Cell Host & Microbe, Volume *30*

Supplemental information

Discovery of ultrapotent broadly neutralizing antibodies from SARS-CoV-2 elite neutralizers

Kanika Vanshylla, Chengcheng Fan, Marie Wunsch, Nareshkumar Poopalasingam, Matthijs Meijers, Christoph Kreer, Franziska Kleipass, Denis Ruchnewitz, Meryem S. Ercanoglu, Henning Gruell, Friederike Münn, Kai Pohl, Hanna Janicki, Tobias Nolden, Simone Bartl, Saskia C. Stein, Max Augustin, Felix Dewald, Lutz Gieselmann, Philipp Schommers, Thomas F. Schulz, Leif Erik Sander, Manuel Koch, Marta Łuksza, Michael Lässig, Pamela J. Bjorkman, and Florian Klein

Figure S1

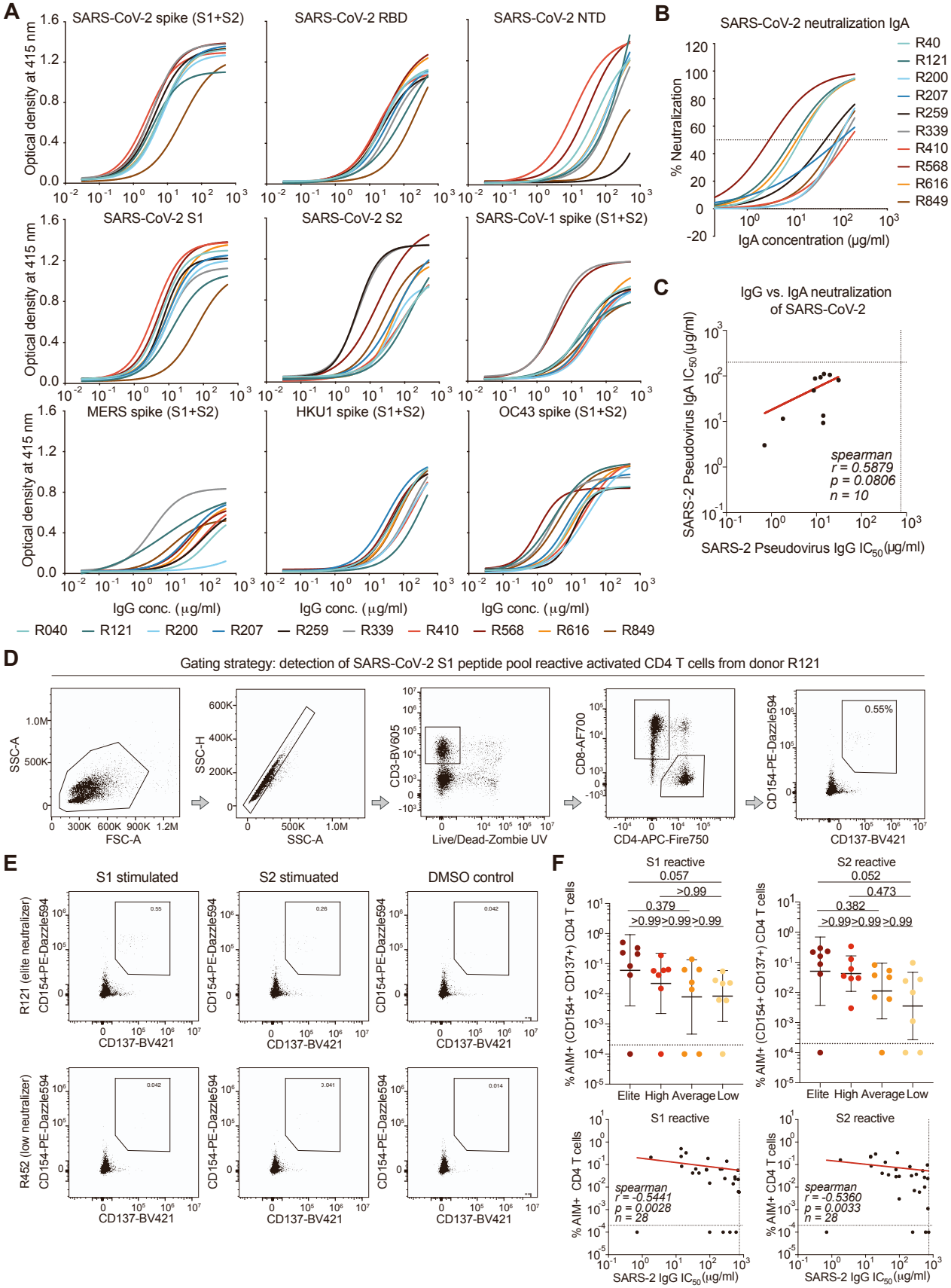

Figure S1: Coronavirus reactivity of elite neutralizers, related to Figure 1

A, ELISA-based binding curves depicting reactivity of elite neutralizer plasma IgG to SARS-CoV-2 spike trimer, RBD, NTD, S1, S2 as well as SARS-CoV-1 trimer, MERS trimer, HKU-1 spike and OC43 spike proteins. **B,** Neutralization curves depicting IgA neutralization from n=10 donor elite neutralizers against SARS-CoV-2 pseudovirus. Mean of two measurements plotted and dotted line represents 50% neutralization. **C,** Correlation plot between plasma purified IgG and IgA against SARS-CoV2 pseudovirus for 10 analyzed elite neutralizers. Dotted line represent limit of detection of assays; 750 μg/ml for IgG and 200 μg/ml for IgA. **D,** Gating strategy for detection of SARS-CoV-2 peptide pool reactive activated CD4 T cells as measured by presence of CD137+/CD154+ activation induced marker (AIM+) CD4 T cells. **E,** Exemplary plots of AIM+ expression on CD4+ T cells (of one elite- and one low-neutralizer after stimulation with S1 and S2 peptide pools as well as unstimulated control (DMSO). **F,** upper panel,T cell reactivity against SARS-CoV-2 S1 and S2 peptide pools in elite-, high-, average- and low-neutralizers (n=7 per group) as measured by activation induced marker (AIM+) CD137+/CD154+ CD4 T cells. Bars show geometric mean with 95%CI and statistical testing was done using the Kruskal-Wallis test. Values plotted below the dotted line did not show any detectable reactivity; lower panel, correlation plots between reactive AIM+ CD4 T cells and SARS-CoV-2 pseudovirus neutralization. Dotted lines denote limit of detection (IC_{50}) or values below dotted line did not show detectable reactivity (%AIM+ CD4 T cells).

