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Supplemental information

Epitope profiling reveals binding signatures of SARS-CoV-2 immune response in natural infection and cross-reactivity with endemic human CoVs

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Supplementary Information

Table S1. Additional demographic information, Related to Table 1

	Mild (n = 14)	Moderate/Severe (n = 5)	Endemic (n = 2)
Mean Age (Mean ± SD)	42.2 ± 14.6	62.9 ± 7.3	64 ± 3.0
Female (n, %)	8 (57.1)	1 (20.0)	0 (0.0)
Race/ethnicity (n, %)			
White, non-Hispanic/Latino	12 (85.7)	2 (40.0)	2 (100.0)
African American, non-Hispanic/Latino	0 (0.0)	0 (0.0)	0 (0.0)
Other, non-Hispanic/Latino	1 (7.1)	3 (60.0)	0 (0.0)
Hispanic/Latino	1 (7.1)	0 (0.0)	0 (0.0)
Insurance Status (n, %)			
Public (or none/self-pay)	2 (14.3)	3 (60.0)	2 (100.0)
Private or both	12 (85.7)	2 (40.0)	0 (0.0)
Number of underlying conditions (n, %)			
≥1	0 (0.0)	3 (60.0)	2 (100.0)
None	14 (100.0)	2 (40.0)	0 (0.0)

