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Supplemental information

SARS-CoV-2 reactive and neutralizing antibodies discovered by single-cell sequencing of plasma cells and mammalian display

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SUPPLEMENTAL ITEMS

Supplementary Figures:

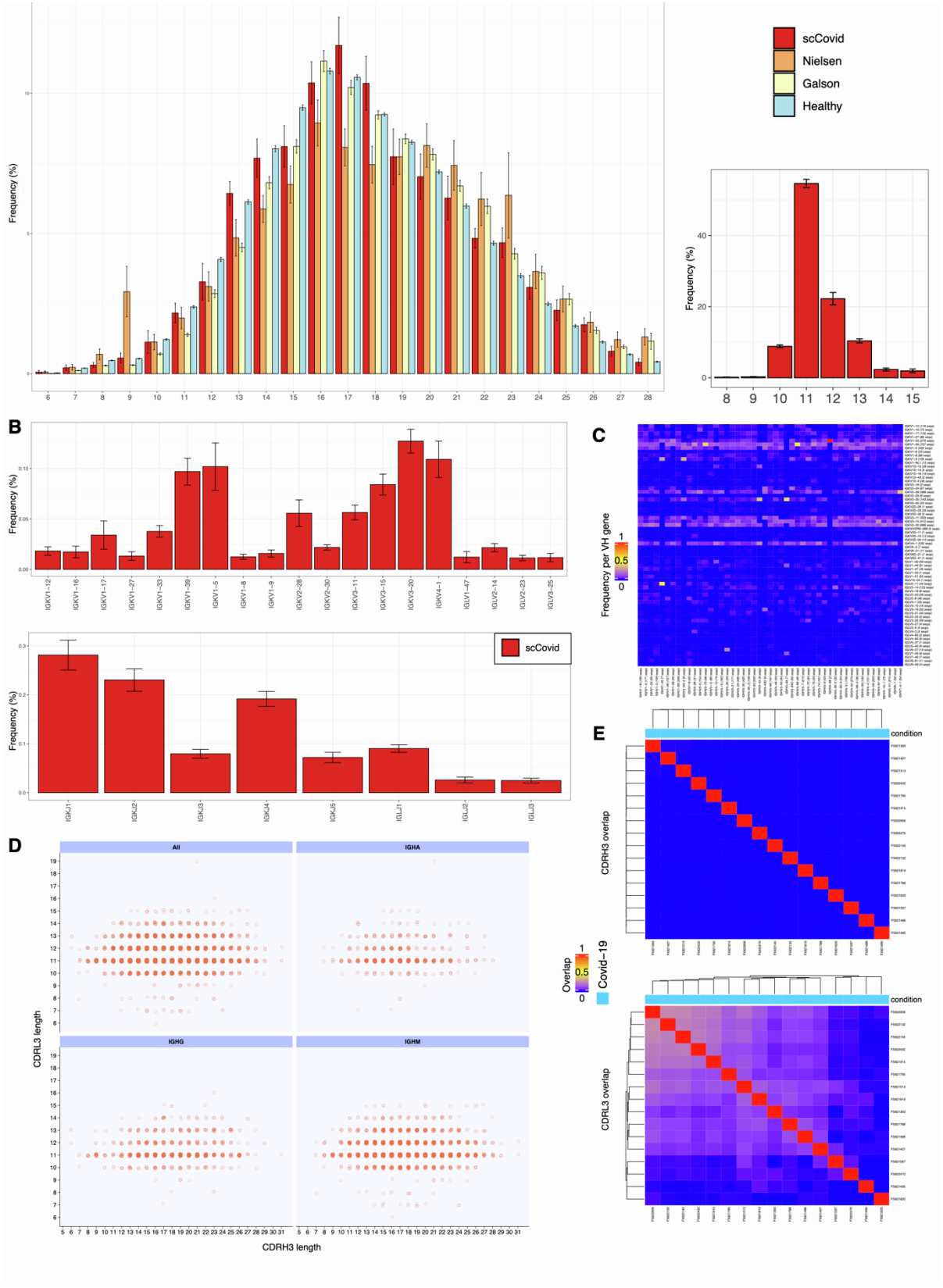

Fig. S1. Additional repertoire features. (A) Length distribution of heavy and light chain CDR3 sequences. (B) Light chain germline gene usage, V (top) and J (bottom). (C) Heavy-Light chain pairing frequencies pooled across

all scCovid repertoires. (D) Lengths of paired CDRH3 and CDRL3s per isotype. (E) Overlap of CDRH3 and CDRL3 sequences across patients. Related to Fig. 3. __ (A,C) Per sample length distribution of heavy and light chain CDR3s per patient sample. (B,D) Isotype usage per patient for heavy and light chains. Related to Fig. 3.

