

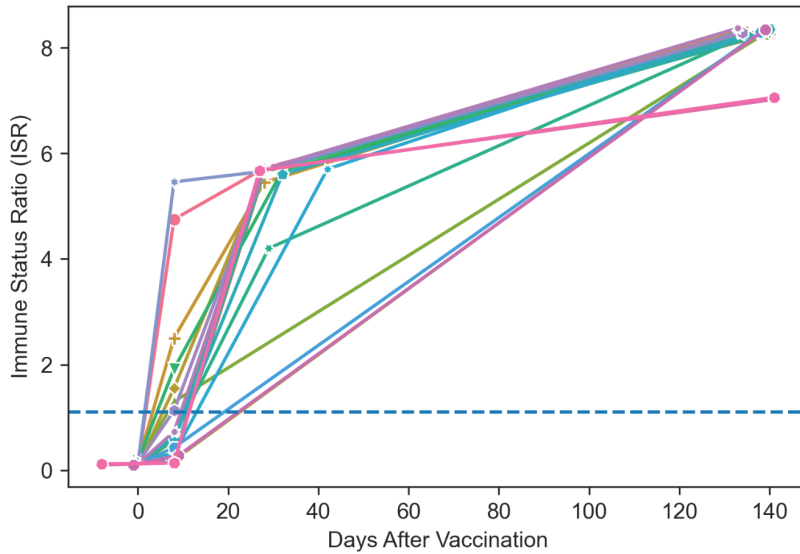
**Supplemental information**

**COVID-19 vaccination elicits an evolving,  
cross-reactive antibody response to epitopes  
conserved with endemic coronavirus spike proteins**

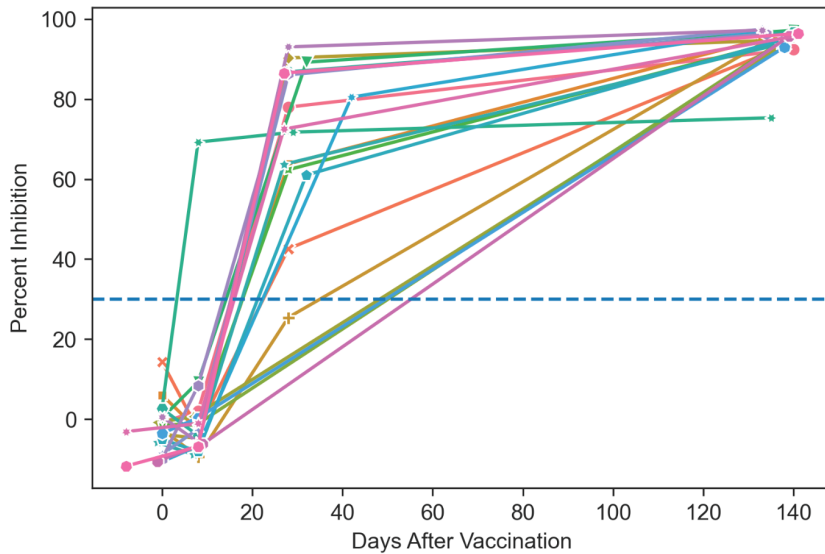
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**Supplemental Information**

