Functional characterization of CD4+ T-cell receptors cross-reactive for SARS-CoV-2 and endemic coronaviruses

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Supplementary Figures

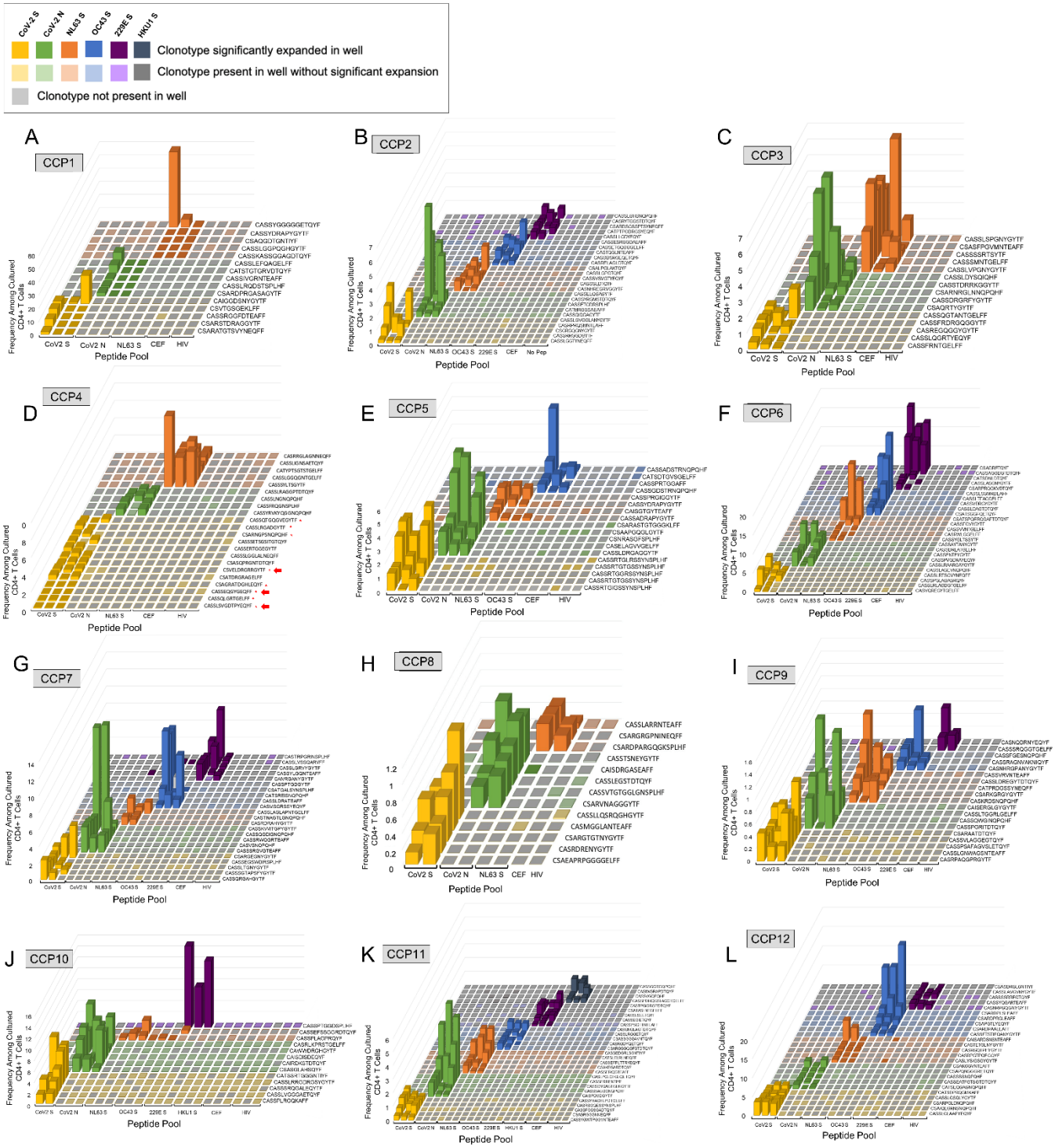

Figure S1*:* SARS-CoV-2 and CCC memory CD4+ T cell responses in COVID-19 convalescent patients. The ViraFEST assay was performed to test for reactivity to SARS-CoV-2 and CCC (HCoV-NL63, HCoV-OC43, HCoV-229E, and HCoV-HKU1) spike glycoprotein pools in COVID-19 convalescent patients (CCPs; A-L). Peptide co-culture was done in triplicate unless otherwise noted. Data are shown as the frequency (%) after culture (y-axis) of antigen-specific CD4+ T cell clonotypes (z-axis) for all peptide pools tested (x-axis). The top five mono-reactive TCR Vβ CDR3 amino acid clonotypes for each CCP are shown. Solid color represents significant (FDR<0.05) clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent color indicates the clonotype was present at low frequency in the well, but did not significantly expand. Grey indicates the relevant TCR clonotype was not detected in that well.