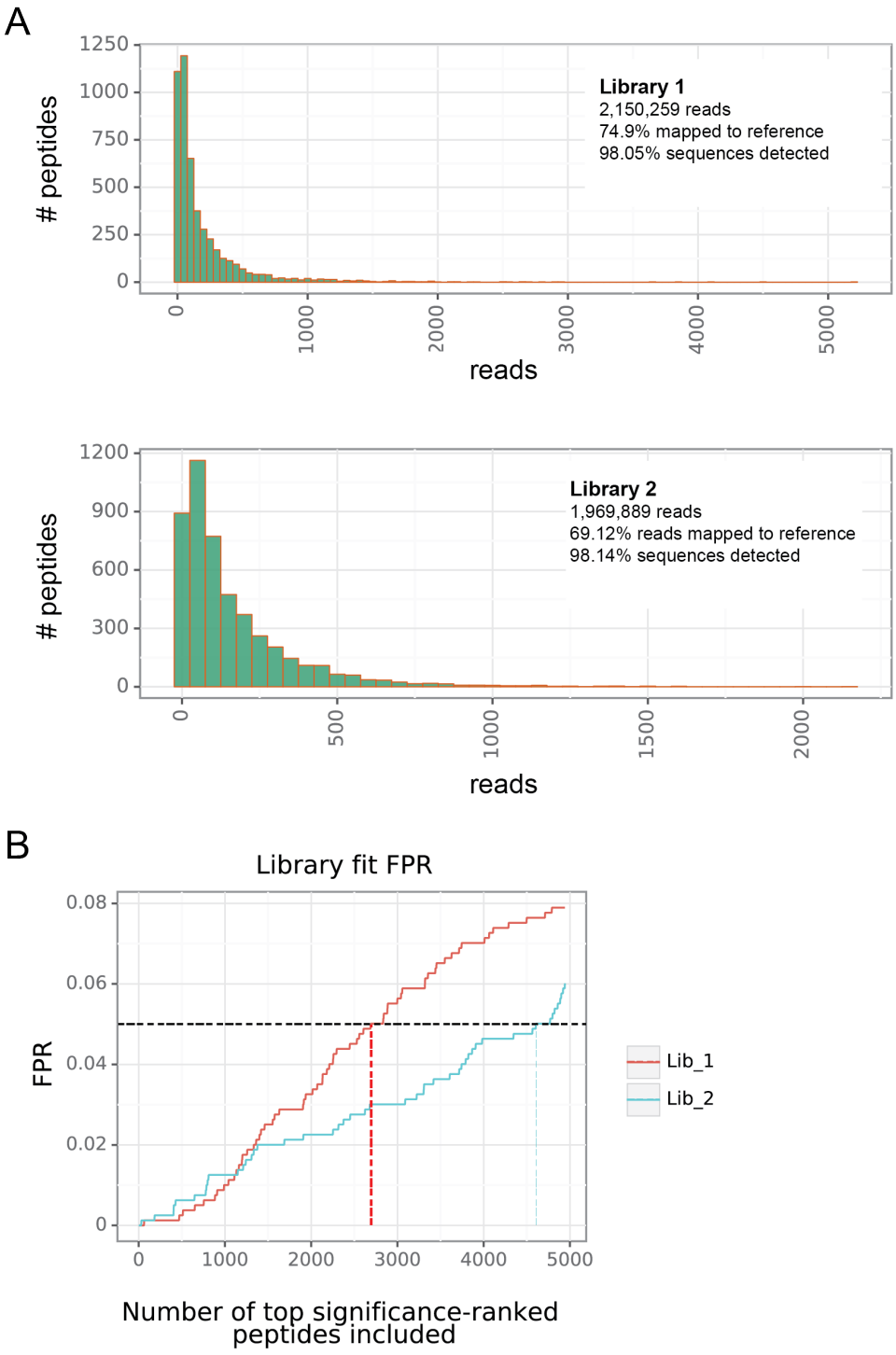
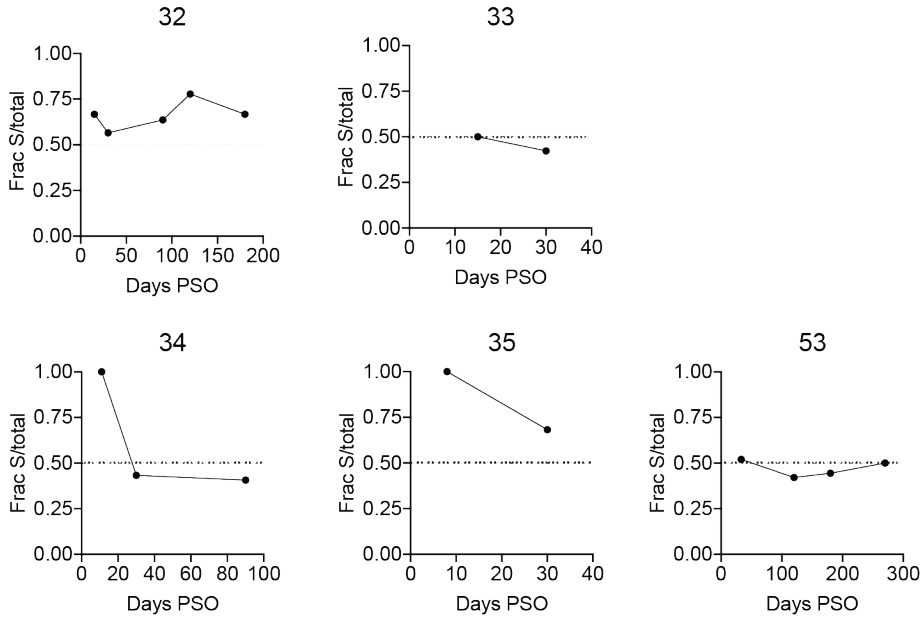


Figure S1. Pan-CoV input phage library coverage and FPR modeling for significance thresholding, Related to Figure 3. (**A**) Number of peptides plotted against number of reads for both independently generated phage libraries. Text inlays show the total number of reads sequenced from the input libraries, the percentage of

reads mapping to the target CoV peptide sequences, and the percentage of peptide sequences with zero reads. (**B**) The number of peptides included in the analysis plotted against the FPR, as calculated from the presence of enriched HIV-1 sequences. A FPR of 0.05 was chosen as a threshold and the number of peptides this cutoff corresponded to is indicated by red and blue lines for Libraries 1 and 2, respectively.