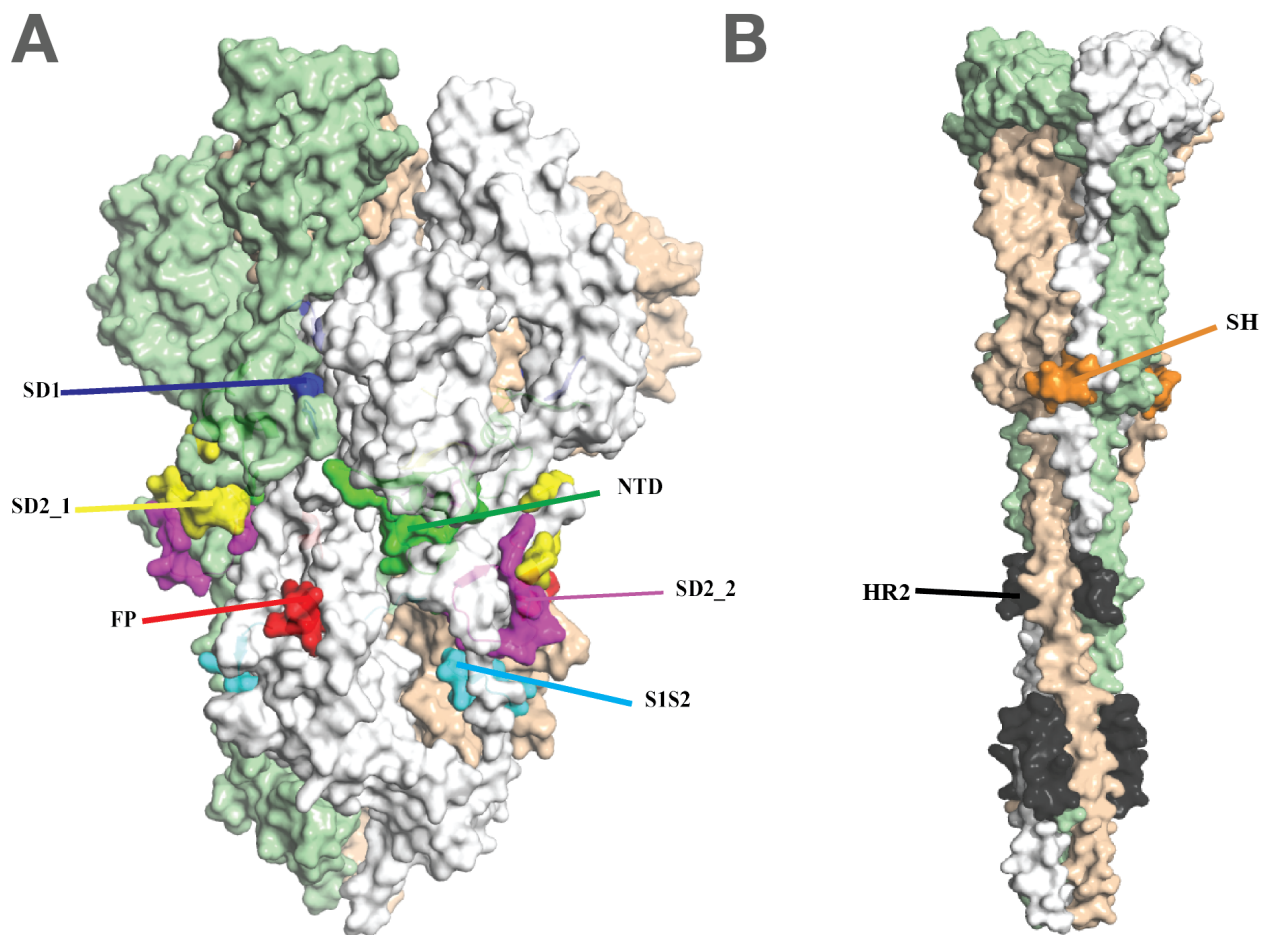
**A**



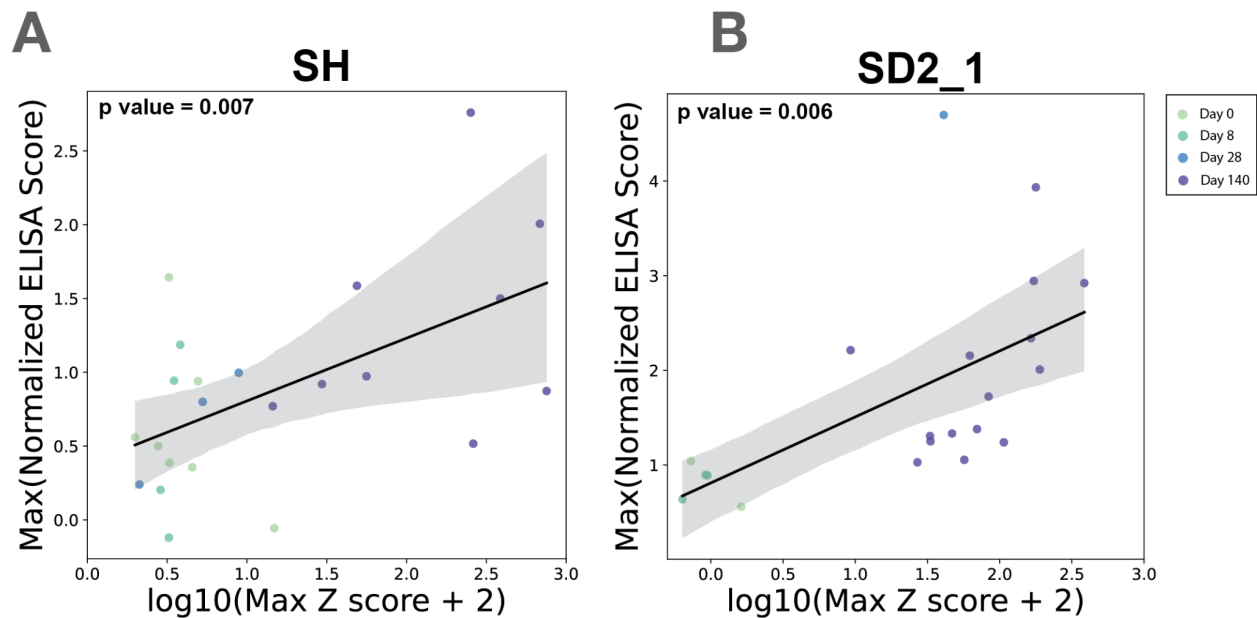
**B**



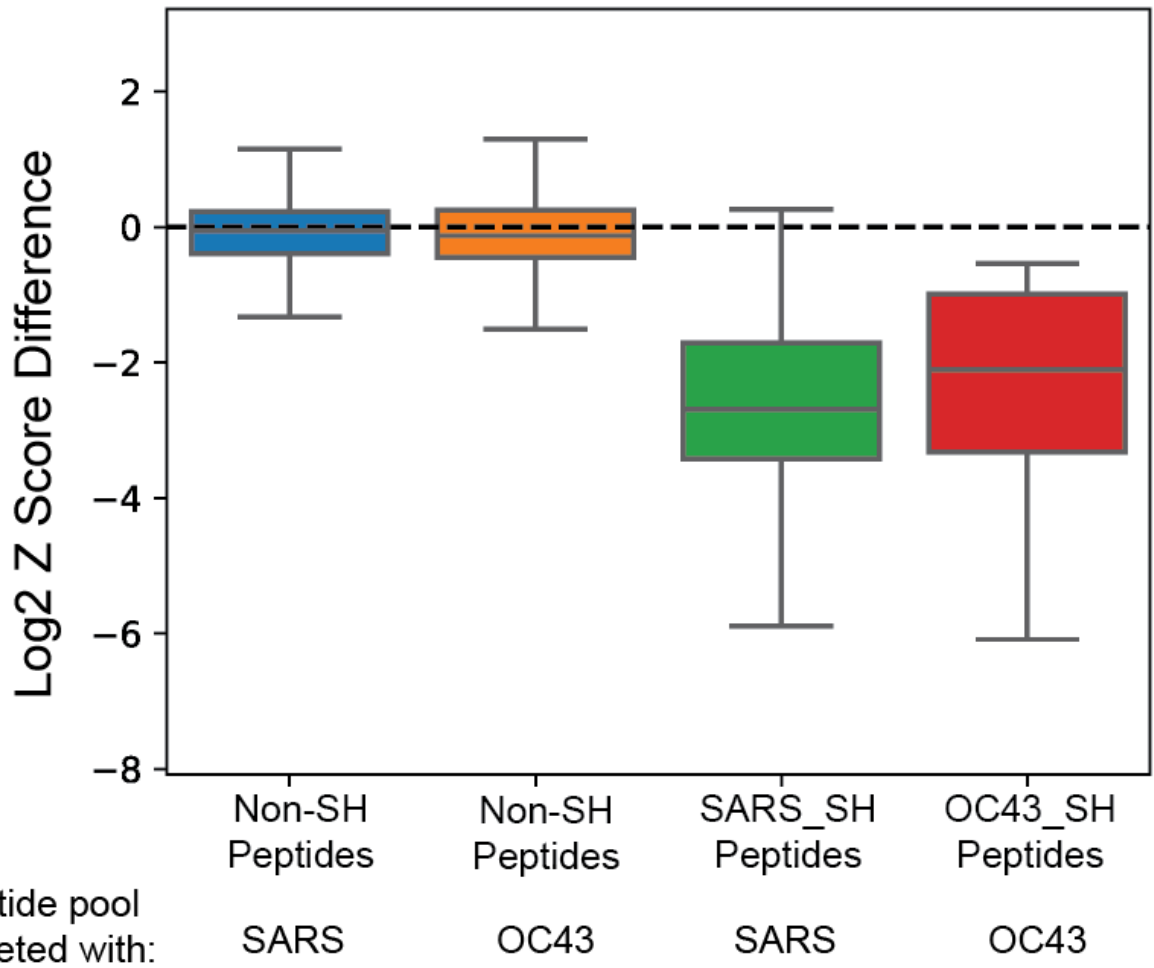
**Supplemental Figure 1: Vaccine-induced increases in SARS-CoV-2 Spike-binding and RBD-inhibiting antibodies over time across the cohort.** (Related to Figure 1). **A.** Magnitude of plasma IgG binding to SARS-CoV-2 Spike over time in vaccinated patients, measured by ELISA (InBios). Positivity threshold (ISR = 1.1) is indicated as dashed blue line. **B.** Magnitude of plasma inhibition of SARS-CoV-2 RBD binding to ACE2 over time in vaccinated patients, measured by competition ELISA (GenScript). Positivity threshold (30% inhibition) is indicated as dashed blue line.



