Cell Reports, Volume *34*

Supplemental Information

Cross-reactivity of SARS-CoV structural protein antibodies against SARS-CoV-2

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SUPPLEMENTAL FIGURES

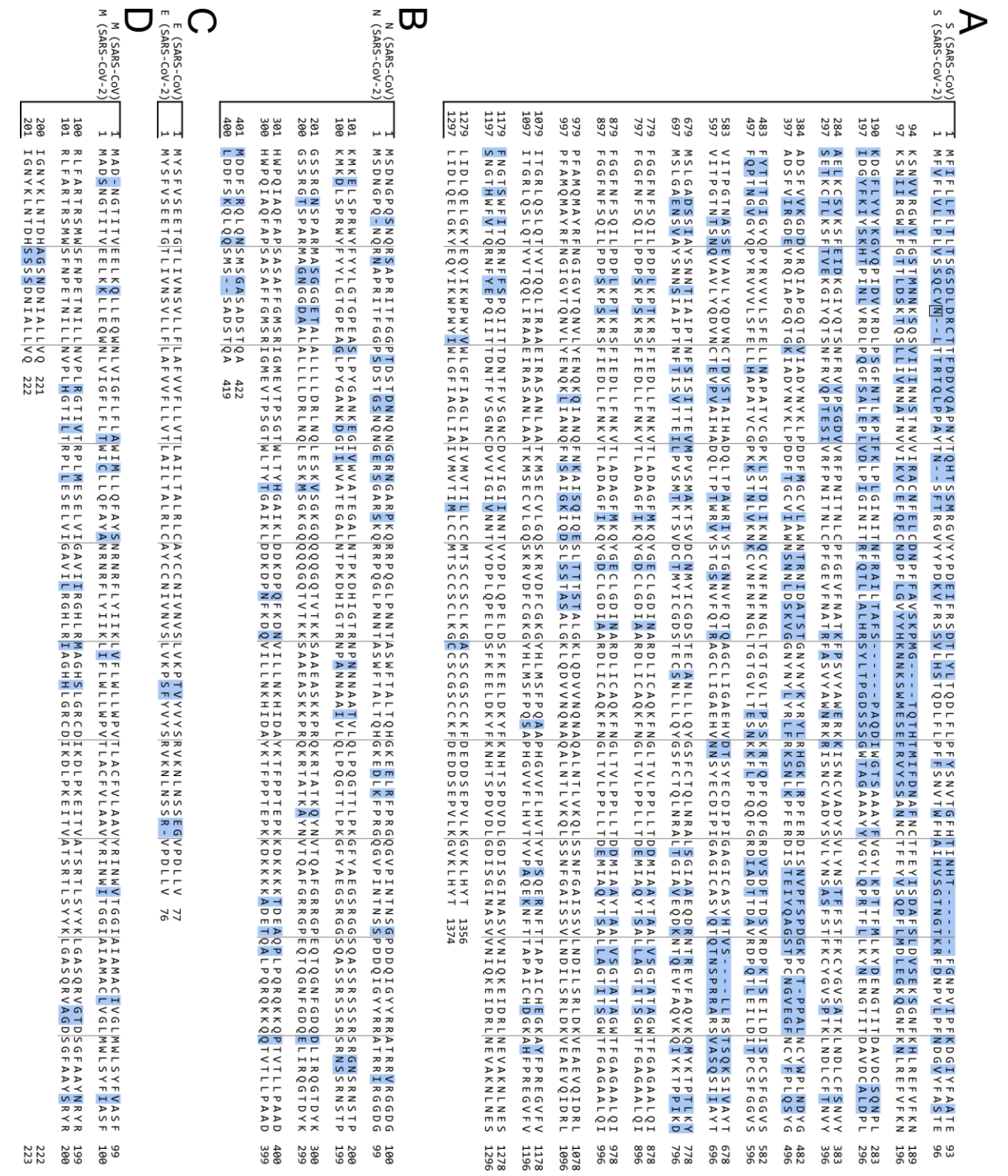

Figure S1: Alignment of structural proteins for SARS-CoV and SARS-CoV-2. Protein sequences were obtained from Uniprot. Differences are highlighted in blue. Grey lines are spaced every 10 characters. For each pair, SARS-CoV is on the top and SARS-CoV-2 is on the bottom. Related to Figure 1.