A



B

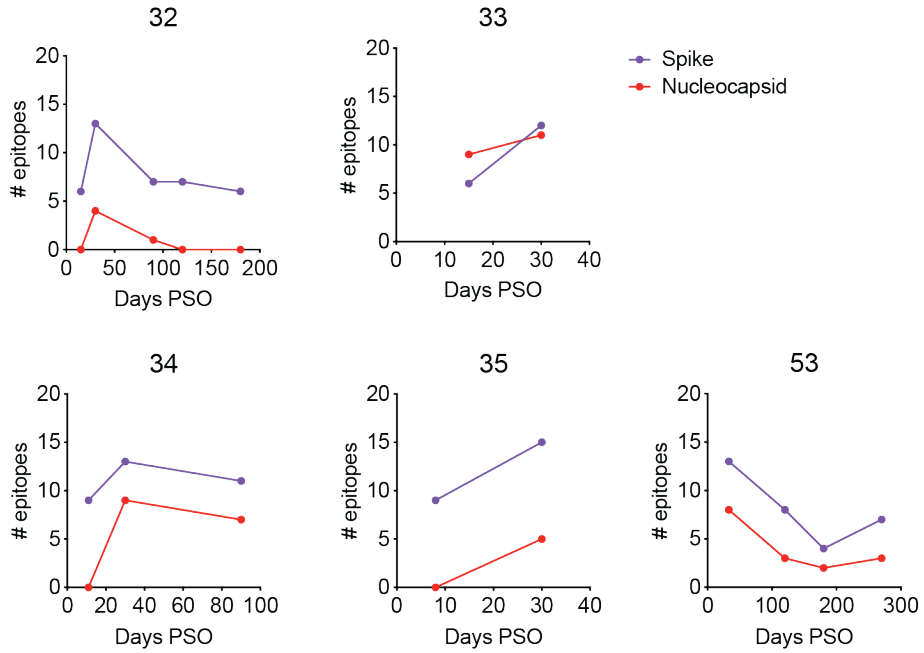


Figure S2. Kinetics of epitope breadth in patients with moderate/severe COVID-19. Related to Figure 3. Antibodies from longitudinal samples were immunoprecipitated and significant epitopes counts were measured using the gamma-Poisson model described previously. The fraction of epitopes from the S protein over the total number of epitopes is plotted in (**A**) and the number of epitopes from the S (purple) and N (red) proteins are

plotted in (**B**) for each available time point from individuals with moderate/severe COVID-19.

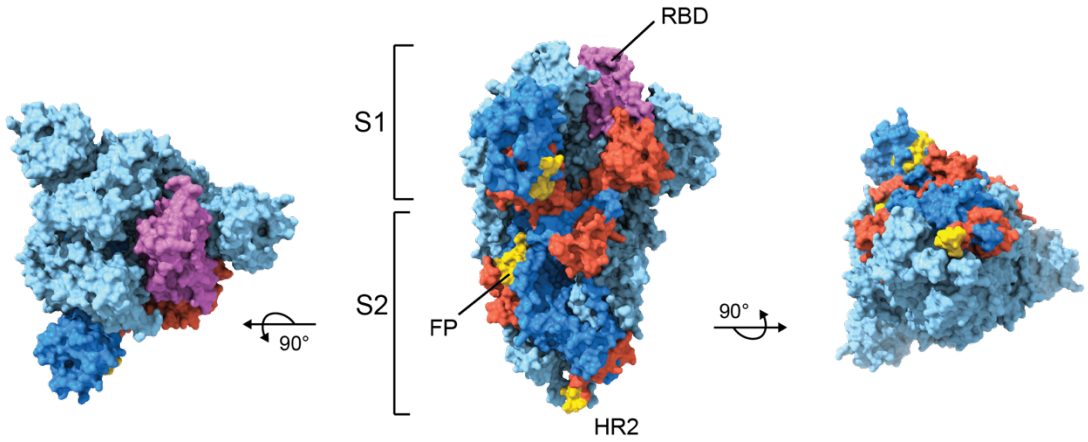


Figure S3. Top epitopes mapped onto the closed Spike trimer cryo-EM structure, Related to Figure 4 and Table 2. Spike epitopes from Table 2 mapped using an existing cryo-EM structure of the Spike trimer in the closed conformation (PDB 6VXX) (Walls et al., 2020). Left, "top" view of S1 subunit trimer; middle, "side" view centered on one S monomer (dark blue); right, "bottom" view. Epitopes (red) are mapped onto one representative monomer (dark blue); the single cross-reactive S epitope found in SARS-CoV-2 unexposed individuals is colored in gold in the S1 subunit. The cross-reactive epitopes in the FP and HR2 regions with high homology to endemic HCoVs (**Fig. 5**) are depicted in gold. The RBD of the monomer in focus is colored in purple.