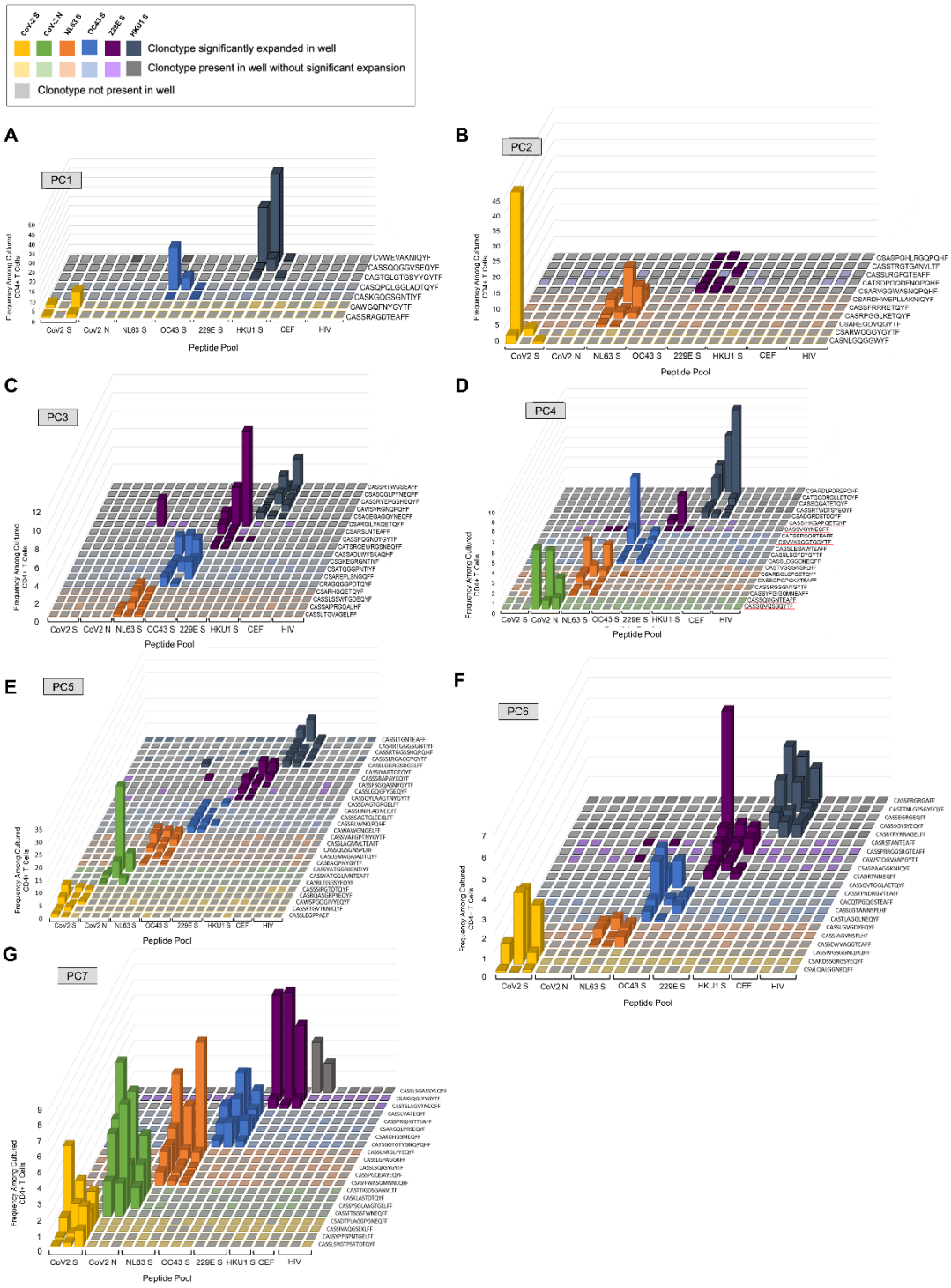

Figure S2*:* Mono-reactive responses to SARS-CoV-2 and CCC in pre-COVID specimens. The Functional Expansion of Specific T cell (FEST) assay was again used to determine the repertoire of SARS-CoV-2 and HCoV-reactive CD4+ T cells in pre-COVID donors (A-G). Peptide co-culture was done in triplicate unless otherwise noted. X-axis represents stimulating peptide conditions; Y-axis is frequency of CD4+ T cell clonotypes in each well. The top significantly expanded mono-reactive TCR Vβ CDR3 amino acid clonotypes for each donor are shown on each plot. This recognition is determined by significant expansion (FDR<0.05) to each peptide pool in triplicate (dark color). Pale colored wells indicate that the TCR Vβ CDR3 was present at low level frequency in the well but without significant expansion. Grey wells show that that CDR3 was not found in that well. SARS-CoV-2 S mono-reactive responses were detected in PC1, PC2, PC5, PC6 and PC7 (A, B, E, F, G). PC3 and PC4 did not mount any response to SARS-CoV-2 S (C, D).