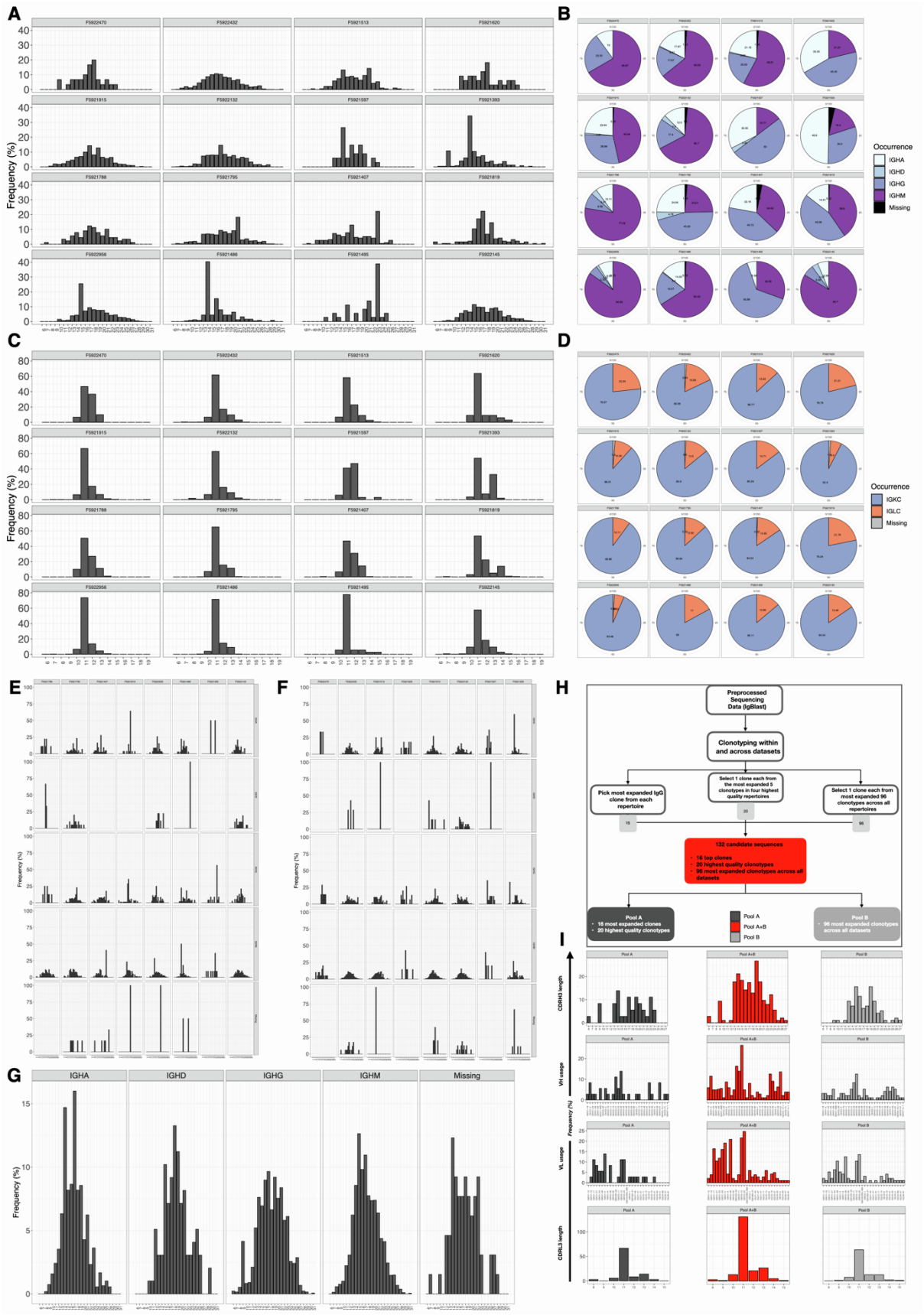

Fig. S2. Per sample statistics. (A,C) Length distribution of heavy and light chain CDR3s per patient sample. (B,D) Isotype usage per patient for heavy and light chains. Related to Fig. 3. (E,F) IGHA, IGHG, IGHM & unknown isotype CDRH3 length distribution for patient samples -470, -432, -513, -620, -915, -132, -597 and -

