Supplementary Materials

Antibody Response to a Nucleocapsid Epitope as a Marker for COVID-19 Disease Severity

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Materials and Methods

Cloning

For phage display of epitopes, the pm1165a phagemid vector as previously described (*42*) as engineered to encode an N-terminal FLAG-tag and a C-terminal fusion to the P8 coat protein of M13-phage. This template, termed FlagTemplate, was used for subcloning of SARS-CoV-2, SARS, MERS, HKU-1 and NL63 epitopes. A vector map of the FlagTemplate (**Fig. S7**), cloning procedures, and a list of oligos (**Table S3**) for Q5 site-directed mutagenesis and Gibson Assembly are provided here.

Putative epitopes for phage display were cloned via Q5 site-directed mutagenesis or Gibson Assembly (New England Biolabs) according to the manufacturer's instructions. For large epitopes (>500 bp), such as Ep17, Gibson Assembly was conducted in two PCR steps with the **FlagTemplate** or pCAGGS containing the SARS-CoV-2 S protein gene (BEI Resources) to generate the vectors and inserts, respectively. The Gibson Assembly (2 µL) or KLD mix (5 µL) was transformed into Nova Blue *E. coli* competent cells, and transformants were plated on a carbenicillin-supplemented (50 µg/mL) agar plate before incubation at 37 °C overnight. Five single colonies were selected to inoculate 4 mL of SOC media in a 15 mL culture tube supplemented with carbenicillin (50 µg/mL). The seed cultures were incubated at 37 °C with shaking at 225 rpm for 8-12 h. Phagemid DNA was isolated using the QIAprep spin miniprep kit according to the manufacturer's instructions. The successful subcloning of the ORF encoding each epitope was verified via DNA sequencing (Genewiz).

Purification and preparation of phage

Phage were propagated and purified using procedures previously described (*43*) with the following changes. A single colony was selected to inoculate 15 mL of 2YT and shaken at 37 °C until the OD_{600} reached 0.6. After incubation at 37 °C for 45 min, 8 mL of the primary culture was used to inoculate 300 mL of 2YT supplemented with carbenicillin (50 µg/mL), kanamycin (20 µg/mL), and isopropyl β-D thiogalactopyranoside (IPTG, 30 µM).

To precipitate the phage, the cultures were centrifuged at 10 krpm (15300 x g) for 10 min at 4 °C. The supernatant was decanted into a centrifuge tube containing 60 mL PEG-8000 (20%, w/v) and NaCl (2.5 M). The tube was inverted 10 times and stored on ice for 30 min followed by an additional centrifugation at 10 krpm (15300 x g) for 20 min at 4 °C. The supernatant was decanted, and tubes were centrifuged for an additional 4 min at 4 krpm (2429 x g) at 4 °C. The pellets were resuspended in PBS (10 mM phosphate, 137 mM NaCl, pH 7.2) with TWEEN 20 (0.05%, v/v) and glycerol (10%, v/v), separated into 1 mL aliquots, flash frozen with liquid nitrogen, and stored at -80 °C. For binding assays via ELISA, the purified phage was thawed on ice, precipitated a second time as before. The quality of each phage preparation was routinely checked by quality control ELISA, termed QC ELISA, to a FLAG peptide fused to the N-terminus of each epitope (**Fig. S7**); additionally, PCR using Oligo69 and Oligo70 followed by DNA sequencing (Genewiz) was performed for every phage preparation. Such quality control allowed for identification of toxic clones; for example, C8, was apparently toxic to *E. coli*, and three protein epitopes failed to express in *E. coli* for unknown reasons. The phage concentration was determined by absorbance at 260 nm using a coefficient of molar absorptivity of 0.003 nM^{-1} cm^{-1} and diluted to 40 nM in PBS.

Patient sample collection

The UC Irvine Experimental Tissue Resource operates under a blanket IRB protocol (UCI #2012-8716) that gives ETR personnel 'Honest Broker' status and enables the collection of any fluid or tissue remnant in excess to that needed for clinical diagnosis and distribution to investigators under the conditions of their own IRB approval. Patients undergoing COVID testing in the Emergency Department or on the inpatient service with confirmed COVID (+) pharyngeal swabs, were followed for their blood collections daily. Specimens collected originally for diagnostic purposes were processed and stored by the hospital laboratory in a

manner compliant with College of American Pathologists (CAP) standards. EDTA-anticoagulated whole blood was stored for 2 days at 4 °C after clinical diagnosis and released for research purposes. Plasma from heparin-anticoagulated blood was centrifuged immediately after collection and preserved at 4 °C for 3-4 days before being released for research use. All COVID (+) specimens were handled under BSL-2 conditions, aliquoted into screw cap cryovials, and stored at -80 °C long term with constant temperature monitoring. Specimens were coded by the ETR with unique de-identifiers, and accompanying clinical information was stripped of PHI, such that investigators could receive specimens under a Non Human Subjects Determination exemption from the UC Irvine IRB. All samples from SARS-CoV-2 infected patients were inactivated by incubation in a water bath at 56 °C for 30 mins (*44*), aliquoted (40 µL each), and stored at -80 °C.

Phage ELISA with plasma

The phage-displayed SARS-CoV-2 epitopes were used in phage ELISAs with patient plasma samples diluted 100-fold in coating buffer (50 mM Na_2CO_3, pH 9.6). After incubation in a 96-well Nunc MaxiSorp flat-bottom microtiter plate with shaking at 150 rpm at 4 °C for 12-18 h, plasma was aspirated by a plate washer (BioTek). Next, the plate was treated with 100 µL per well of ChonBlock Blocking/Sample Dilution Buffer (Chondrex, Inc.) for 1 h with shaking at 150 rpm at room temperature and washed three times with wash buffer (0.05% v/v Tween-20 in PBS). The epitope displaying phage and controls were diluted to 1 nM in ChonBlock Blocking/Sample Dilution Buffer and 100 µL were added to each well before incubating for 2 h with shaking (150 rpm) at room temperature. The plate was then washed three times with wash buffer. The primary antibody, anti-M13-HRP (Creative Diagnostics), was diluted 1:5000 in ChonBlock Secondary Antibody Buffer and 100 µL was added per well; the plate was incubated for 1 h at 150 rpm and room temperature. Following three washes with wash buffer, 1-Step Ultra TMB-ELISA Substrate Solution (100 µL per well, ThermoScientific) was added. Absorbance of TMB substrate was measured twice at 652 nm by UV-Vis plate reader (BioTek) after 5 and 15 min of incubation.