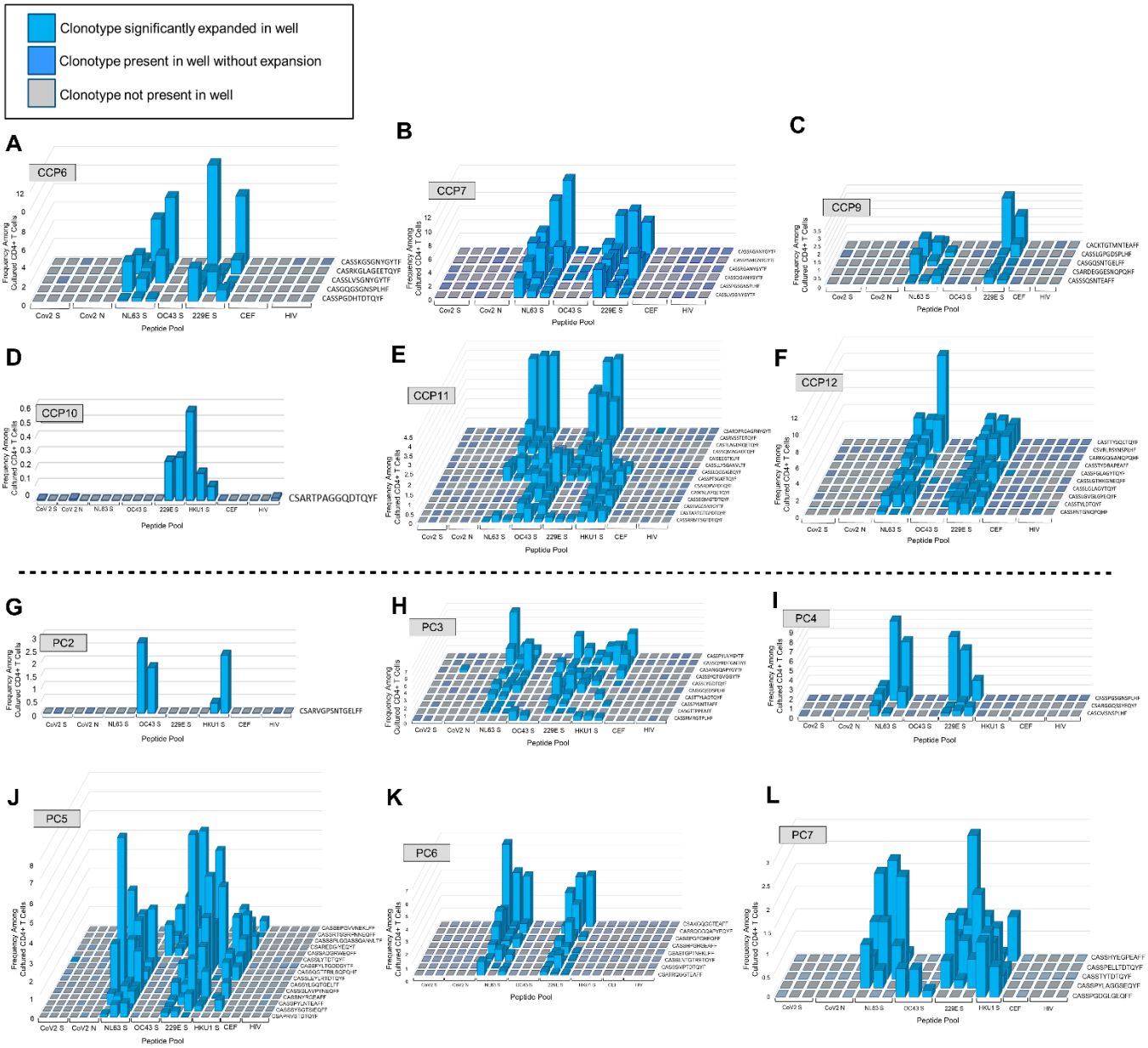

Figure S3: Cross-reactive CD4+ T cell responses in CCPs. CD4+ T cell responses cross-reactive for CCC are shown for CCP6 (A), CCP7 (B), CCP9 (C), CCP10 (D), CCP11 (E), CCP12 (F) during the COVID-19 pandemic (G), and the pre-treatment specimens obtained from PC2 (G), PC3 (H), PC4 (I), PC5 (J), PC6 (K), and PC7 (L). Peptide stimulations were performed in triplicate unless otherwise noted. Data are shown as the frequency (%) after culture (y-axis) of antigen-specific CD4+ T cell clonotypes (z-axis) for all peptide pools tested (x-axis). Solid color represents significant (FDR<0.05) clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent color indicates the clonotype was present at low frequency in the well, but did not significantly expand. Grey indicates the relevant TCR clonotype was not detected in that well.

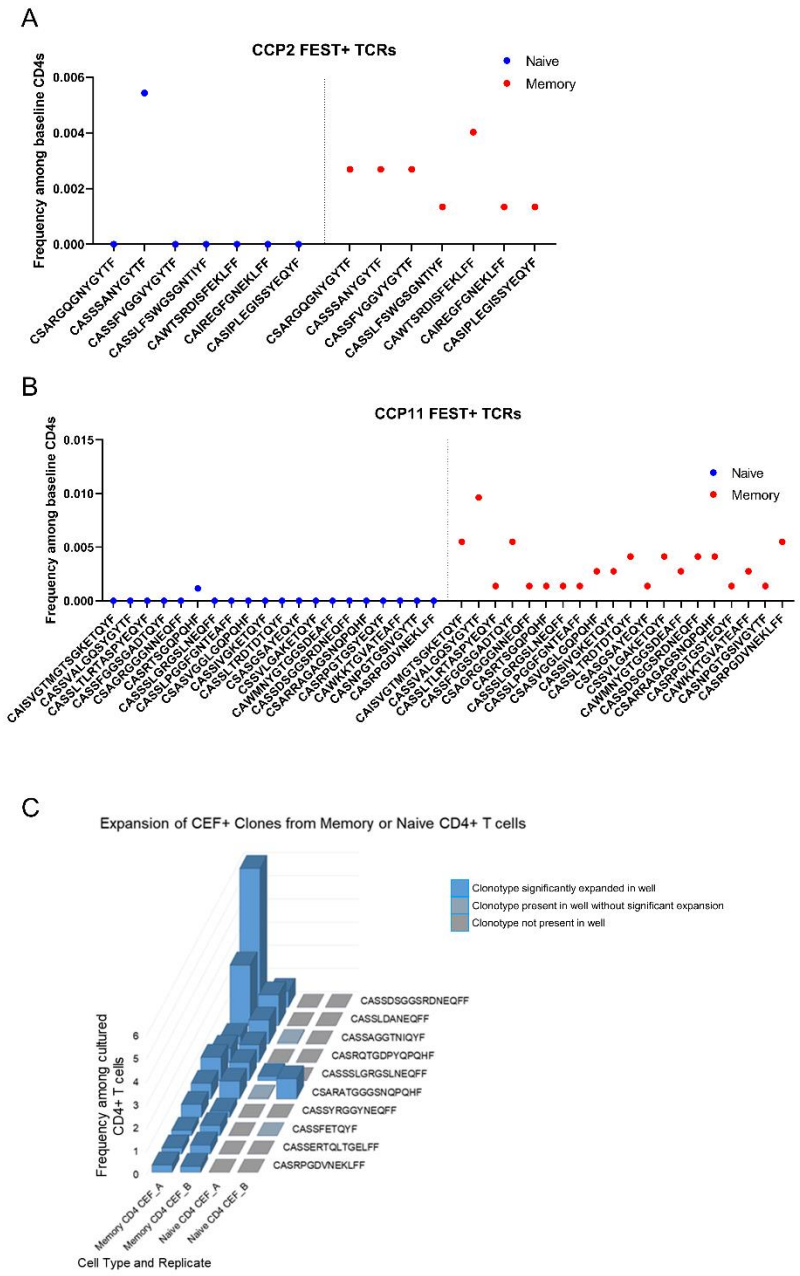

Figure S4: CD4+ ViraFEST identifies memory CD4+ T-cells. Naïve and memory CD4+ T cells were isolated from cryopreserved PBMC from two COVID-19 convalescent donors (CCP2 (A), CCP11 (B) and deep TCR-sequenced. All SARS-CoV-2 cross-reactive, SARS-CoV-2 mono-reactive, and CEF-specific TCR clonotypes as determined by the ViraFEST assay were queried (**Figure 2H, S1B, S1K)**. Only clonotypes found in at least one population are shown. Clonotype frequency of naïve or memory cells is shown on the y-axis with specific FEST+ Vβ CDR3 sequences shown on the x-axis (A, B). Naïve and memory CD4+ T cells were isolated from CCP11 cryopreserved PBMC by magnetic bead separation. 10^5 of each were plated at a 1:1 ratio with autologous T cell-depleted PBMC and stimulated with a CMV, EBV, flu (CEF) peptide pool in duplicate for 10 days as in the ViraFEST assay, followed by TCR sequencing. Data are shown as the frequency (%) after culture (y-axis) of antigen specific CD4+ T cell clonotypes (z-axis) for cell type replicates tested (x-axis). Solid color represents significant (FDR<0.05) clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent color indicates the clonotype was present at low frequency in the well but did not significantly expand. Grey indicates the relevant TCR clonotype was not detected in that well.