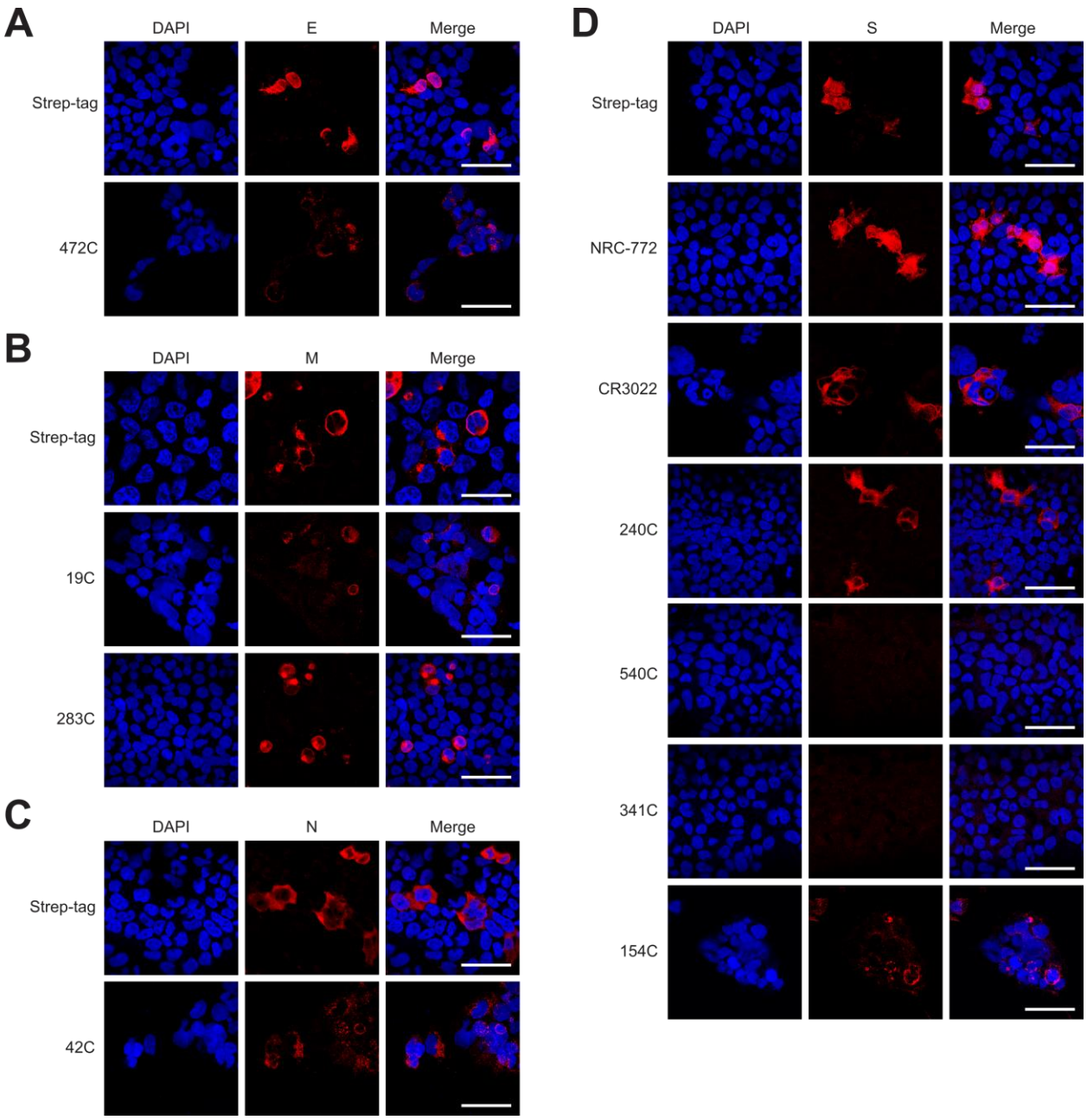

Figure S2: Immunofluorescence of SARS-CoV-2 structural proteins using SARS-CoV antibodies. Representative immuno-fluorescence images of HEK 293T cells transiently transfected with SARS-CoV-2 structural proteins. 24 h post-transfection, cells were fixed and stained with the listed SARS-CoV antibodies: (A) Envelope, (B) Membrane, (C) Nucleocapsid, and (D) Spike proteins. All proteins are strep-tagged and control stained with anti-strep-tag antibody or the indicated antigen-specific antibody (Red). Scale bars 50 μm. DAPI (Blue) was used to visualize cell nuclei. Related to Figure 2.