**Supplemental Figure 2: 3D model of SARS-CoV-2 Spike protein in the pre- and post-fusion conformations highlighting epitope regions recognized by the vaccine response.** (Related to Figure 1.) A. Pre-fusion 3D model of SARS-CoV-2 Spike protein trimer (PDB:6VYB) with the epitopes identified in Figure 1 highlighted. Dark blue=SD1, yellow=SD2\_1, red=FP, green=NTD, purple=SD2\_2, aqua=SIS2. B. Post-fusion 3D model of SARS-CoV-2 Spike S2 trimer (PDB:6XRA) with the relevant epitopes identified in Figure 1 highlighted. Orange=SH, Black=HR2. HR2 appears as two segments as there is an unstructured region that is not well defined in the middle of the epitope. The FP epitope is in a disordered loop at the bottom of the structure, and therefore is not shown here. Epitopes were rendered on space-filling models created using The PyMOL Molecular Graphics System, Version 2.5.2 Schrödinger, LLC.



**Supplemental Figure 3: Correlated signal between PepSeq and peptide ELISA analysis of the same peptides.** (Related to Figure 1.) Subsets of samples from the vaccinated cohort were selected across a range of PepSeq Z scores (x-axis) for a cross-reactive epitope (A: SH, n=22) and a non-cross-reactive epitope (B: SD2\_1, n=20) and assayed by peptide ELISAs using the corresponding chemically-synthesized peptides (y-axis). Datapoints are colored by timepoint post-vaccine and show a significant correlation for each epitope (p values generated using Pearson correlation test are shown in upper-left corners).