Figure S2

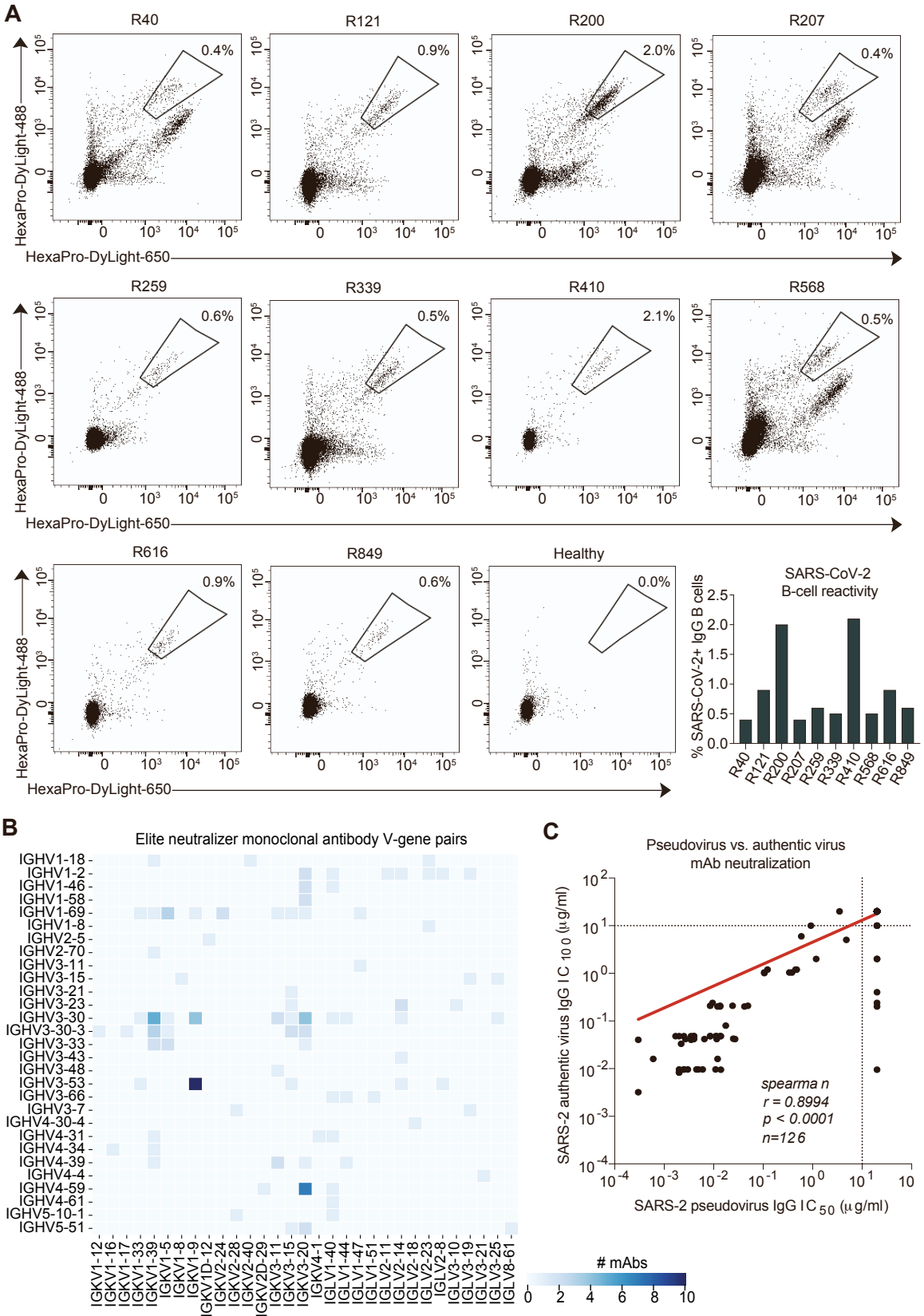

Figure S2: Fraction of SARS-CoV-2 reactive B cells from elite neutralizers and features of isolated mAbs, related to Figures 2 and 3

A, FACS plots and graph (lower right) showing the SARS-CoV-2 spike-reactive fraction amongst IgG+ B cells in elite neutralizers. **B,** Heat map illustrating the frequency of heavy and light V-gene combinations of the n=126 elite neutralizer derived mAbs produced and studied in detail. **C,** Correlation plot between SARS-CoV2 pseudovirus and authentic virus neutralization for n=126 tested mAbs. Dotted lines represent limit of detection of assays of 10 μg/ml IgG.