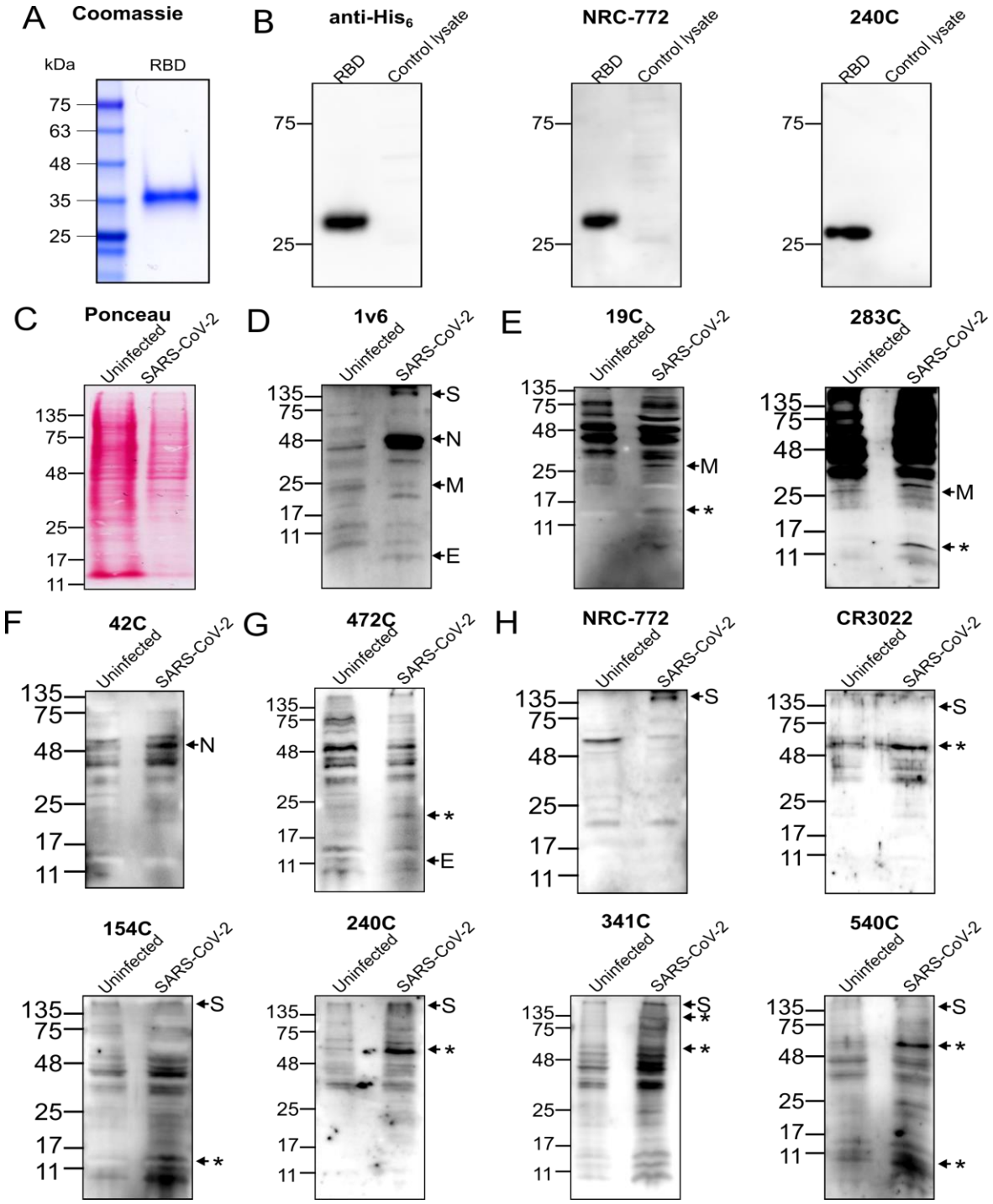

Figure S3: SARS-CoV structural proteins show cross-reactivities with SARS-CoV-2 by immunoblotting. (A) Coomassie stain of purified RBD used in B. (B) purified SARS-CoV-2 spike RBD protein and control wild-type HEK 293T lysate probed with anti-S monoclonal antibodies. (C) Ponceau staining of SARS-CoV-2 infected and uninfected Vero E6 cell lysate. Western blots with SARS-CoV-2 lysates stained with (D) convalescent human serum (E) anti-M (F) anti-N (G) anti-E and (H) anti-S SARS-CoV monoclonal antibodies. Arrows indicate expected molecular weight and * indicates expected alternate bands based on western blot results from previous reports (Gordon et al., 2020). Shown are representative images of 2 to 3 independent experiments. Related to Figure 3.

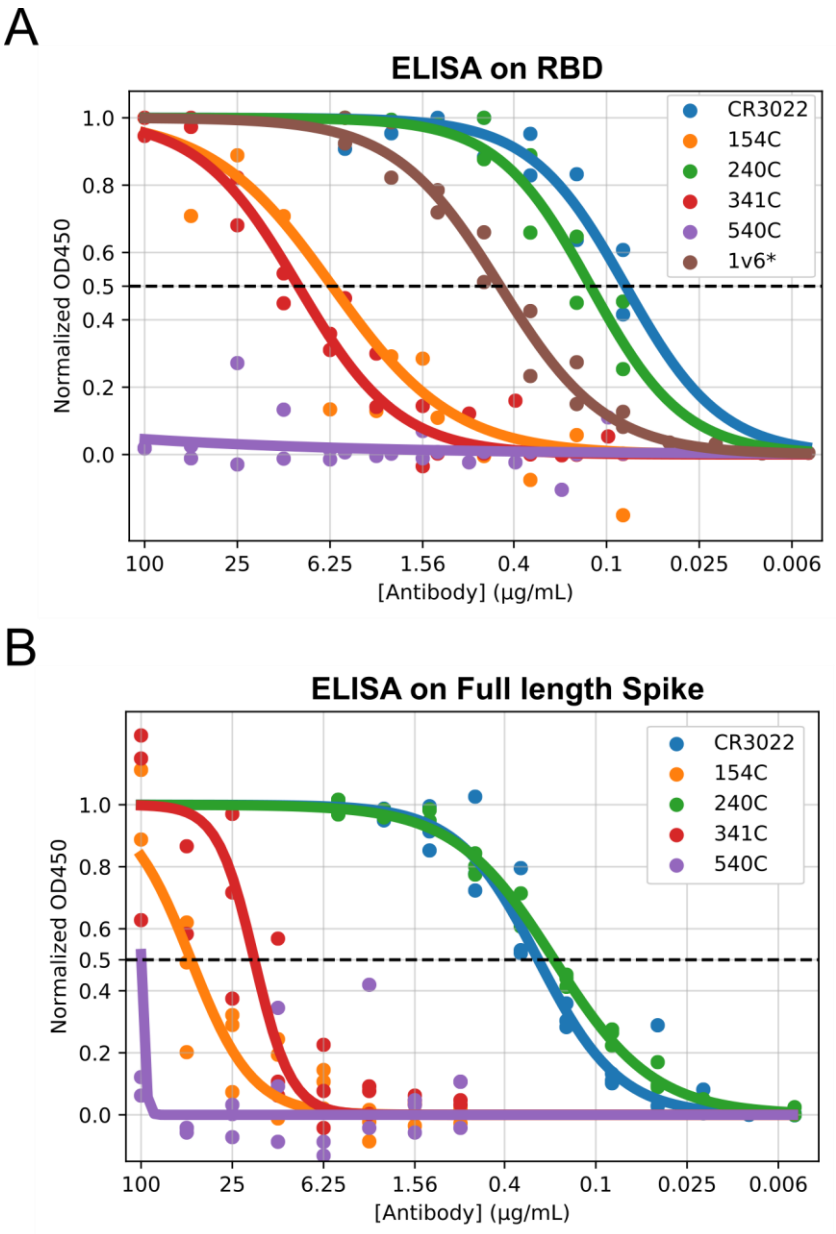

Figure S4: SARS-CoV Spike monoclonal antibodies show cross-reactivity by ELISA (extended data). ELISA against (A) RBD coated at 2 µg/mL and (B) full length spike coated at 2 µg/mL and then probed with the indicated monoclonal antibody, or 1v6 human convalescent serum. Each point represents the mean of 3 technical replicates from a single experiment (2 technical replicates for 1v6, CR3022, 154C, and 240C on RBD); data from 3 independent experiments is shown (n=3). Data were normalized according to the maximum signal seen for each secondary antibody in each experiment. *1v6 is convalescent serum used to validate the assay. A stock concentration of 1 mg/mL was used to facilitate calculations for reference purposes, but this does not represent an accurate EC_{50} value. Related to Figure 3.