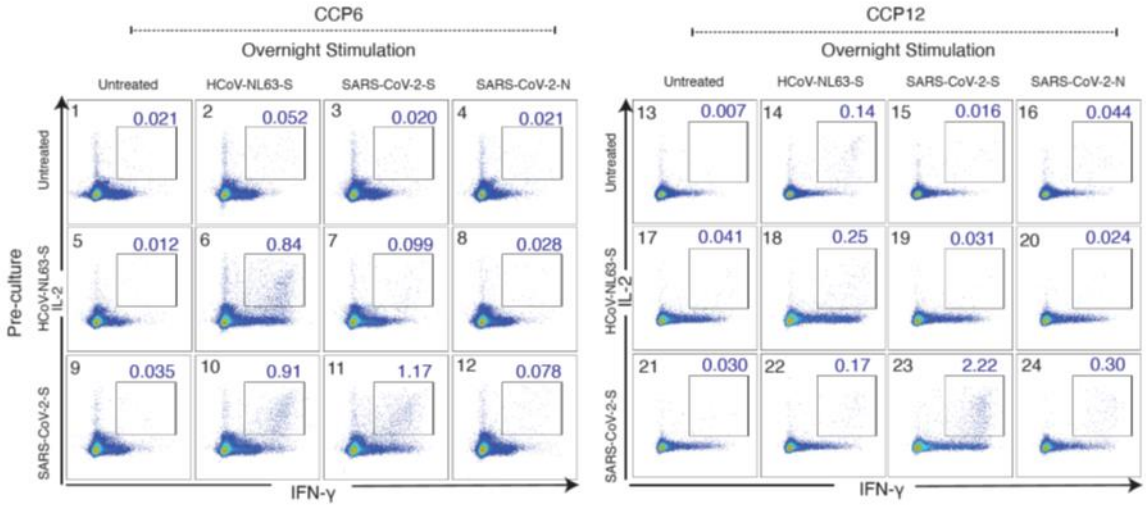

Figure S5. Cross-recognition of HCoV-NL63 and SARS-CoV-2 S protein peptide pools by CCP6 and CCP12. PBMCs from CCP6 (A) and CCP12 (B) were precultured with peptide pools (shown in rows) for 10–12 days and then stimulated for 12 hours with peptide pools (shown in columns). The percentage of cells that co-expressed IL-2 (y axis) and IFN-γ (x axis) is shown above the gated box in the upper right corner of each plot.

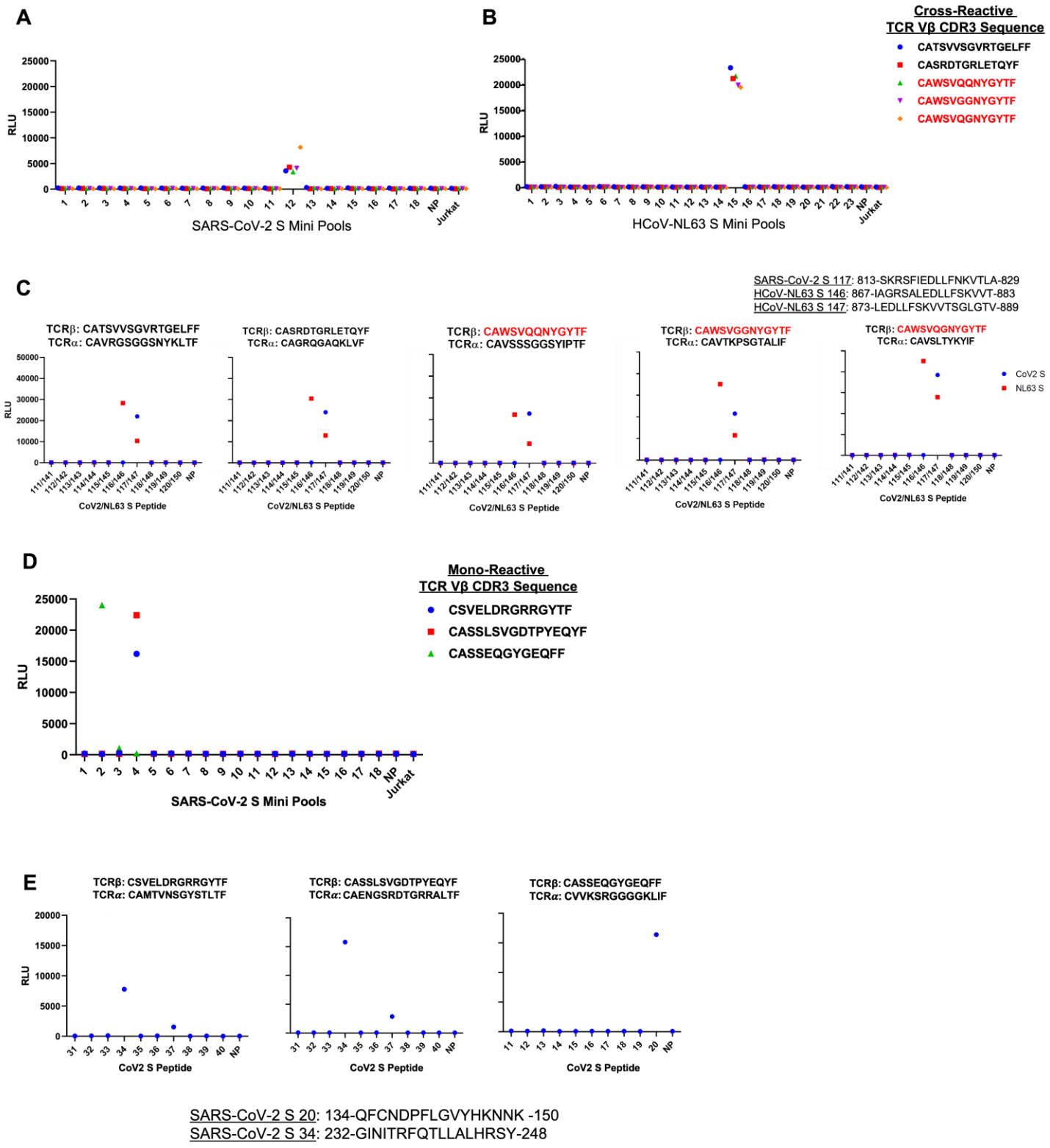

Figure S6: Cross-reactive and mono-reactive clonotypes recognize different SARS-CoV-2 epitopes. The reactive 17-mer peptide was defined for five cross-reactive (A-C) and three mono-reactive (D and E) TCRs identified in CCP4. Single cell TCRseq was performed on cultured cells from CCP4 to enumerate the cognate alpha chain corresponding to the mono-reactive TCR beta chain. TCRs were cloned into a jurkat NFAT luciferase reporter system. TCR-transfected CD4+ jurkat cells were screened for reactivity to mini-pools of 10-peptides each (A, B, D). After the positive mini pool was identified, TCR-transfected jurkats were screened for reactivity to each individual peptide within the positive mini pool (**Table S2**) to determine the exact stimulating 17-mer (C, E).