Figure S3

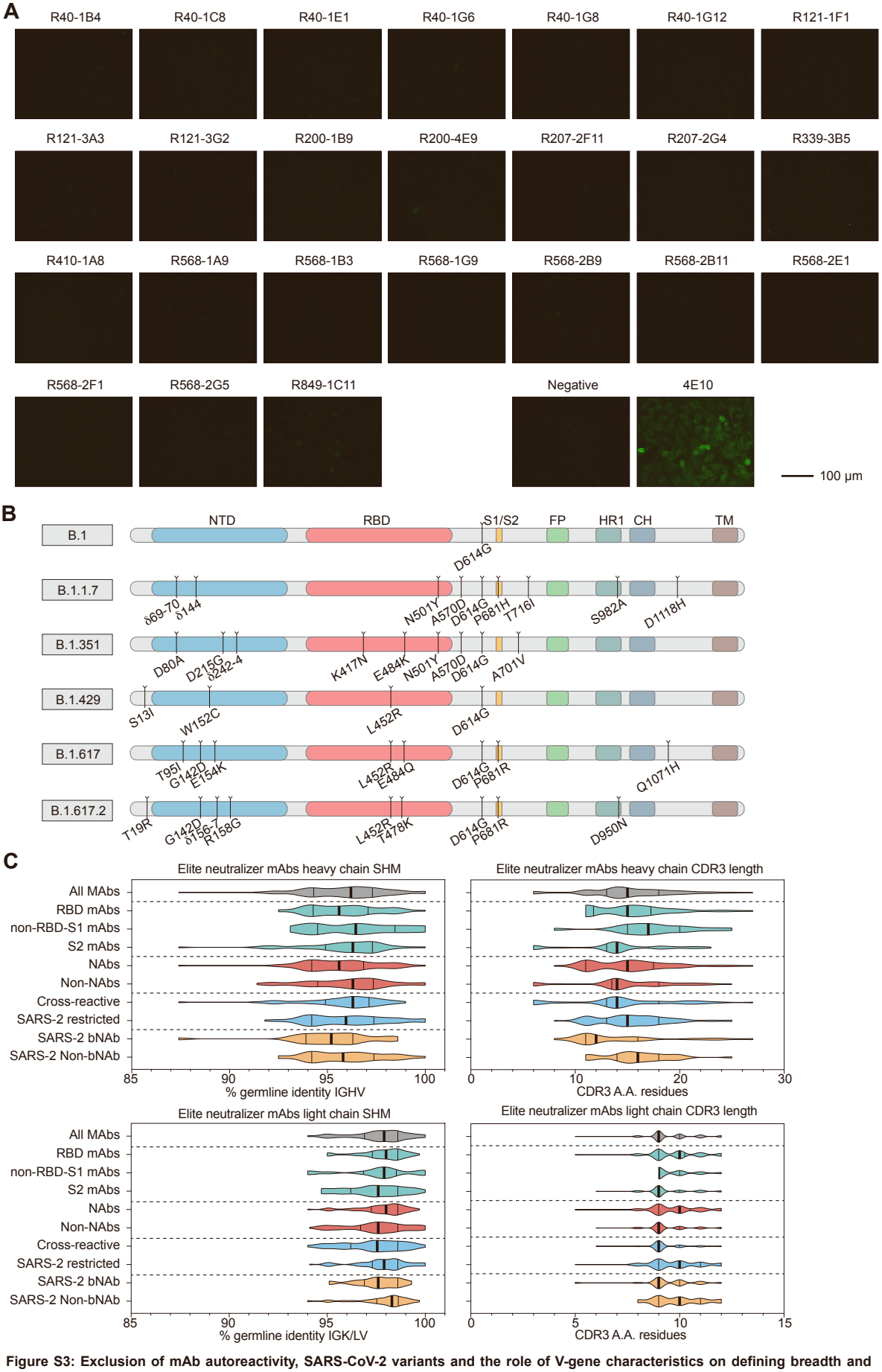

Figure S3: Exclusion of mAb autoreactivity, SARS-CoV-2 variants and the role of V-gene characteristics on defining breadth and potency of isolated NAbs, related to Figures 3 and 4

A, HEp-2 cell assay to screen for autoreactivity of selected mAbs tested at 100 µg/ml. **B,** Schematic of the SARS-COV-2 spike domains highlighting the residues mutated in the VOCs or VOIs used in the study. **C,** Analysis of the role of heavy chain (top panel) and light chain (lower panel) V-gene somatic hypermutation rate (left) and CDR3 length (left) in influencing spike binding patterns or SARS-CoV-2 pseudovirus neutralization potency of the mAbs (n=126).