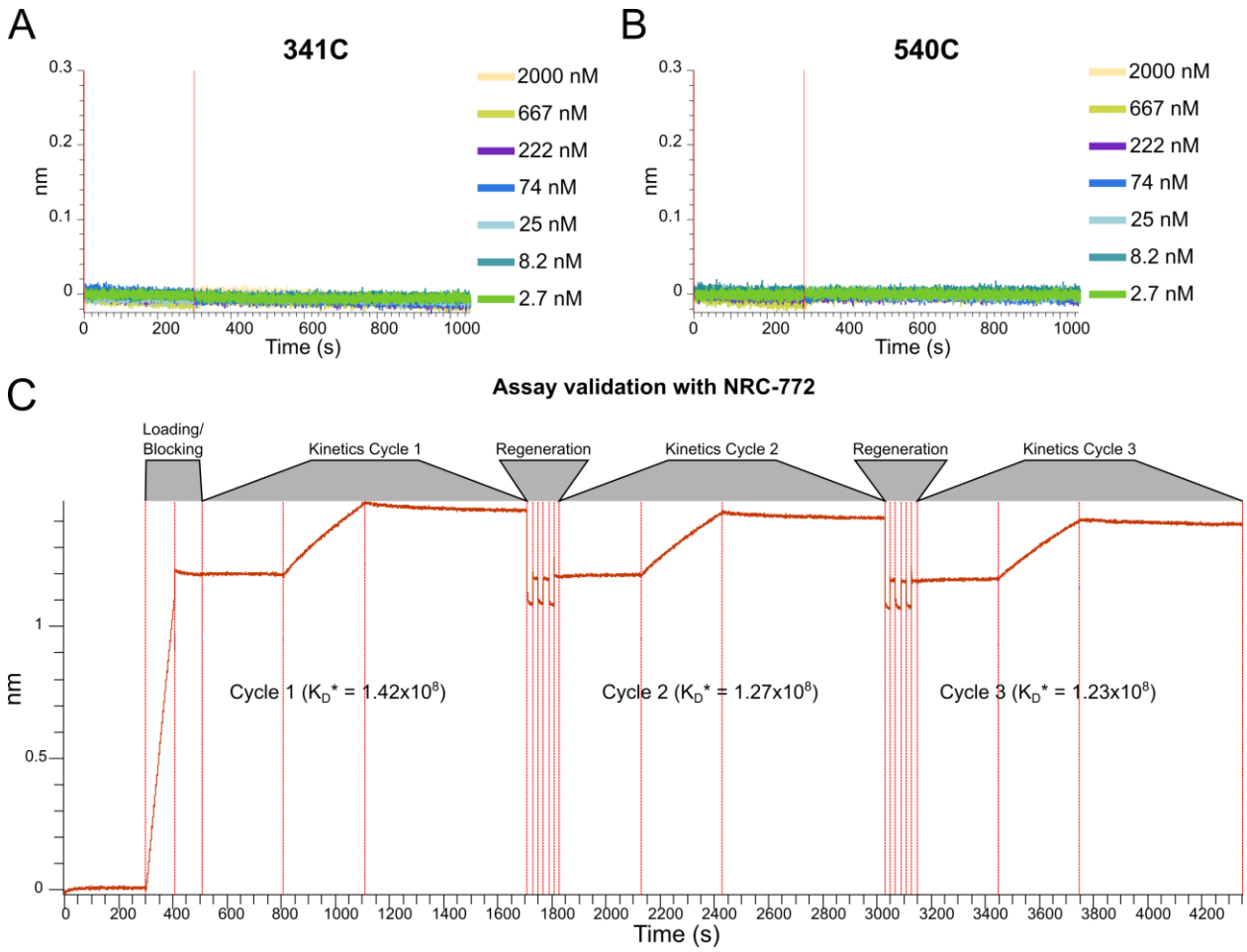

Figure S5: Binding Kinetics of Spike specific antibodies against the RBD of the SARS-CoV-2 (BLI extended data). Negative binding curves for antibodies (A) 341C and (B) 540C. (C) NRC-772 rabbit polyclonal antibody used for method validation. Curves show minimal loss of signal with multiple regeneration cycles as well as stable K_D values, demonstrating stability of RBD under regeneration conditions (3x cycles of 20 seconds in 10mM Glycine pH 1.7). NRC-772 serum used at 1:50 dilution in kinetics buffer. *K_D values assume 1 mg/mL initial concentration in order to facilitate K_D calculation only as a reference between cycles and does not represent an accurate affinity measurement. Related to Figure 4.