Serum coronavirus antigen microarray (COVAM)

COVAM included 61 antigens across respiratory virus subtypes including 11 antigens from SARS-CoV-2 expressed in either baculovirus or HEK-293 cells as previously detailed (**Table S4**) *(4)*. These antigens were provided by Sino Biological U.S. Inc. as either catalog products or custom synthesis service products. The antigens were printed onto microarrays, probed with human sera, and analyzed as previously described (*45-47*). Briefly, lyophilized antigens were reconstituted with sterile water to a concentration of 0.1 mg/mL protein in PBS, and printing buffer was added. Antigens were then printed onto ONCYTE AVID nitrocellulose-coated slides (Grace Bio-Labs) using an OmniGrid 100 microarray printer (GeneMachines). The microarray slides were probed with human sera diluted 1:100 in 1X Protein Array Blocking Buffer (GVS Life Sciences, Sanford, ME) overnight at 4°C and washed with TTBS buffer (20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20 in ddH2O adjusted to pH 7.5 and filtered) three times for 5 mins each. A mixture of human IgG and IgA secondary antibodies conjugated to quantum dot fluorophores Q800 and Q585 respectively was applied to each of the microarray pads and incubated for 2 h at room temperature, and pads were then washed with TTBS three times for 5 mins each and dried. The slides were imaged using an ArrayCam imager (Grace Bio-Labs) to measure background-subtracted median spot fluorescence. Non-specific binding of secondary antibodies was subtracted using a saline control. The mean fluorescence of the 4 replicate spots for each antigen was used for analysis.

Statistical analysis

The ELISA data were analyzed in GraphPad Prism 8. Since the total antibody content differs from person to person, the raw absorbance values for every patient sample were normalized and represented as the ratio as compared to a negative control. Analysis of

variance (ANOVA) with Dunnett's multiple comparisons was performed to determine if values were statistically significant. Correlations between COVAM IgG/IgM and ELISA were determined by plotting normalized values on an XY graph and performing a non-parametric correlation analysis using a Spearman's rank correlation coefficient test.

For data visualization of clinical patient data, trends in data were evaluated using Knime Analytics Platform software. GraphPad Prism was used to calculate column statistics including mean, standard deviation, SEM, p-values, Odds Ratios, and Likelihood Ratios defined as sensitivity / (1 - specificity). ANOVA with Tukey's multiple comparisons test was used to evaluate antibody response and disease severity between patients with αEp9 Abs, non-Ep9, αN Abs, or non αN Abs. Comparisons of patients with αEp9 Abs and non-αEp9 Abs were conducted using unpaired, two-tailed, parametric t-tests. Contingency graphs were statistically evaluated using Fisher's exact test, for groups with binary categorization, and Chi-squared test for groups with multiple categories. Different datasets were fitted with linear or non-linear regression methods, the fit with the higher R^2 value was chosen. Correlations between two clinical parameters (e.g., IL-6 and AST) were evaluated using the Pearson coefficient or Spearman coefficients (r) for linear or non-linear regressions, respectively; r-values between 1.0-0.7 were considered strong correlations, r-values between 0.7 and 0.5 were considered a moderate correlation, and values below 0.5 were considered a weak correlation (*48*). The significance of the correlation was evaluated based on p-value <0.05.

Supplementary Text

Table S1. Phage-displayed putative epitopes of SARS-CoV-2 and Ep9 orthologous sequences from SARS, MERS, HKU-1, and NL63.

Epitope	Virus	Protein	Residues*	Amino Acid Sequence	Sequence Length	Ref.
Ep1	SARS-CoV-2	S	287-317	DAVDCALDPLSETKCTLKSFTVEKGIYQTSN	31	(*13*)
Ep2	SARS-CoV-2	S	802-819	FSQILPDPSKPSKRSFIE	18	(*13*)
Ep3	SARS-CoV-2	S	15-30	CVNLTTRTQLPPAYTN	16	(*14*)
Ep4	SARS-CoV-2	S	1056-1070	APHGVVFLHVTYVPA	15	(*12*)
Ep5	SARS-CoV-2	M	1-24	MADSNGTITVEELKKLLEQWNLVI	24	(*13*)
Ep6	SARS-CoV-2	M	132-151	PLLESELVIGAVILRGHLRI	20	(*13*)
Ep7	SARS-CoV-2	M	97-11	IASFRLFARTRSMWS	15	(*12*)
Ep8	SARS-CoV-2	N	41-61	RPQGLPNNTASWFTALTQHGK	21	(*13*)
Ep9	SARS-CoV-2	N	152-172	ANNAAIVLQLPQGTTLPKGFY	21	(*13*)
Ep10	SARS-CoV-2	N	264-278	ATKAYNVTQAFGRRG	15	(*12*)
Ep11	SARS-CoV-2	E	52-66	VKPSFYVYSRVKNLN	15	(*12*)
Ep12	SARS-CoV-2	S	524-598	VCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIAD TTDAVRDPQTLEILDITPCSFGGVSVI	75	(*13*)
Ep13	SARS-CoV-2	S	601-640	GTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGS	40	
Ep13*	SARS-CoV-2	S	601-640	GTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGS	40	(*49*)
Ep14	SARS-CoV-2	S	61-76	NVTWFHAIHVSGTNGT	16	
Ep15	SARS-CoV-2	S	373-390	SFSTFKCYGVSPTKLNDL	18	
Ep16	SARS-CoV-2	N	354-400	NKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADL	47	(*13*)
Ep17	SARS-CoV-2	S	319-529	RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYS VLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQ TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLK PFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRV VVLSFELLHAPATVCGPKK	211	
Ep18	SARS-CoV-2	S	488-507	CYFPLQSYGFQPTNGVGYQP	20	
Ep19	SARS-CoV-2	S	429-448	FTGCVIAWNSNNLDSKVGGN	20	
Ep20	SARS-CoV-2	S	448-466	NYNYLYRLFRKSNLKPFER	19	
Ep21	SARS-CoV-2	S	467-487	DISTEIYQAGSTPCNGVEGFN	21	
sEp9	SARS	N	153-173	NNNAATVLQLPQGTTLPKGFY	21	
mEp9	MERS	N	141-161	NNDSAIVTQFAPGTKLPKNFH	21	
hEp9	HKU-1	N	166-186	TTQEAIPTRFPPGTILPQGYY	21	
nEp9	NL63	N	119-136	NQKPLEPKFSIALPPELS	18	

*Residue numbering from protein sequences deposited in GenBank. Specifically, the accession numbers were as follows: S protein (YP_009724390.1), M protein (YP_009724393.1), N protein (YP_009724397.2) and E protein (YP_009724392.1) from SARS-CoV-2 and N protein from SARS (NP_828855.1), MERS (YP_009047211.1), HKU-1 (YP_173242.1), or NL63 (YP_003771.1).