**Supplemental Figure 4: Specificity of the PepSeq assay relative to chemically-synthesized peptides.** (Related to Figures 1 and 3.) 25 samples were assayed by PepSeq, with or without prior epitope-specific antibody depletion using beads bearing chemically-synthesized SH peptides of either the SARS or OC43 sequences. For each of the 4 columns/bars, the difference in PepSeq signal between the non-depleted and depleted samples (y-axis) is shown for all peptides in the indicated category that have non-depleted Z-scores  $\geq 10$ . Median PepSeq signal reductions of 2-3 log<sub>2</sub> units are observed for peptides matching the depleted sequences (2 right columns), whereas no such reductions are seen for the non-depleted peptides (2 left columns).

**Supplemental Table 1 Vaccinated Participant Biodata**

Sex	Age range	Period between doses (days)	Collection timepoints (days from first vaccine dose)			
			~Day 0	~Day 8	~Day 28	~Day 140
Female	30-39	28	0	8	28	140
Female	30-39	28	0	8	28	138
Male	30-39	28	0	8	28	133
Male	40-49	28	0	8	28	135
Female	30-39	28	0	8	28	138
Male	50-59	32	-1	7	28	138
Female	18-29	28	0	8	NC	139
Male	40-49	28	0	8	28	140
Male	18-29	32	0	8	32	140
Male	60+	29	0	8	29	135
Female	18-29	28	0	8	28	140
Female	18-29	27	-1	7	27	137
Male	18-29	32	0	8	32	138
Female	40-49	45	0	8	42	134
Male	18-29	28	0	8	NC	138
Female	30-39	28	0	8	28	133
Female	50-59	28	-2	8	28	134
Female	30-39	28	0	8	28	133
Female	60+	28	-1	10	25	NC
Female	60+	28	-8	8	27	141
Male	60+	28	-8	8	27	141

NC= Not Collected

All timepoints are expressed in days relative to the day on which the first vaccine dose occurred. Day 28 collection occurred before the second vaccine dose.

**Supplemental Table 2 Convalescent Participant Biodata**

Sex	Age range	Collection timepoint (days from positive diagnosis)
Male	50-59	38
Female	30-39	121
Female	40-49	85
Female	40-49	68
Female	30-39	69
Female	30-39	53
Female	50-59	94
Female	18-29	29
Female	40-49	127
Female	30-39	81
Female	40-49	78
Male	18-29	44
Female	60+	135
Female	50-59	118
Male	30-39	59
Female	18-29	50
Female	30-39	40
Female	40-49	66
Female	30-39	119
Female	18-29	70
Female	18-29	99
Male	40-49	67
Female	50-59	74
Female	18-29	79
Female	50-59	111
Male	30-39	49
Female	30-39	92
Male	60+	142
Male	40-49	69
Female	50-59	50
Male	30-39	65
Female	50-59	20

Female	30-39	34
Male	50-59	20
Female	60+	30
Female	60+	28
Female	18-29	24
Female	50-59	17
Female	30-39	28
Female	50-59	25
Female	50-59	25
Female	18-29	30
Female	30-39	24
Female	50-59	21
Male	30-39	31

Positive diagnosis determined by PCR. Collection timepoint is expressed in days relative to the day on which the patient tested PCR positive for COVID-19.