393 (E) and -788, -795, -407, -819, -956, -486, -495 and -145 (F). (G) Bulk statistics. CDRH3 length distribution per isotype. (H) Candidate sequences were selected from all samples in three steps. Initially the top IgG clone in each sample was identified and selected. This was followed by the selection of 20 sequences through determining the most expanded clone within the top 5 clonal lineages observed in four high quality patient repertoires (as determined by QC and number of cells detected). Finally, one clone from the top 96 clonal lineages observed across all repertoires (not previously selected) was added to the set of selected sequences. (I) CDRH3 and -L3 length distribution for the selected clonal lineages, as well as the V_H/V_L gene usage, split into the respective pools, Pool A (black), Pool B (grey) and Pool A+B (red). Related to Figures 3 and 4.

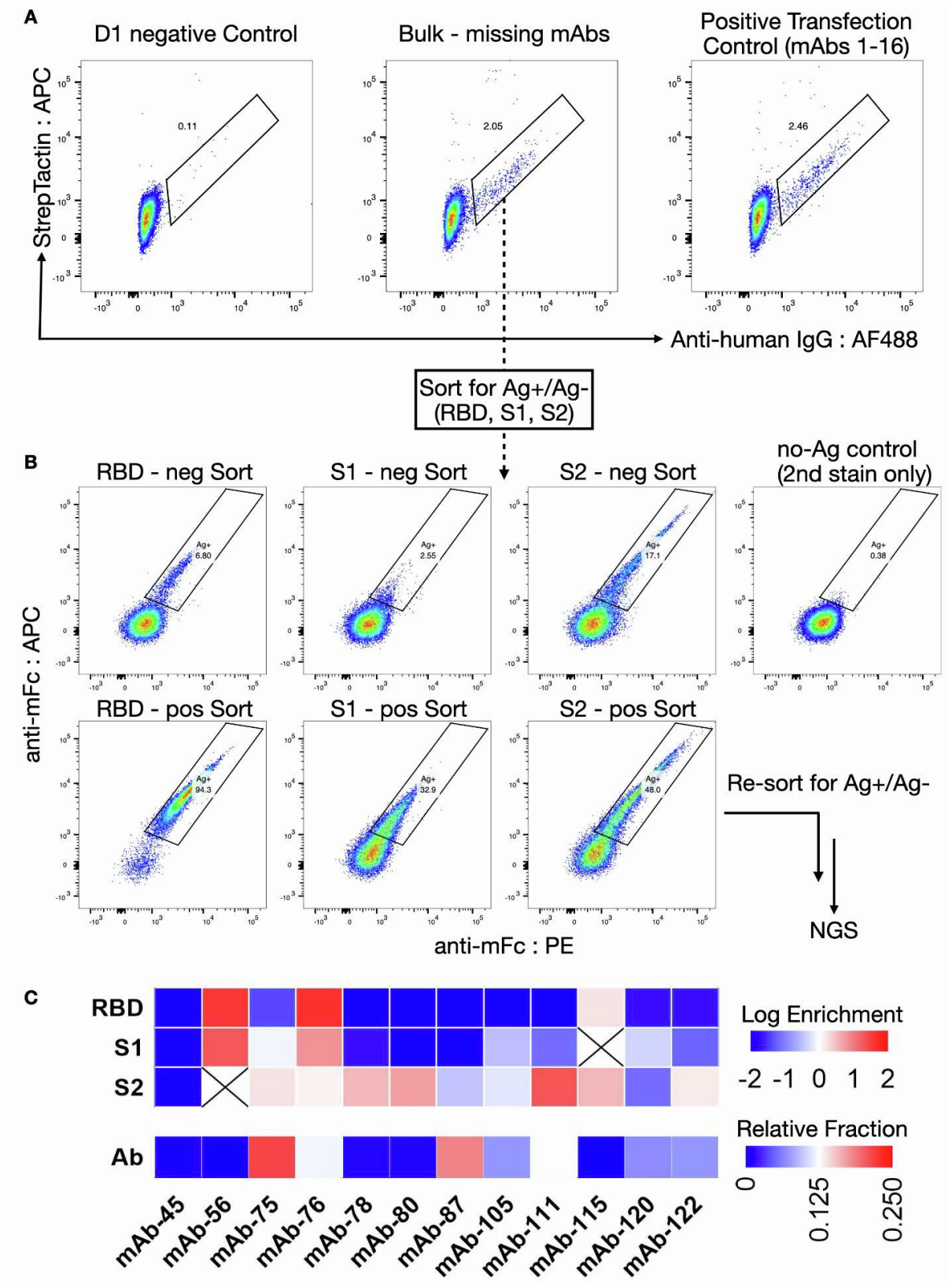

Fig. S3. Re-transfection on previously missing mAbs (A) Negative control (left), bulk transfection of 13 missing mAbs (middle), mAb1-16 bulk transfection positive control. (B) FACS plots of Ag+ and Ag- sorted populations for either RBD, S1 or S2. After one additional resort, genomic DNA was extracted and (C) deep sequenced to determine antigen specificity. Related to Fig. 4 and 5.