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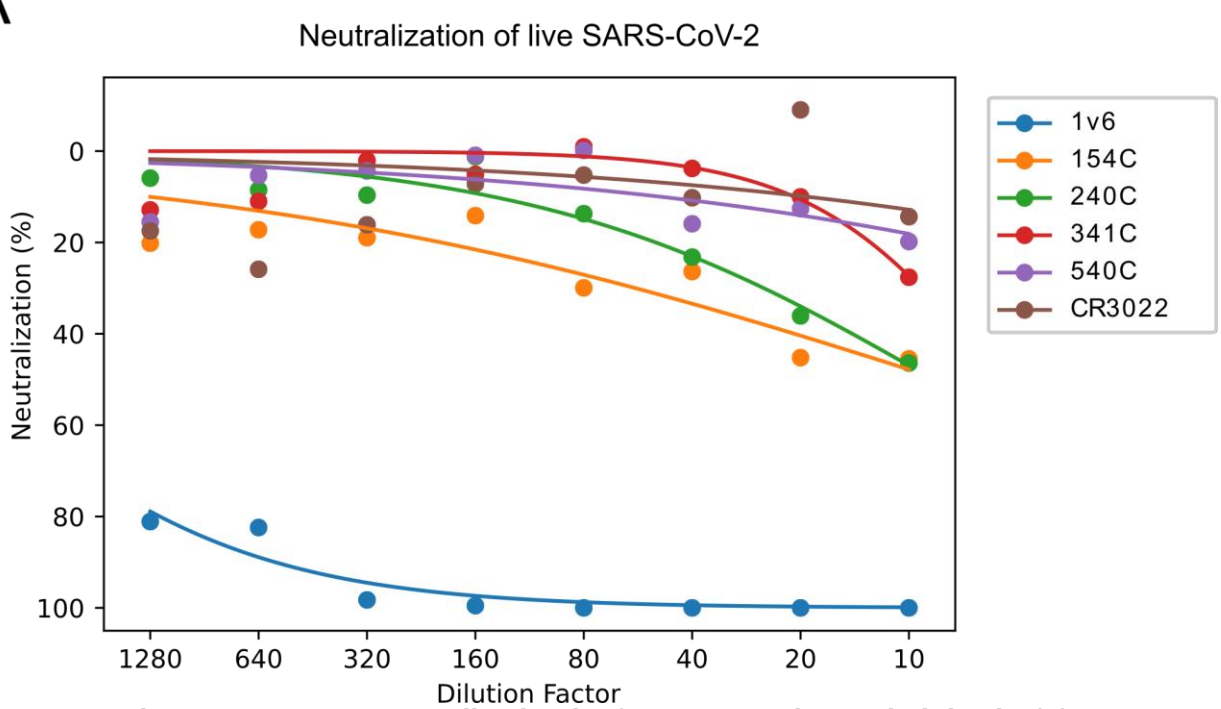

Figure S6: Live SARS-CoV-2 neutralization by focus assay (extended data). (A) Vero E6 cells were infected with approximately 30 pfu/well of live SARS-CoV-2 which was pre-incubated for 1 hour with the indicated final dilutions of antibodies before the addition of overlay media and 48 hours of incubation. Each point represents the average of three biological replicates (n=3), each in technical triplicate. Monoclonal antibodies 154C, 240C, 341C, 540C, and CR3022 stock concentrations were 1 mg/mL. Related to figure 4.