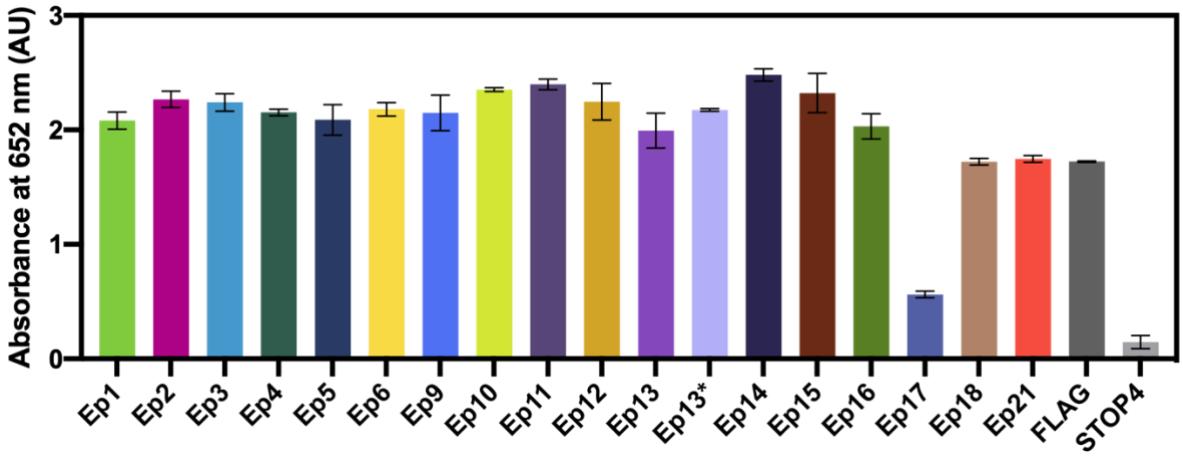

Fig. S1. Quality control ELISA (QC ELISA) for phage-displayed, epitope candidates. Anti-FLAG antibodies (1:1000 in coating buffer) were immobilized on a microtiter plate. Subsequent steps followed the ELISA protocol provided here. Error bars represent SEM (n = 3).

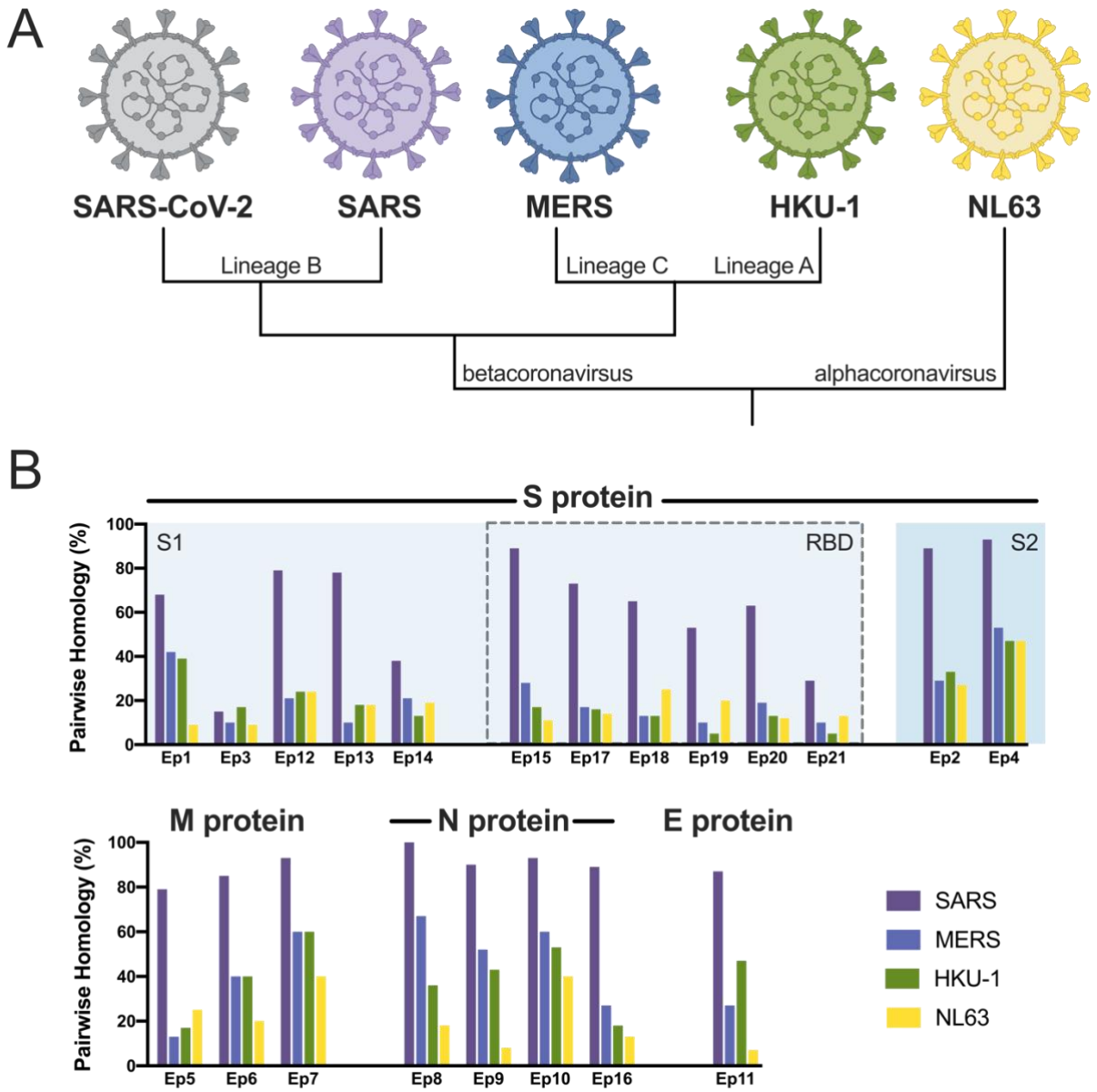

Fig. S2. Epitope homology of SARS-CoV-2 with four phylogenetically related coronaviruses known to infect humans. A) Evolutionary lineages of the human coronaviruses investigated here, including the highly pathogenic (SARS-CoV-2, SARS, and MERS) and the less virulent (HKU-1 and NL63). B) The pairwise homology (% amino acid identity) between SARS-CoV-2 and the indicated coronavirus. Labels (top) indicate the proteins and domains (e.g., S1) from which the epitopes are derived.