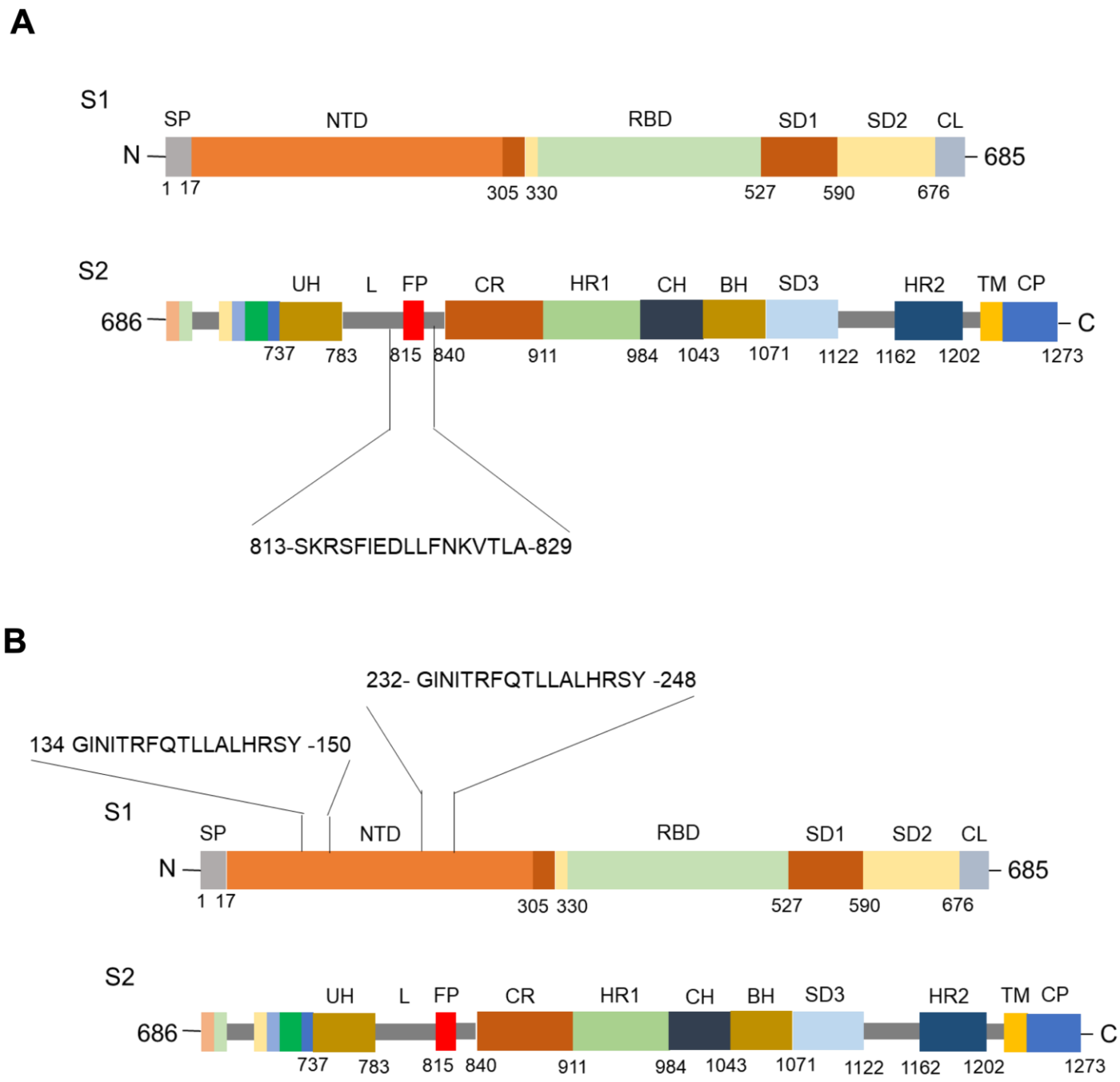

Figure S7: Stimulating epitope regions in SARS-CoV-2 spike glycoprotein. The position in the SARS-CoV-2 proteome is shown for regions eliciting CD4+ T cell cross-reactivity (A) and mono-reactivity (B). N-terminal-domain (NTD), structural domain signal peptide (SP), receptor-binding domain (RBD), subdomain (SD), cleavage loop (CL), upstream helix (UH), fusion peptide (FP), connecting region (CR), heptad repeat (HR), central helix (CH), β-hairpin (BH), transmembrane region (TM), and cytoplasmic part (CP).

