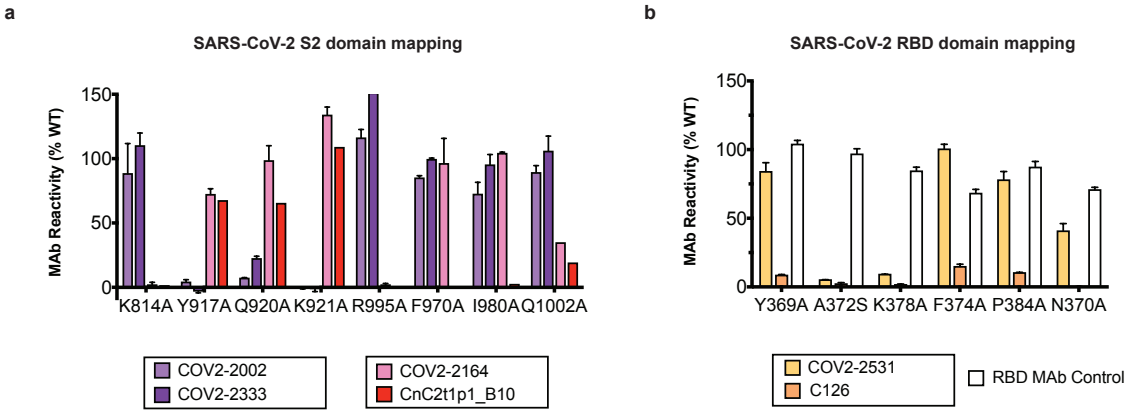

Figure S6. Primary data for alanine mutagenesis screening, Related to Figure 3. Binding values for mAbs on the SARS-CoV-2 S protein alanine scan library. The binding values at critical mutant clones for

a. Group 1 (COV2-2002 in light purple and COV2-2333 in dark purple) and Group 2 (COV2-2164 in pink and CnC2t1p1_B10 in red)antibodies are shown as a percentage of mAb binding to wild-type (WT) SARS-CoV-2 spike protein and are plotted with the range (highest-minus lowest binding value) of at least two measurements.
b. Group 3 (COV2-2531 in light orange and C126 in dark orange) antibodies are shown as a percentage of mAb binding to wild-type (WT) SARS-CoV-2 spike protein and are plotted with the range (highest-minus lowest binding value) of at least two measurements.

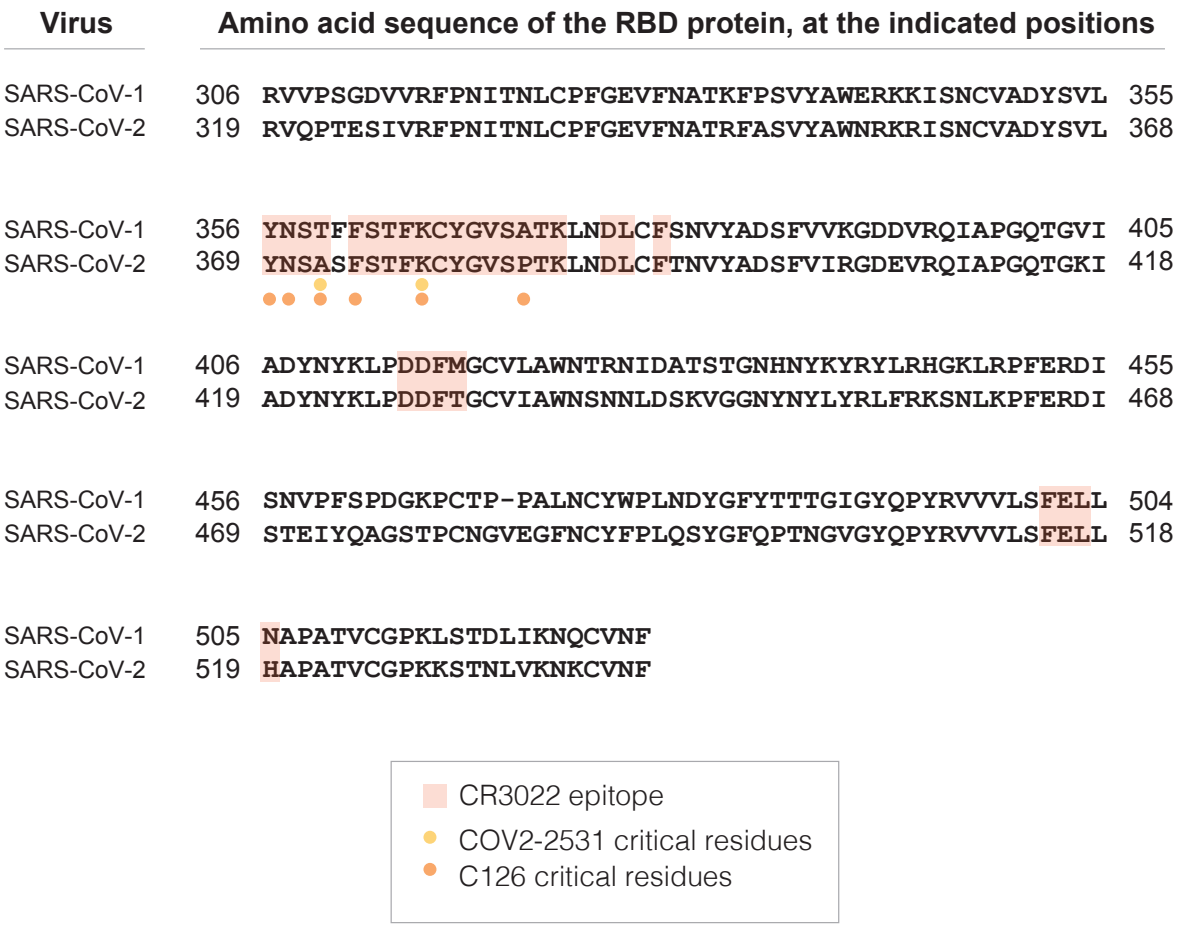

Figure S7. Overlay of CR3022 structure with Group 3 antibodies when bound to RBD, Related to Figure 3.

a. The structures for the RBD domains for both SARS-CoV-2 and SARS-CoV-1 were overlaid. The epitope of rCR3022 is highlighted in orange (from Yuan *et al.*). Light orange dots denote the binding residues for mAb COV2-2531, and dark orange dots denote the binding residues for C126. Figure adapted from previous study[48].

Group 1 Germline Revertant:

HC: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYY
VDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVGSSSWYFDYWGQGTLVTVSS

LC: SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPER
FSGSNSGNTATLTISGTQAMDEADYYCQAWDSSTGVFGGGTKLTV

Group 2 Germline Revertant:

HC: QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANY
AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARTSHYDSSGSYFEYWGQGTLVTVSS

LC: EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPSLTFGGGTKVEI

Group 3 Germline Revertant:

HC: QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYYSGSTNYN
PSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARATWLRDAFGIWGQGTMVTVSS

LC: NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVP
DRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNVVFGGGTKLTVL

Figure S8. Germline revertant sequences for each public clonotype group, Related to Figure 4. The corresponding heavy and light chain germline revertant sequences of each public clonotype are listed.