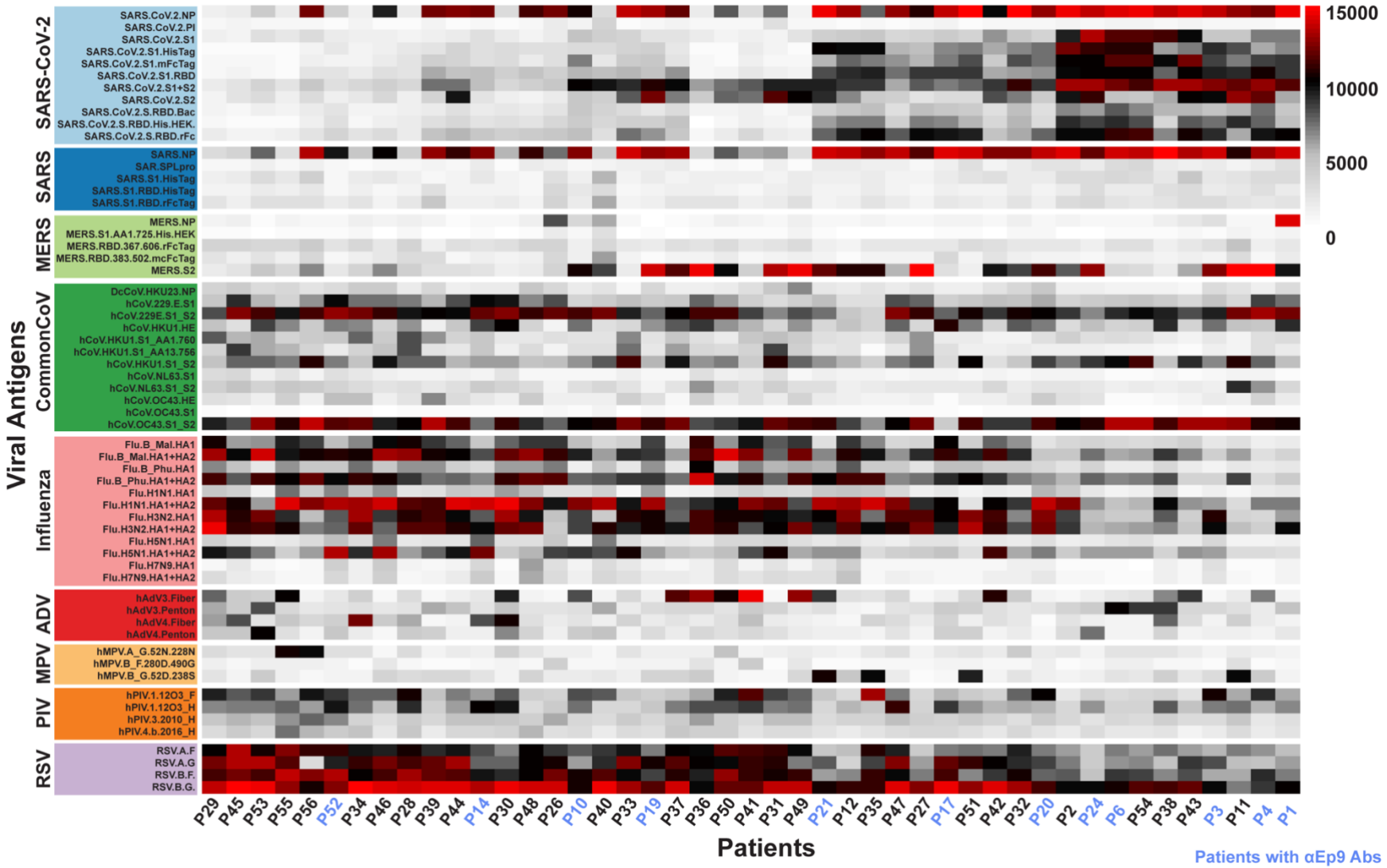

Fig. S3. COVAM data showing the variation in IgG seroreactivity of patient plasma. The heatmap shows normalized signal intensity from plasma samples (n = 45). Plasma samples are in columns and sorted left to right by increasing average intensity to differentially reactive IgG, and viruses are in rows sorted by decreasing average seroreactivity.