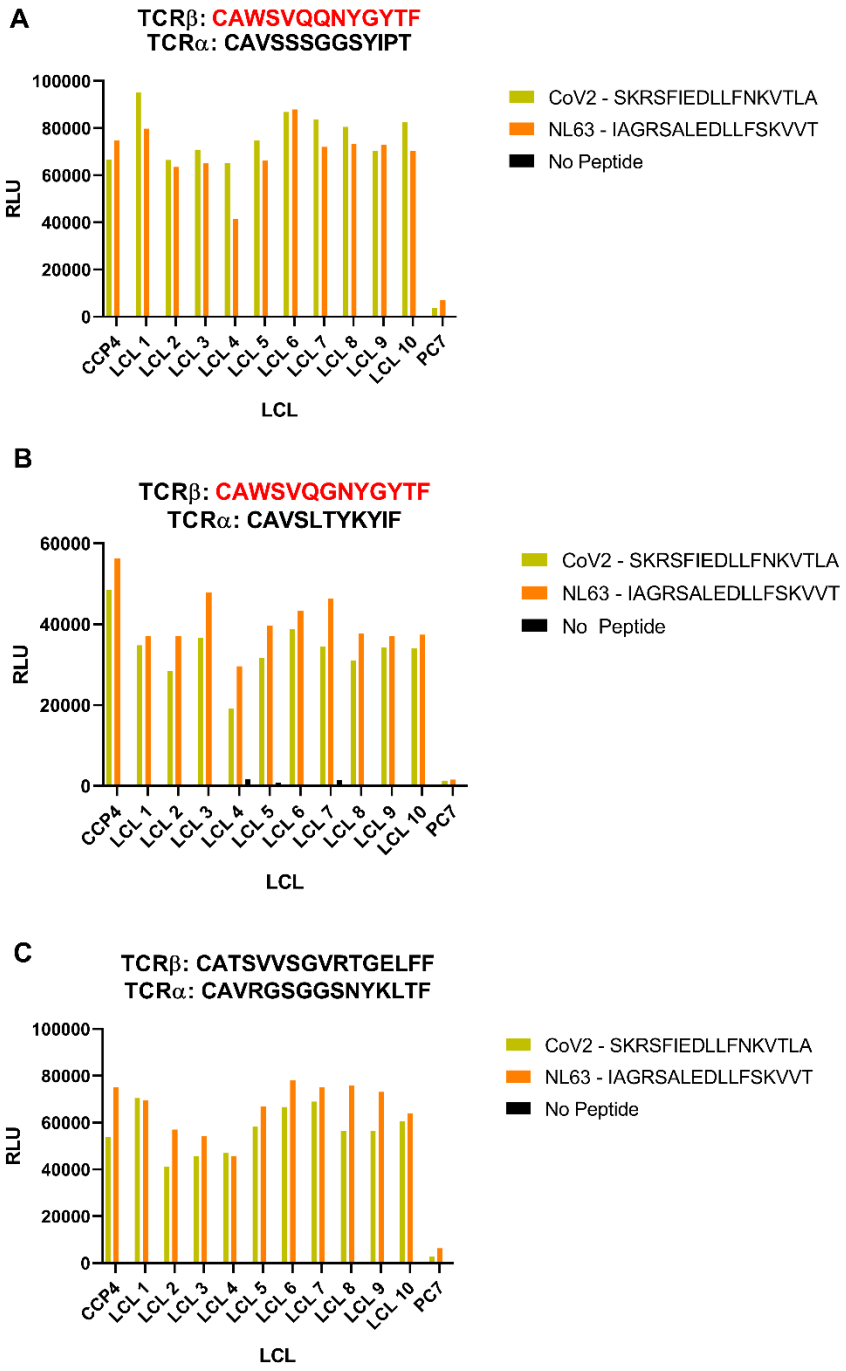

Figure S8: Restricting HLA alleles for SARS-CoV-2 S and HCoV-NL63 S cross-reactive epitopes. (A, B) Shared cross-reactive TCR-expressing Jurkats were cultured with patient matched (CCP4) and un-matched LCL (1-10, PC7) with the SARS-CoV-2 or HCoV-NL63 minimal S epitope. (C) Unshared CCP4 cross-reactive clone recognizes peptides in same restriction pattern. All RLU is normalized to TCR-expressing Jurkat alone cultured with peptide. For all TCR-Jurkat/LCL cocultured with SARS-CoV-2 S and HCoV-NL63-S, PC7 was determined to be an outlier by ROUT (Q = 1%). TCR Vβ-CAWSVQGNYGYTF/LCL cocultured without peptide LC4, LC5, and LCL7 were determined to be outliers by ROUT (Q = 1%). There were no outliers for TCR Vβ – CAWSVQQNYGYTF/LCL or TCR Vβ-CATSVVSGVRTGELFF/LCL cocultured without peptide.

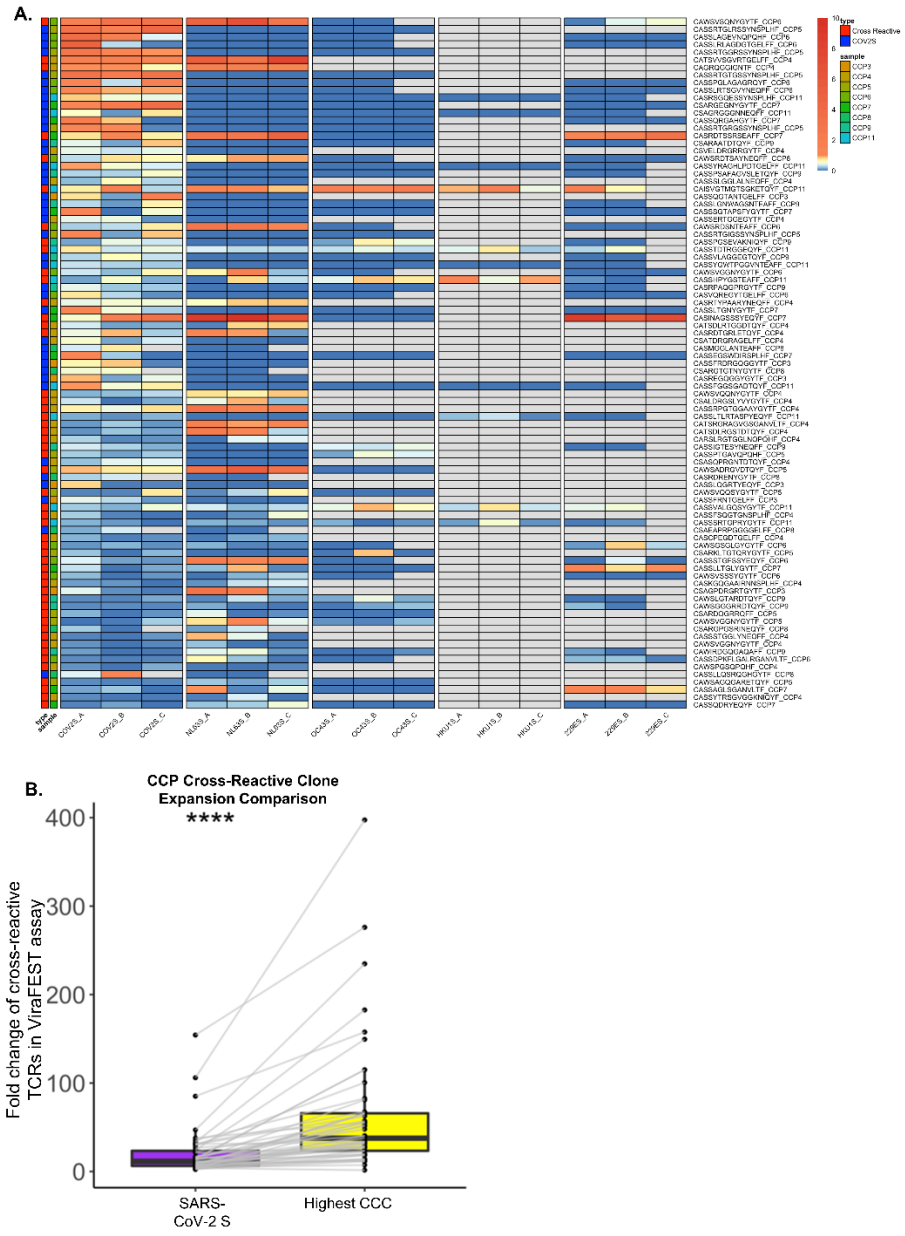

Figure S9. Functional activity of SARS-CoV-2 S cross-reactive CD4+ T cell responses. A heatmap was generated to visualize the frequency of SARS-CoV-2/CCC cross-reactive (n=51; red) and SARS-CoV-2 mono-reactive (n=40; blue) T cell clonotypes in each triplicated culture well (annotated by A, B, C) stimulated by SARS-CoV-2 S (labeled as COV2S) or other CCC S pools (NL63 S, OC43 S, HKU1 S and OC43 S). Each row represents a single TCR Vβ CDR3 clonotype and each column represents a peptide pool. Rows were ordered by SARS-CoV-2 S fold change in a descending order as shown in Figure 5A. Red represents high frequency and blue represents low frequency (A). The average fold change was calculated to compare overall functional avidity of cross-reactive clones in CCPs for SARS-CoV-2 S relative to CCC S (n=51), where the highest fold change across any recognized CCC was used for each clonotype (B). To account for clones that were only present in one condition, a pseudocount of 1 was added to all clonotype counts and frequency was recalculated. Each dot represents a cross reactive clonotype from a sample with grey line indicating a pair. Wilcoxon signed-rank test was performed to compare the fold change of cross-reactive clones in response to S vs. SARS-CoV-2 S. ****: P≤0.0001.