**Supplemental Table 3 PepSeq Coronavirus Peptides**

Epitope	Target Virus	Assay Peptides	Core	Core Coordinates in SARS-CoV-2 Spike
NTD	SARS-CoV-2	TITDAVDCALDPLSEKCTLKSF VEKGIY	TITDAVDCALDPLSEKCTLKSF TVEKGIY	284-313
SD1	SARS-CoV-2 SARS-CoV-2	GVLTESNKKFLPFQQFGRDIADT TDAVRDP FLPFQQFGRDIADTTDAVRDPQT LEILDIT	QQFGR	563-567
SD2_1	SARS-CoV-2	EVPVAIHADQLTPTWRVYSTGSN VFQTRAG	EVPVAIHADQLTPTWRVYSTGSN VFQTRAG	619-648
SD2_2	SARS-CoV-2 SARS-CoV-2 SARS-CoV-2	GSNVFQTRAGCLIGAEHVNNSY ECDIPIGA AGCLIGAEHVNNSYECDIPIGAGI CASYQT IGAEHVNNSYECDIPIGAGICASY QTQTNS	IGAEHVNNSYECDIPIGA	651-668
S1S2	SARS-CoV-2 SARS-CoV-2 SARS-CoV-2	NRALSGIAVEQDKNTQEVFAQV KQIYKTPA QDKNTQEVFAQVKQIYKTPPIKD FGGFNFS QLNRALTGIAVEQDKNTQEVFAQ VKQIYKT	VKQIYKT	785-791



	SARS-CoV-2 SARS-CoV-2	VKQIYKTPPIKDFGGFNFSQILPD PSKPSK NTQEVFAQVKQIYKTPPIKDFGG FNFSQIL		
FP	SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2	GSDCNTVSSRSAIEDLLFNKVR SDVGFVE NFSQILPDPSKPSKRSFIEDLLFNK VTLAD SKPSKRSFIEDLLFNKVTLADAG FIKQYGD IEDLLFNKVTLADAGFIKQYGDC LGDIAAR PDPSKPSKRSFIEDLLFNKVTLAD AGFIKQ FGGFNFSQILPDPSKPSKRSFIEDL LFNKV	EDLLFN	819-824
	OC43 229E 229E 229E HKU1 HKU1 HKU1 OC43 OC43	DINFSPVLGCLGSDCNKASTRSAI EDLLFD YNLSSVIPSLPRSGSRVAGRSAIE DILFSK RVAGRSAIEDILFSKVVTSGGLTV DADYKK PSLPTSGSRVAGRSAIEDILFSKLV TSGLG GPHCGSSSRSFEDLLFDKVKLS DVGVEA FKSLVGCLGPHCGSSSRSFEDLL FDKVKL FEDLLFDKVKLSDVGFVEAYNN CTGGSEIR IEDLLFDKVKLSDVGFVEAYNNC TGGAEIR CLGSECSKASSRSAIEDLLFDKV KLSVGF	EDLLFD,EDILFS	
SH	SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2 SARS-CoV-2	FKEELDKYFKNHTSPDVDLGD GINASV SFKEELDKYFKNHTSPDVDLGD GINASV TVYDPLQPELDSFKEELDKYFKN HTSPDVD GIVNNTVYDPLQPELESFKEELD KYFKNHT DVGIVNNTVYDPLQPELDSFK EELDKYF IVNNTVYDPLQPELDSFKEELDK YFKNHTS	EELDKY	1150-1155
	OC43 OC43 OC43 OC43 OC43	APDVMLNISTPKLPDFKEELDQW FKNQTSV VNYTKAPYVMLNTSIPNLPDFKE ELDQWFK LNISTPNLHDFKEELDQWFKNQ	EELDQW	

	OC43 OC43 OC43	LVAPDLS SIPNLPDFREELDQWFKNQTSVA PDLSEFDY ISTPNLPDFKEELDQWFKNHTSV APNLSLD AVNYTKAPYVMLNTSTPNLPDF REELDQWF PNLPDFKEELDQWFKNQTSVAPD LSLDYIN TKAPYVMLNTSIPNLPDFKEELD QWFKNQTS		
HR2	SARS-CoV-2	GDISGINASVVNIQKEIDRLNEVA KNLNES	GDISGINASVVNIQKEIDRLNEVA KNLNES	1167-1196