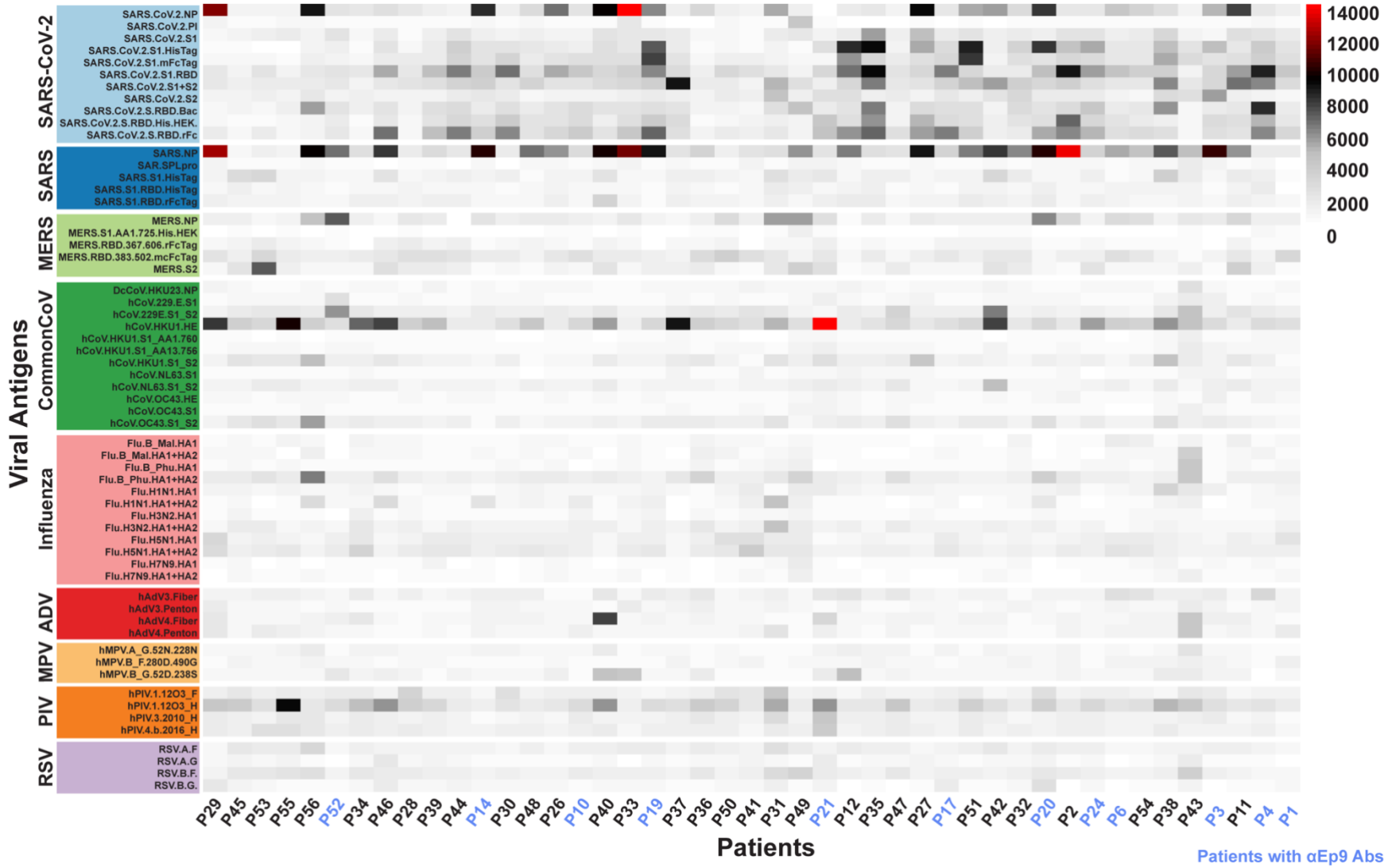

Fig. S4. Variation in IgM seroreactivity of patient plasma. Heatmap showing normalized signal intensity from plasma samples (n = 45). Plasma samples are in columns and sorted left to right by increasing average intensity to differentially reactive IgM, and viruses are in rows sorted by decreasing average seroreactivity.

Table. S2. Demographics and clinical characteristics of COVID-19 patients categorized by αEp9 Abs response.

Characteristics	No αEp9 Abs (n=63)	αEp9 Abs (n=23)	p-value
Demographics			
Age (± SD)	49.75 (± 18.45)	47.26 (± 18.45)	0.5668
Gender F: M (%)	21:42 (44.4/66.7)	10:13 (43.5/56.5)	0.4502
Ethnicity n, (%) (Hispanic: Asian: Caucasian: Black: Other)	15 (65.2): 4 (17.4): 3 (13.0): 1 (4.3): 0 (0)	39 (61.9): 8 (12.7): 9 (14.3) :3 (4.8) :4 (6.3)	0.7760
BMI (± SD)	28.89 (± 6.445)	32.06 (± 7.896)	0.0642
Preconditions, n (%)			
Hypertension	23 (36.5)	10 (43.5)	0.6203
Diabetes	21 (33.3)	6 (26.1)	0.6065
CVD	6 (9.5)	2 (8.7)	1.0000
CAD	6 (9.5)	2 (8.7)	1.0000
CKD/ESRD	6 (9.5)	2 (8.7)	1.0000
Asthma/COPD	8 (12.7)	3 (13.0)	1.0000
Obesity	24 (38.1)	13 (56.5)	0.1461
Cancer	2 (3.17)	3 (13.0)	0.1163
Symptoms, n (%)			
Total Days of Symptoms	9.8 (± 8.98)	17 (± 10.13)	0.0059**
Cough	43 (68.3)	15 (65.2)	0.7997
Dyspnea/SOB	28 (44.4)	11 (47.8)	0.8108
Myalgia/Fatigue	17 (27.0)	8 (34.8)	0.5926
Headache	12 (19.0)	2 (8.7)	0.3349
Chest pain	7 (11.1)	3 (13.0)	1.0000
Anosmia	4 (6.3)	2 (8.7)	0.6561
Stroke-like Symptoms	0	2 (8.7)	0.0692
Abdominal pain	3 (4.8)	0	0.5611
Pulmonary symptoms^ (Pneumonia: Other: None)	16 (25.4): 36 (52.38): 8 (12.7)	13 (56.5): 7 (30.4): 1 (4.3)	0.0142*
Severity, n (%)			
Asymptomatic	3	0	0.5611
Non-severe: Severe^^	51:12 (n, severity 19.0%)	10:13 (n, severity 56.5%)	0.0013**
Days in Hospital	5.79 (± 8.01)	10.95 (± 10.74)	0.0183*
Days in ICU	12.63 (± 13.19) n=11	12.50 (± 6.93), n=12	0.8004
Days on ventilator	14.00(± 3.96), n=6	12.86 (± 5.40), n=7	0.7934

Results are presented as mean ± standard deviation (SD) or patient number (n) and percentage of population (%). P-values for continuous variables are calculated using unpaired, two-tailed T-tests. P-values for categorical variables use Fisher's exact test for single value parameters, and Chi-squared test for multi-group variables. *****, ****** p-values < 0.05, 0.01, respectively.

^ Pulmonary symptoms are based descriptive reports of X-ray and CT scans. "Other" pulmonary symptoms include, but are not limited to, atelectasis, pleural scarring, pleural effusion, pulmonary edema, mild peribronchial thickening.
^^ non-severe include ER and In-patients only, severe includes patients in the ICU, on the ventilator or death.

BMI = body mass index, CVD = cardiovascular disease, CAD = coronary artery disease, CKD = chronic kidney disease, ESRD = end-stage renal disease, SOB = shortness of breath, COPD = chronic obstructive pulmonary disease