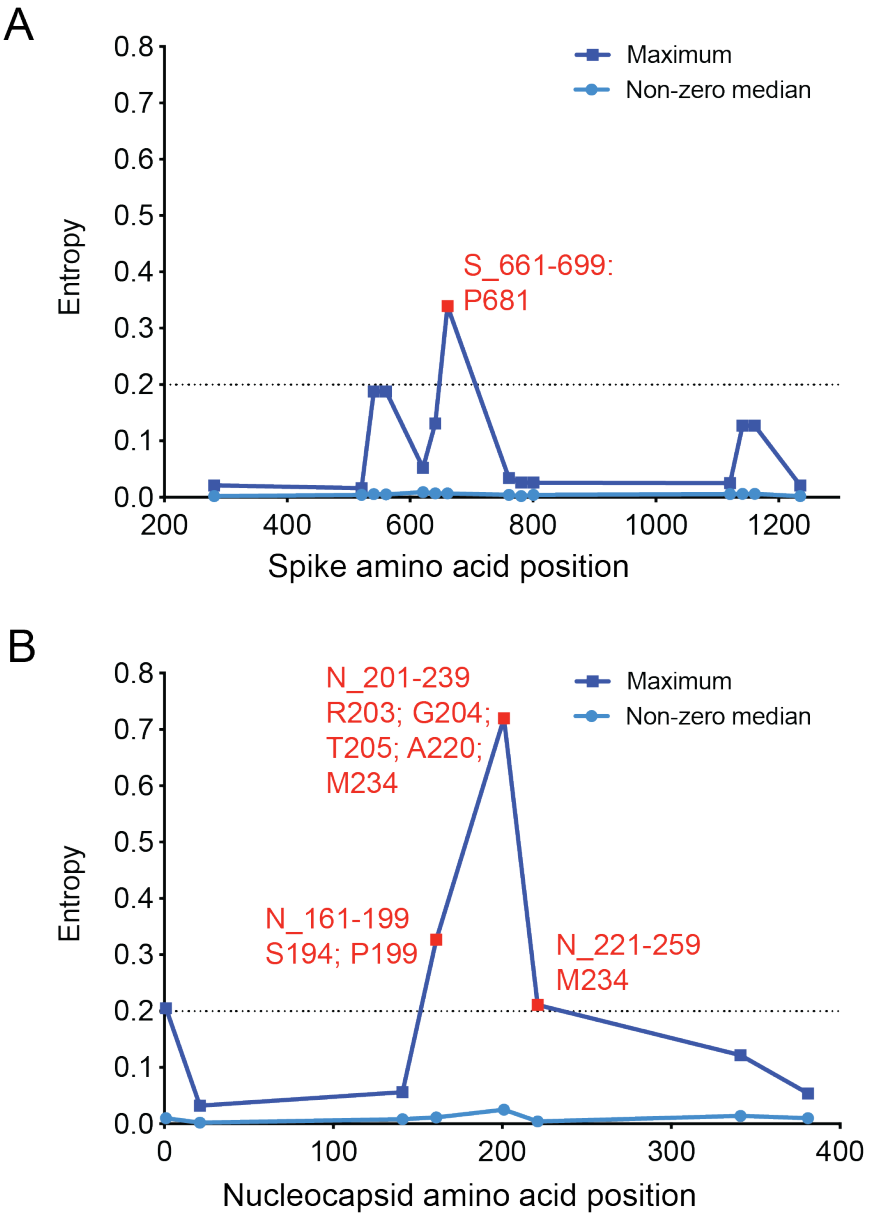


Figure S4. Mutational entropy within significant epitopes from Spike and Nucleocapsid, Related to Table 2. Shannon entropy data were downloaded from Nextstrain (nextstrain.org). Entropy for each amino acid within each significant epitope is plotted as either the maximum value within the peptide, or the median value among all non-zero values (non-zero median). Entropy within significant peptides is plotted for the Spike protein in (**A**) and Nucleocapsid protein in (**B**). Epitopes with a maximum entropy in the 99th percentile (> 0.2) are denoted by red squares and the residues contributing to high entropy within those peptides are indicated.