Figure S4

* Emerging escape variants identified and functionally tested in this study. Note that D614G is designated B.1 based on pangolin lineage.

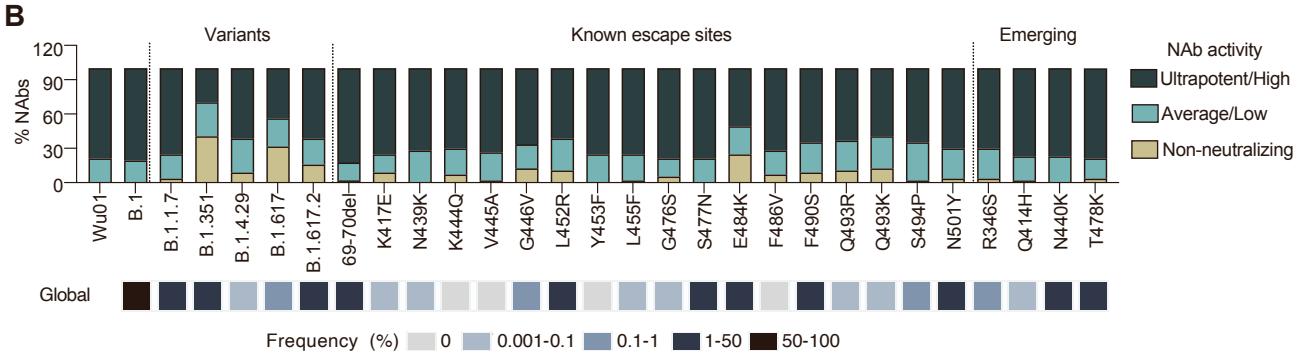

Figure S4: Frequency of known and emerging escape variants and potency of NAbs against them, related to Figure 5
A, Plots show the frequency distribution of the variants and escape sites from sequences downloaded on July 21st 2021. Frequencies are corrected for the collection date and total case counts in the region (see Methods). Countries with high vaccination rates (>60%) include UK and Israel. Countries and regions with low vaccination rates (<30%) include South America, Japan, India, Russia, Indonesia, Thailand, Iran Bangladesh, Vietnam, Africa. B, Plot depicting fraction of isolated NAbs which show ultrapotent/high neutralization (IC_{50} < 0.2 μg/ml, olive green), average/low neutralization (IC_{50} 0.2-10 μg/ml, light green) or complete escape (IC_{50}>10 μg/ml, golden) against the corresponding pseudovirus variant tested along with the respective global frequencies of respective variants (panel below).