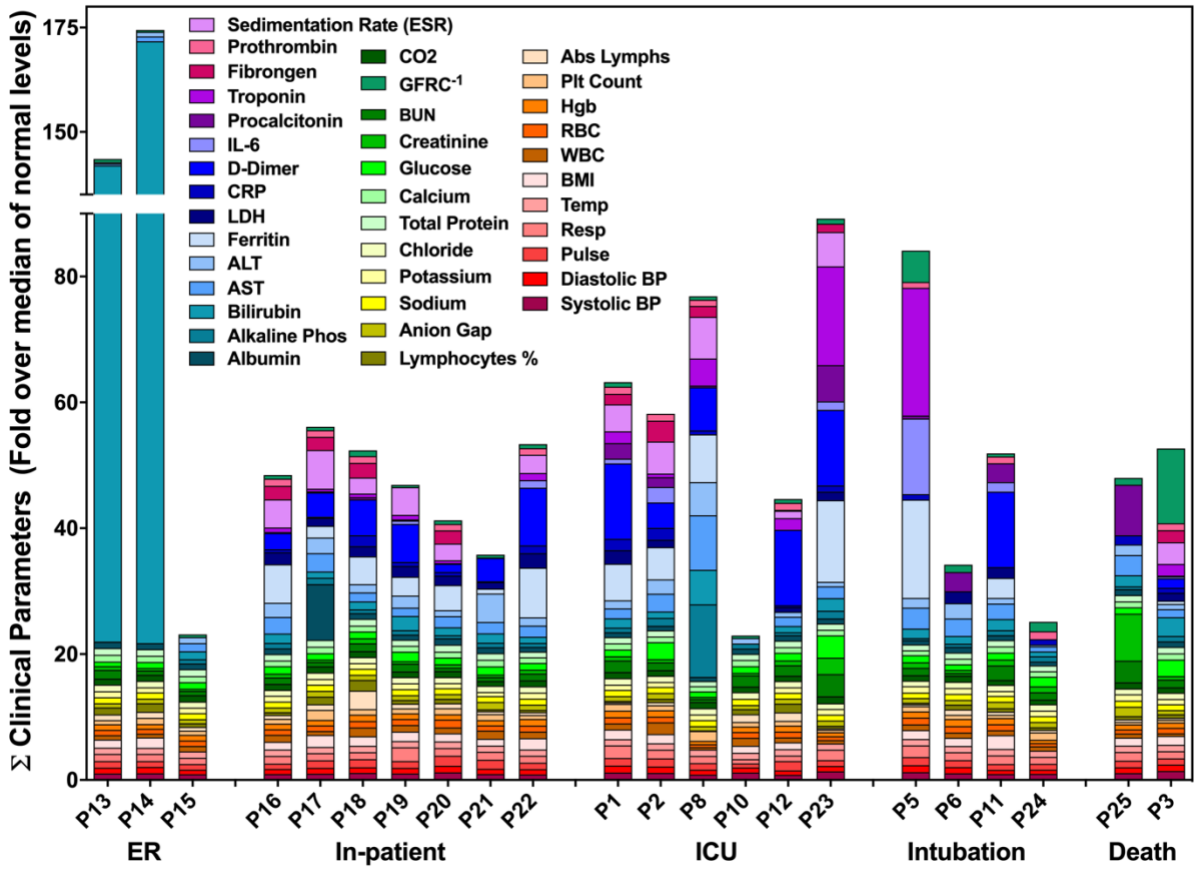

Fig. S5. Comparison of disease severity and clinical parameters of patients with αEp9 Abs. The data shown represents the fold change of each clinical parameter over the mean of the normal range. The sum of all the fold changes of the clinical parameters for each Ep9-responsive patient is binned according to COVID-19 disease severity. For facile visualization and comparison of clinical biomarkers between Ep9-reponsive patients, the values of each parameter were normalized to fold over the mean of healthy values. No significant trends in clinical parameters (color indicated) were observed with increased disease severity or relative to patients lacking αEp9 Abs.

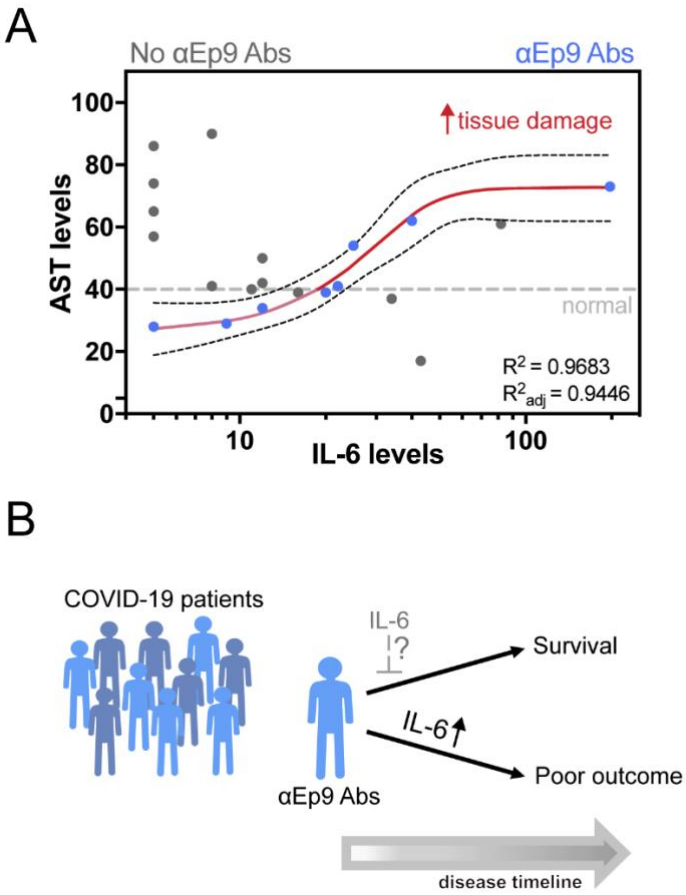

Fig. S6. Association of COVID-19 patients having αEp9 Abs with inflammatory cytokine and tissue damage markers. A) Association between the inflammatory cytokine, IL-6, and the tissue damage marker, aspartate transaminase (AST), shows a sigmoidal curve fit for patients with αEp9 Abs, R^2 = 0.9683, Spearman's correlation coefficient = 1.0, p< 0.0001. **B**) Schematic of patients with αEp9 Abs with increasing IL-6 levels leading to poor outcomes. We hypothesize patients with αEp9 Abs could benefit from IL-6 inhibition early in the disease, such as monoclonal antibody drugs targeting IL-6 or its receptor (IL6R), to disrupt a cytokine storm and reduce severe outcomes.

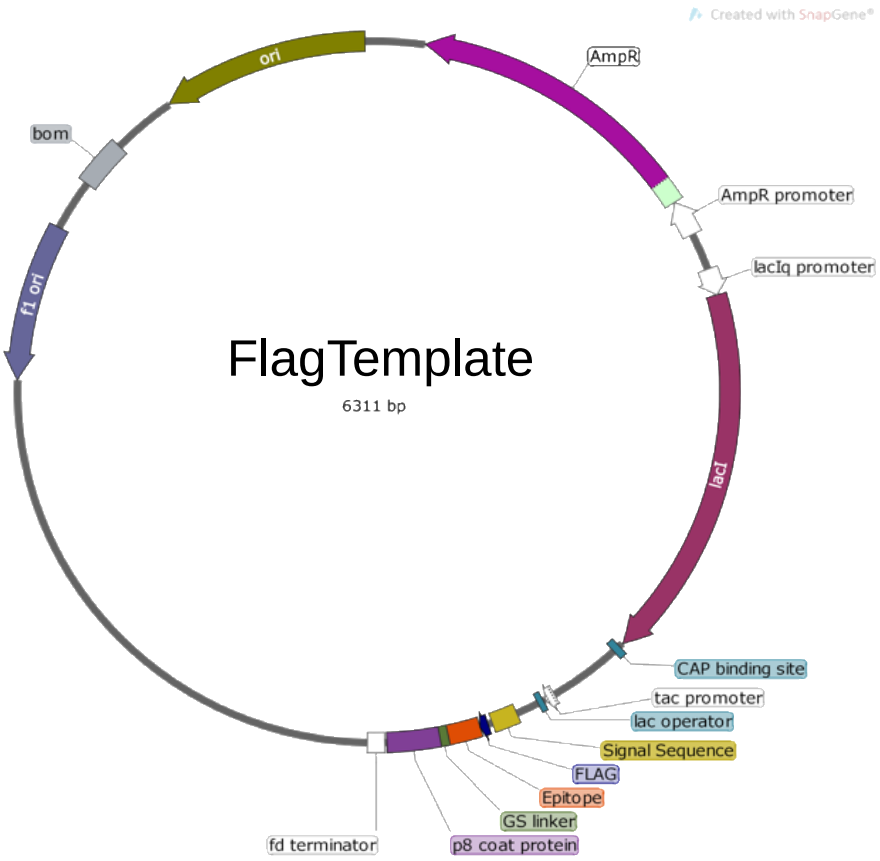

Fig. S7. Schematic of the FlagTemplate phagemid used for cloning phage-displayed epitopes. The phagemid, termed FlagTemplate, for the subcloning of SARS-CoV-2, SARS, HKU-1 and NL63 epitopes encodes an N-terminal FLAG tag, followed by a GSG linker to the epitope before a C-terminal GGGSGSSS linker to the P8 coat protein of M13-phage.