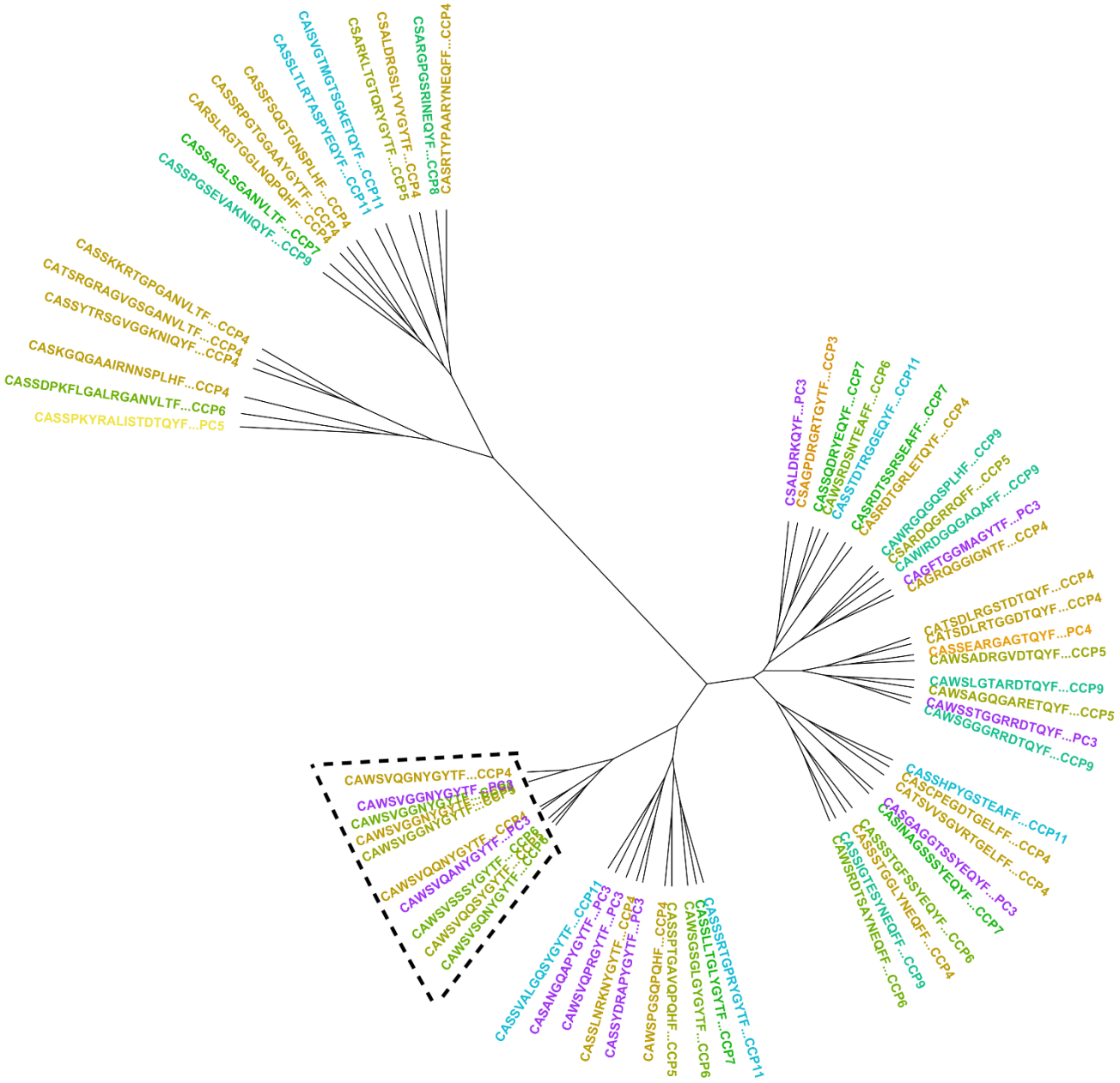

Figure S10: SARS-CoV-2/CCC cross-reactive TCRs share sequence homology among patients. Unrooted phylogenetic tree for all cross-reactive clonotypes. Each leaf represents a cross-reactive clonotype in a sample and different colors were used to distinguish patient samples. The black box highlights area of high sequence homology across multiple patients as shown in Figure 7.