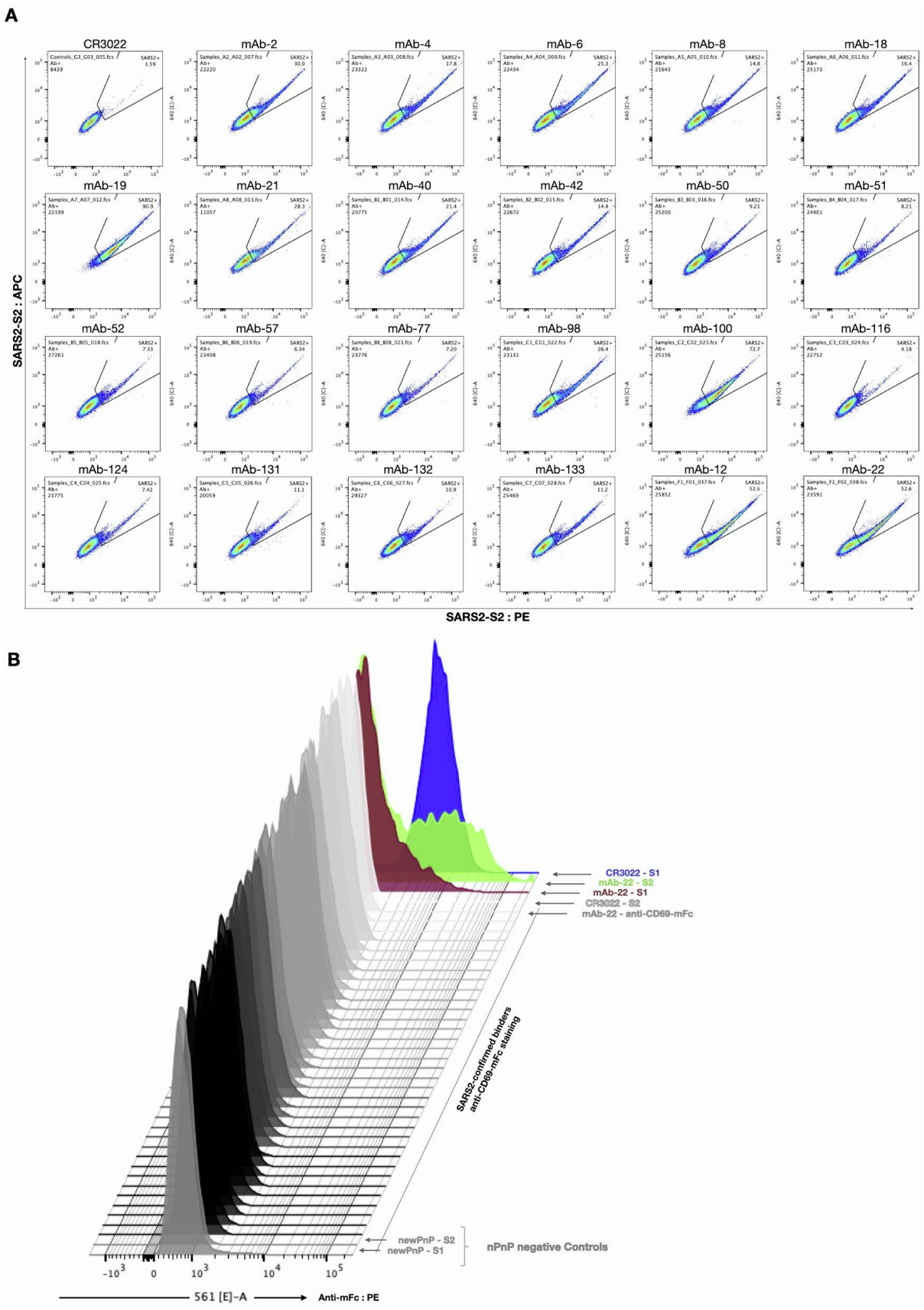

Fig. S4. Background controls. (A) Double staining of selected S2 binders. Low S2 binding mAbs were double stained for both SARS-CoV-2: S2-mFc plus anti-mouse IgG1:APC and :PE to check for PE related background and false positivity. (B) Background determination for unrelated antigen-mFc**.** Select SARS-CoV-2 binders were

additionally stained with anti-hCD69 (mouse IgG1), followed by the previously used anti-mouse Fc secondary staining (here shown: anti-mFc : PE). Related to Fig. 5.