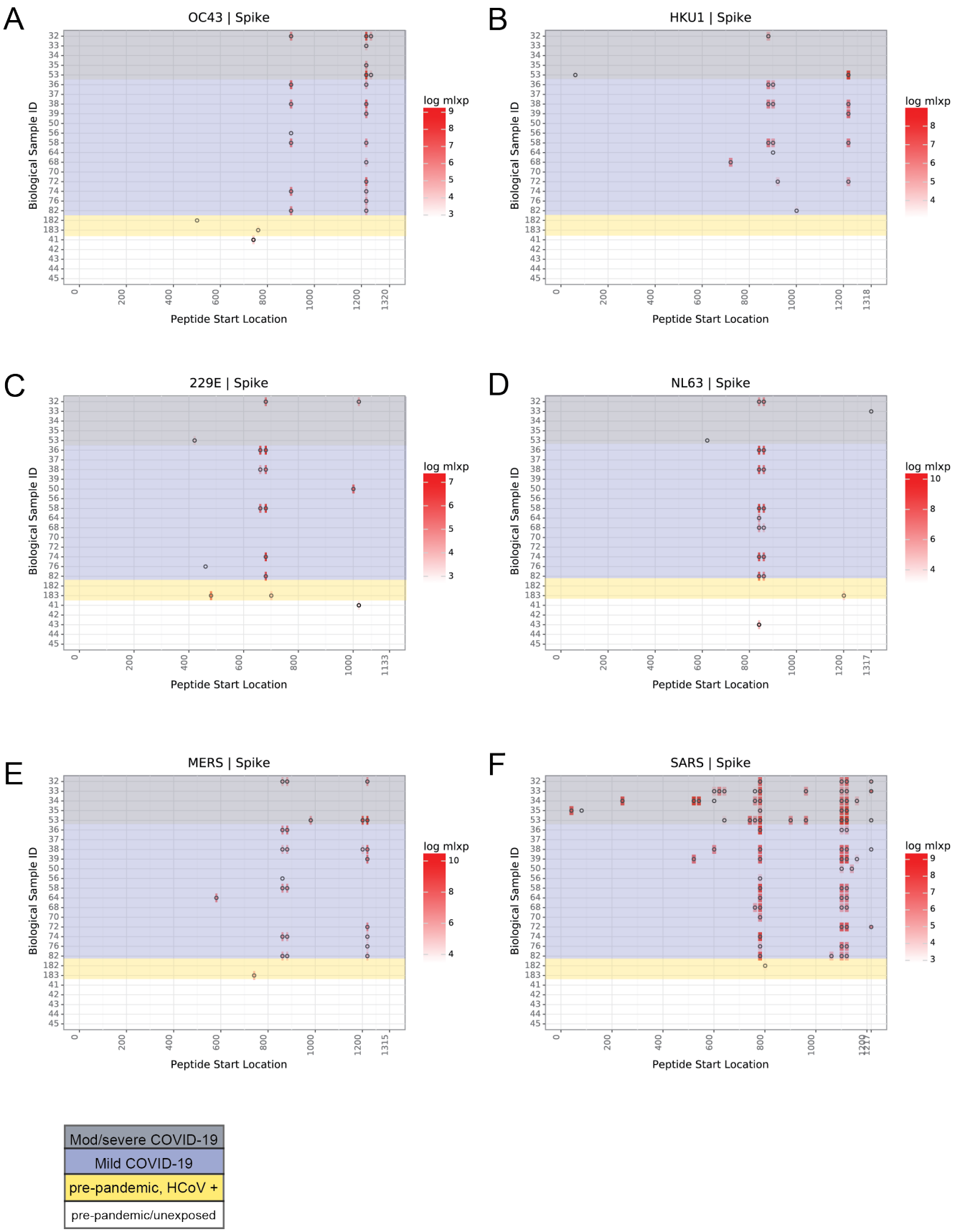

A
OC43 | Spike
B
HKU1 | Spike
C
229E | Spike
D
NL63 | Spike
E
MERS | Spike
F
SARS | Spike
Biological Sample ID
Peptide Start Location
log mlxp
Mod/severe COVID-19
Mild COVID-19
pre-pandemic, HCoV +
pre-pandemic/unexposed