Table S3. Oligos used for cloning of phage-displayed putative epitope for SARS-CoV-2 and ortholog Ep9 epitope from SARS, MERS, HKU-1, and NL63.

Oligo#	Sequence (5' to 3') with insertions denoted in lowercase and substitutions in bold	Product	Mutagenesis	Rounds
Oligo1	aggaagtggaggtggaggatccgggagctccagcCCGAGGGTGACGATCCCG	FlagTemplate	Q5	1
Oligo2	ttatcatcgtcatctttataatcaaccaatgcataGCCGAGGCGGAAAACATC			
Oligo3	attaaagtcgttcaccgtcgaaaaaggaatctatcagacctctaacGGTGGAGGATCCGGGAGC	Ep1	Q5	1
Oligo4	gtacactttgtttcactcagtggatctaatgcacaatcgaccgcatcTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo5	gagcaagccttctaagcgctctttcattgaaGGTGGAGGATCCGGGAGC	Ep2	Q5	1
Oligo6	gggtcaggcaggatctgcgagaatccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo7	tacacagttacctcccgcgtatacaaatGGTGGAGGATCCGGGAGC	Ep3	Q5	1
Oligo8	cgagttgtcaagttcacacatccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo9	ttcttgcacgtgacgtatgtgcctgctGGTGGAGGATCCGGGAGC	Ep4	Q5	1
Oligo10	caccactccatggggcgctccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo11	ggagctgaaaaaactgttggaacaatggaaccttgtaatcGGTGGAGGATCCGGGAGC	Ep5	Q5	1
Oligo12	tctacggtaatcgtaccgttcgagtccgccattccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo13	tggagctgtgatcttacgcggacacctgcgtatcGGTGGAGGATCCGGGAGC	Ep6	Q5	1
Oligo14	atcactaattctgattccaacaggggtccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo15	ttcgcacgcactcgctccatgtggtctGGTGGAGGATCCGGGAGC	Ep7	Q5	1
Oligo16	caagcggaagctcgcaattccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo17	gcttcgtggttcactgcgcttacccagcacggaaagGGTGGAGGATCCGGGAGC	Ep8	Q5	1
Oligo18	tgtattattaggcagcccttgagggcgtccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo19	tcaagggactaccttgcccaaggggttctatGGTGGAGGATCCGGGAGC	Ep9	Q5	1
Oligo20	ggtaattgtaacacgattgcagcgttattagcTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo21	gtaacccaagcgttcggtcgccgcgggGGTGGAGGATCCGGGAGC	Ep10	Q5	1
Oligo22	attatacgcttttgtagctccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo23	gtctactctcgtgtaaaaaacttgaatGGTGGAGGATCCGGGAGC	Ep11	Q5	1
Oligo24	gtaaaaggaaggcttcactccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo25	tttcaactttaatggcctgacggggaccggagtcctgactgaatccaatGGTGGAGGATCCGGGAGC	Ep12	Q5	3
Oligo26	ttgacacacttatttttaaccaggtttgttgacttcttcggcccgcatacTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo27	agacgctgttcgtgacccacagactctggagattttggacattacacctGGTGGAGGATCCGGGAGC			
Oligo28	gttgtatcggcgatgtcgcgtccaaattgctggaacggcagaaatttttttATTGGATTCAGTCAGGACTCCG			
Oligo29	tgtctccgtcatcGGTGGAGGATCCGGGAGC			
Oligo30	cctccgaatgaacaAGGTGTAATGTCCAAAATCTCCAGAG			
Oligo31	ctgtatcaggatgtcaattgcacagaagtcGGTGGAGGATCCGGGAGC	Ep13	Q5	2
Oligo32	tacagcaacttggttacttgtgtttgtcccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo33	cccacttggcgcgtctacagcacaggcagtGGTGGAGGATCCGGGAGC			
Oligo34	cgtcagttggtcggcgtggatggctaccggGACTTCTGTGCAATTGACATCCTGATAC			
Oligo35	GCTGTACCAG**ggc**GTGAACTGTA	Ep13*	Q5	1
Oligo36	ACTGCCACCTGATTGCTG			
Oligo37	tatccacgtatcgggtacgaatggaacgGGTGGAGGATCCGGGAGC	Ep14	Q5	1
Oligo38	gcgtggaaccacgtcacatttccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo39	tggagtgtctcccactaaattgaacgaccttGGTGGAGGATCCGGGAGC	Ep15	Q5	1
Oligo40	tagcatttgaaggttgagaacgatccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo41	tcccccgacggagccgaagaaggataagaaaaaaaaaGGTGGAGGATCCGGGAGC	Ep16	Q5	2
Oligo42	aaggttttatacgcatcaatgtgtttatttccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo43	acttccacagggaacgacactgccaaagggatttGGTGGAGGATCCGGGAGC			
Oligo44	tgcagtacagtggcagcattgttatttccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			