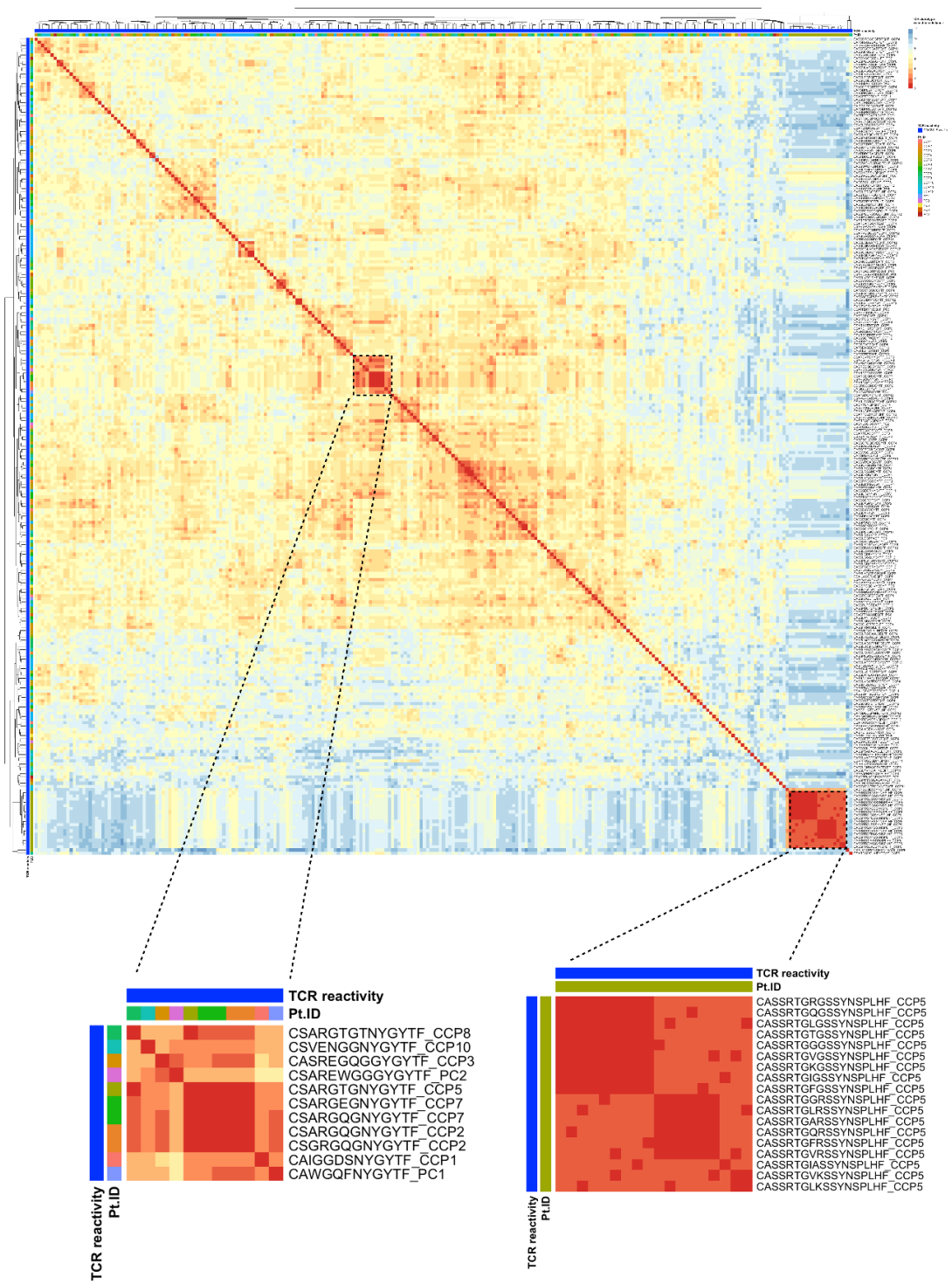

Figure S11: SARS-CoV-2 S mono-reactive TCRs share sequence homology within and among patients. Heatmap of sequence homology for SARS-CoV-2 S mono-reactive clones. Each row/column represents a SARS-CoV-2 S mono-reactive clonotype in a sample. The color represents the levels of sequence homology using levenshtein distance, with red indicating high degree of sequence homology and blue for less sequence similarity. Different colors were used to distinguish patient samples in the color sidebar. Area of interest showing high sequence homology across patients were amplified in bottom left and area of high sequence homology within patients were amplified in bottom right.

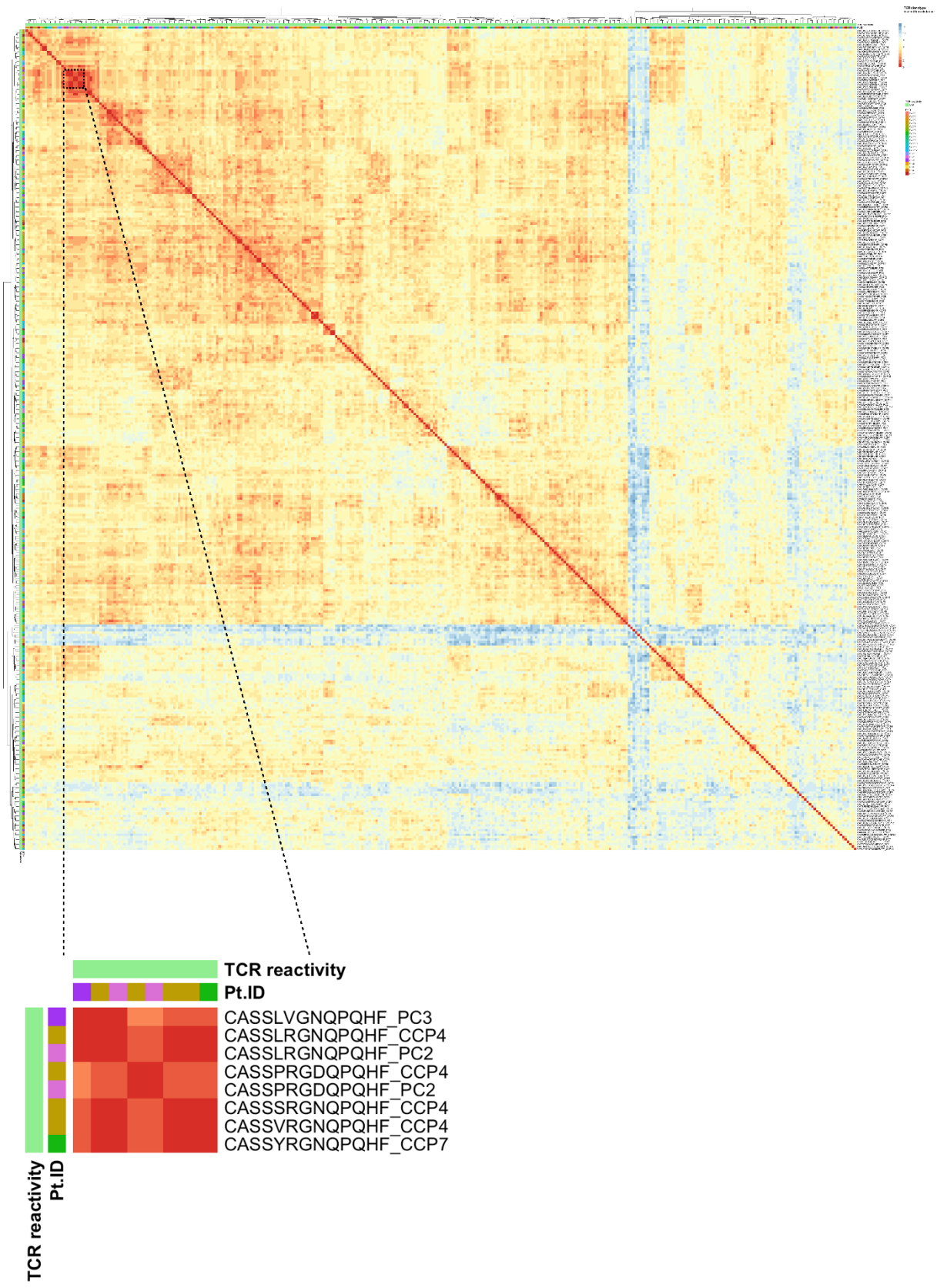

Figure S12: CEF TCRs share sequence homology among patients. Heatmap of sequence homology for CEF clones. Each row/column represents a CEF clonotype in a sample. The color represents the levels of sequence homology using levenshtein distance, with red indicating high degree of sequence homology and blue for less sequence similarity. Different colors were used to distinguish patient samples in the color sidebar. Area of high sequence homology across patients were amplified in the bottom.