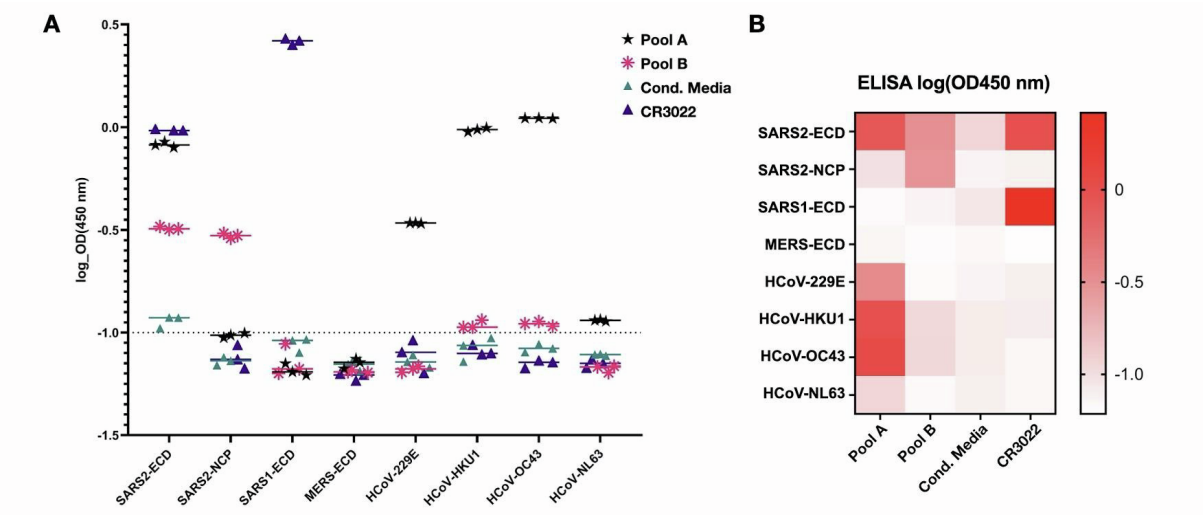

Fig. S5. Cross-specificity ELISA to other human coronavirus antigens. (A) Log OD450-570 nm values given for all antigens in response to Pool A or B supernatant, conditioned medium or CR3022 supernatant. Coating antigens on the X-axis. (B) Heatmap displaying the results from A. Conditioned media of the founder nPnP cell line was used to control for supernatant dependent background signal. Related to Fig. 4 and 5.