Figure S5. Spike protein epitope profiles for non-SARS-CoV-2 HCoVs, Related to Figure 5. Epitopes from HCoV-OC43 (**A**), HCoV-HKU1 (**B**), HCoV-229E (**C**), HCoV-NL63 (**D**), MERS-CoV (**E**), and SARS-CoV (**F**) are mapped across the S protein. Bottom legend shows grouping of the COVID-19 and unexposed sample populations by color. Red rectangles correspond to the significance of the hit, as indicated by the log mlxp gradient to the right of each profile.

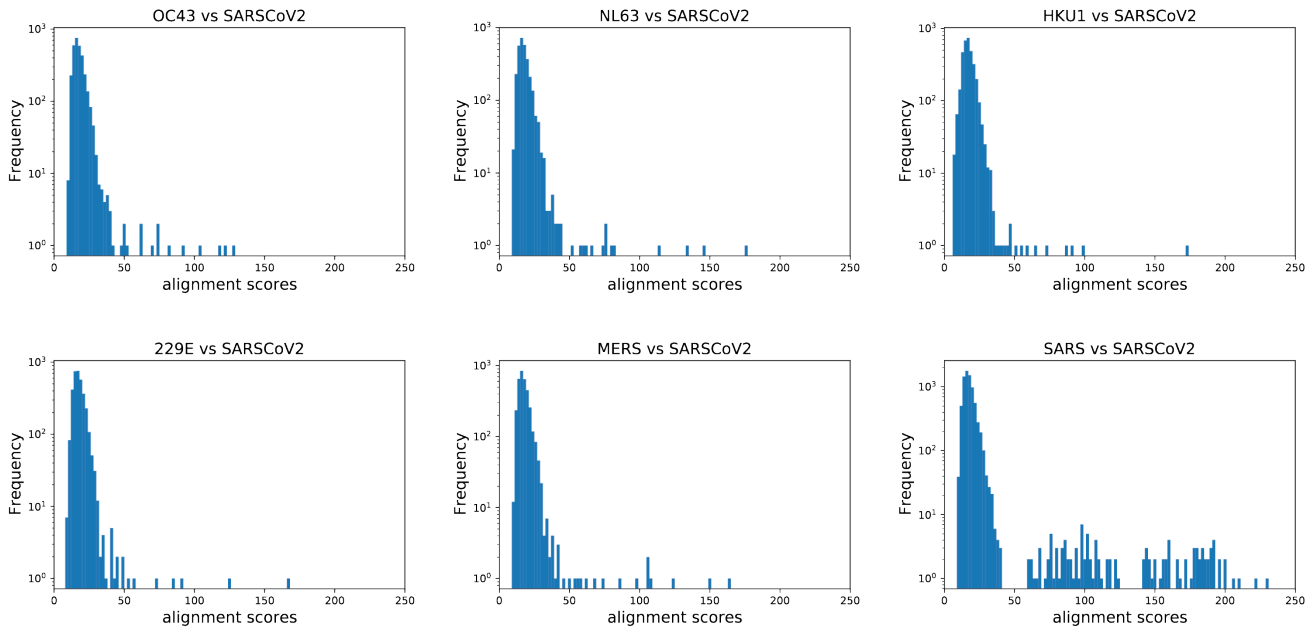


Figure S6. Smith-Waterman alignment scores for significant epitopes from all HCoVs, Related to Figure 6. Pairwise local alignment scores between significant SARS-CoV-2 and other commonly circulating or pathogenic HCoV peptides plotted against peptide pair frequency.