Oligo45	GGTGGAGGATCCGGGAGCTCCAGC	GA Vector	-	-
Oligo46	TTTATCATCGTCATCTTTATAATCAACCAATGCATAAGCCGAGGC			
Oligo47	CTGTATGTGGGCCCAAAAAGGGTGGAGGATCCGGGAGCTC	Ep17	GA	
Oligo48	GGCTGTACGCGTCCACTTCCTTTATCATCGTCATCTTTATAATCAACCAATGCATAAG			
Oligo49	ACGATGATAAAGGAAGTGGACGCGTACAGCCCACTGAAAG			
Oligo50	GAGCTCCCGGATCCTCCACCCTTTTTGGGCCCACATACAGTCG			
Oligo51	caaccgacaaatggtgtgggttaccagccgGGTGGAGGATCCGGGAGC	Ep18	Q5	1
Oligo52	gaaaccatatgactgcaagggaaaataacaTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo53	taataacttggattccaaagtaggaggcGGTGGAGGATCCGGGAGC	Ep19	Q5	1
Oligo54	gaattccaggcaataacacacccagtgaaTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo55	gaaatctaacttgaaaccgtttgaaaggGGTGGAGGATCCGGGAGC	Ep20	Q5	1
Oligo56	cggaaaagcctgtaaagatagttgtaattTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo57	cacgccttgcaatggggtagagggttttaatGGTGGAGGATCCGGGAGC	Ep21	Q5	1
Oligo58	gaccctgcctggtagatctccgttgagatgtcTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo59	caaccgacaaatggtgtgggttaccagccgGGTGGAGGATCCGGGAGC	Ep22	Q5	1
Oligo60	gaaaccatatgactgcaagggaaaataacaTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo61	acttccacagggaacgacactgccaaagggatttGGTGGAGGATCCGGGAGC	sEp9	Q5	1
Oligo62	tgcagtacagtggcagcattgttatttccacttccTTTATCATCGTCATCTTTATAATCAACCAATGC			
Oligo63	tccgggtacaaagttaccaaagaacttccacGGTGGAGGATCCGGGAGC	mEp9	Q5	1
Oligo64	gcaaattgagtaactatcgctgaatcattgttTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo65	tcccggaactattttaccccaaggatactatGGTGGAGGATCCGGGAGC	hEp9	Q5	1
Oligo66	gggaatctagtgggaatcgcctcctgagtagtTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo67	agtattgccttgccacctgagttatctGGTGGAGGATCCGGGAGC	nEp9	Q5	1
Oligo68	aaatttcggttcaagcggcttttgattTCCACTTCCTTTATCATCGTCATCTTTATAATC			
Oligo69	TTTTGCGCCGACATCATAACGGTTCT	pm1165a_P8	-	-
Oligo70	TATGGGGTTTTGCTAAACAACTTTCAACAG			

List of oligos used in Q5 site-directed mutagenesis (Q5) or Gibson Assembly (GA) for the cloning of putative epitopes Ep1-22 for phage display. For epitope constructs between 100-210 bp in length, multiple rounds of Q5 were denoted.

Table. S4. Antigens used in the COVAM experiment.

Virus	Subtype	Strain	Protein	Gen Bank	Expression	Construct	Source	Cat. #
CoV	Beta	SARS-CoV-2	N		Baculovirus	N-(AA)-His-C	Sino	40588-V08B
CoV	Beta	SARS-CoV-2	S1-RBD		HEK293	N-(AA)-mFc-C	Sino	40592-V05H
CoV	Beta	SARS-CoV-2	S1		HEK293	N-(AA)-His-C	Sino	40591-V08H
CoV	Beta	SARS-CoV-2	S1		HEK293	N-(AA)-mFc-C	Sino	40591-V02H
CoV	Beta	SARS-CoV-2	S1		HEK293	N-(AA)-Fc-C	Sino	40591-V05H1
CoV	Beta	SARS-CoV-2	S2		Baculovirus	N-(AA)-His-C	Sino	40590-V08B
CoV	Beta	SARS-CoV-2	S1+S2		Baculovirus	N-(AA)-His-C	Sino	40589-V08B1
CoV	Beta	SARS	PLpro	AAX16193.1	E. coli	N-(AA1541-1859)-His-C	Sino	40524-V08E
CoV	Beta	SARS	S1-RBD	AAX16192.1	Baculovirus	N-(AA306-527)-Fc-C	Sino	40150-V31B2
CoV	Beta	SARS	S1-RBD	AAX16192.1	Baculovirus	N-(AA306-527)-His-C	Sino	40150-V08B2
CoV	Beta	SARS	S1	AAX16192.1	Baculovirus	N-(AA1-667)-His-C	Sino	40150-V08B1
CoV	Beta	SARS	N	NP_828858.1	Baculovirus	N-(AA1-422)-His-C	Sino	40143-V08B
CoV	Beta	MERS	N	AFS88943.1	Baculovirus	N-(AA1-413)-His-C	Sino	40068-V08B
CoV	Beta	MERS	S1-RBD	AFS88936.1	Baculovirus	N-(AA383-502)-Fc-C	Sino	40071-V05B
CoV	Beta	MERS	S1-RBD	AFS88936.1	Baculovirus	N-(AA383-502)-rFc-C	Sino	40071-V31B
CoV	Beta	MERS	S1-RBD	AFS88936.1	Baculovirus	N-(AA367-606)-rFc-C	Sino	40071-V31B1
CoV	Beta	MERS	S1-RBD	AFS88936.1	Baculovirus	N-(AA367-606)-His-C	Sino	40071-V08B1
CoV	Beta	MERS	S1	AFS88936.1	HEK293	N-(AA1-725)-His-C	Sino	40069-V08H
CoV	Beta	MERS	S1	AFS88936.1	Baculovirus	N-(AA1-725)-His-C	Sino	40069-V08B1
CoV	Beta	MERS	S1+S2	AFS88936.1	Baculovirus	N-(AA1-1297)-His-C	Sino	40069-V08B
CoV	Beta	MERS	S2	AFS88936.1	Baculovirus	N-(AA726-1296)-His-C	Sino	40070-V08B
CoV	Alpha	NL63	S1	A0A1L2YVI8	HEK293	N-(AA19-717)-His-C	Sino	40600-V08H
CoV	Alpha	NL63	S1+S2	A0A1L2YVI8	Baculovirus	N-(AA19-1296)-His-C	Sino	40604-V08B
CoV	Alpha	229E	S1	A0A1L7B942	HEK293	N-(AA16-536)-His-C	Sino	40601-v08H
CoV	Alpha	229E	S1+S2	A0A1L7B942	Baculovirus	N-(AA16-1115)-His-C	Sino	40605-V08B
CoV	Beta	HKU-1	S1	YP_173238.1	HEK293	N-(AA1-760)-His-C	Sino	40021-V08H
CoV	Beta	HKU-1	S1	Q0ZME7	HEK293	N-(AA13-756)-His-C	Sino	40602-V08H
CoV	Beta	HKU-1	S1+S2	Q0ZME7	Baculovirus	N-(AA13-1295)-His-C	Sino	40606-V08B
CoV	Beta	HKU-1	HE	Q0ZME7	HEK293	N-(AA16-394)-His-C	Sino	Custom
CoV	Beta	HKU23-368F	N	AHN64796.1	HEK293	N-(AA1-448)-His-C	Sino	40458-V08B
CoV	Beta	OC43	S1	AVR40344.1	HEK293	N-(AA13-533)-His-C	Sino	Custom
CoV	Beta	OC43	S1+S2	AVR40344.1	Baculovirus	N-(AA13-1304)-His-C	Sino	40607-V08B