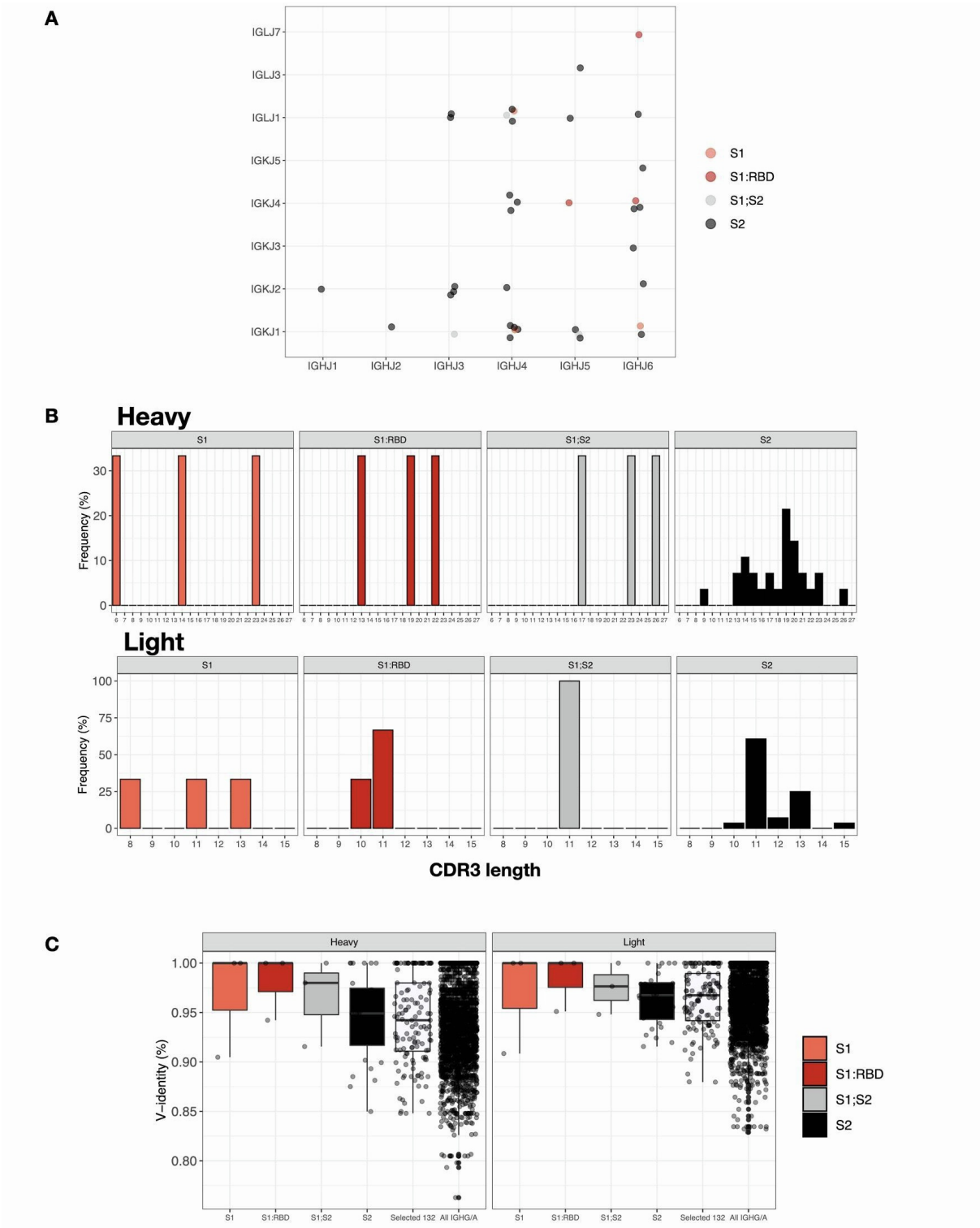

Fig. S6. Extended sequence features of SARS-CoV-2 reactive antibodies. (A) Length distribution of heavy and light chain CDR3s, followed by (B) V- and J-gene usage, and (C) the V-gene identity (level of somatic hypermutation) grouped according to antigen reactivity. Related to Fig. 5.