Figure S5

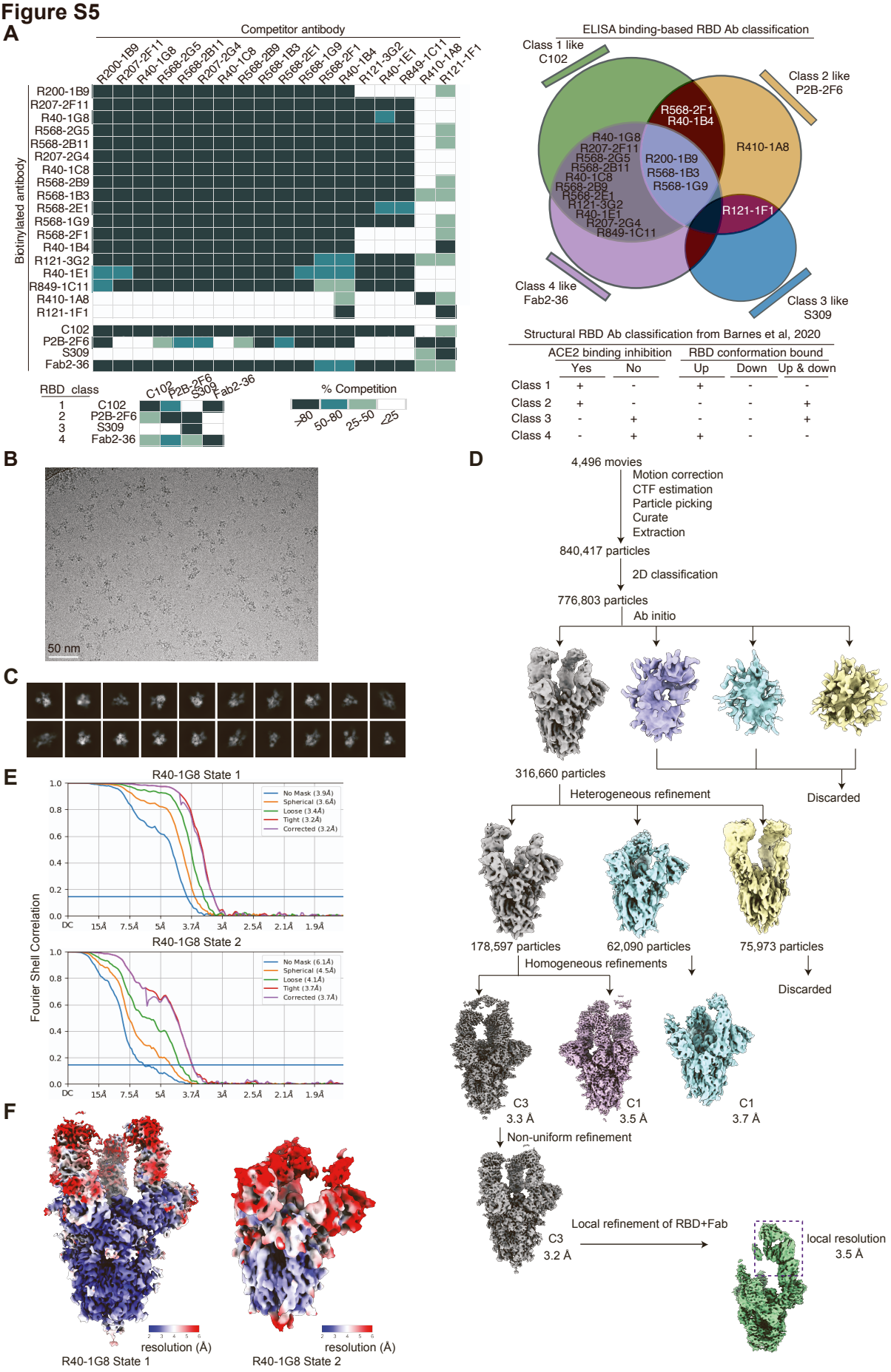

Figure S5: RBD class-mapping of bNAbs and CryoEM data processing and validation of R40-1G8 in complex with SARS-CoV-2 S protein, related to Figure 6.

A, Competition ELISA-based RBD epitope mapping of 18 RBD bNAbs with 100% breadth along with 1 mAb each from the 4 known RBD-binding epitope classes based on structural mapping. **B,** Representative micrograph (scale bar, 50 nm) **C,** 2D classes **D,** workflow of single-particle data processing **E,** Fourier shell correlation (FSC) plots and **F,** local resolution estimations for R40-1G8 in complex with SARS-CoV2-S. Two states of R40-1G8-SARS-CoV2-S complex were resolved, with one state having all 'up' RBDs, and the second state having 1 'up' RBD with R40-1G8 bound, 1 'down' RBD with R40-1G8 bound and 1 flexible 'up' RBD with no antibody binding.

Figure S6

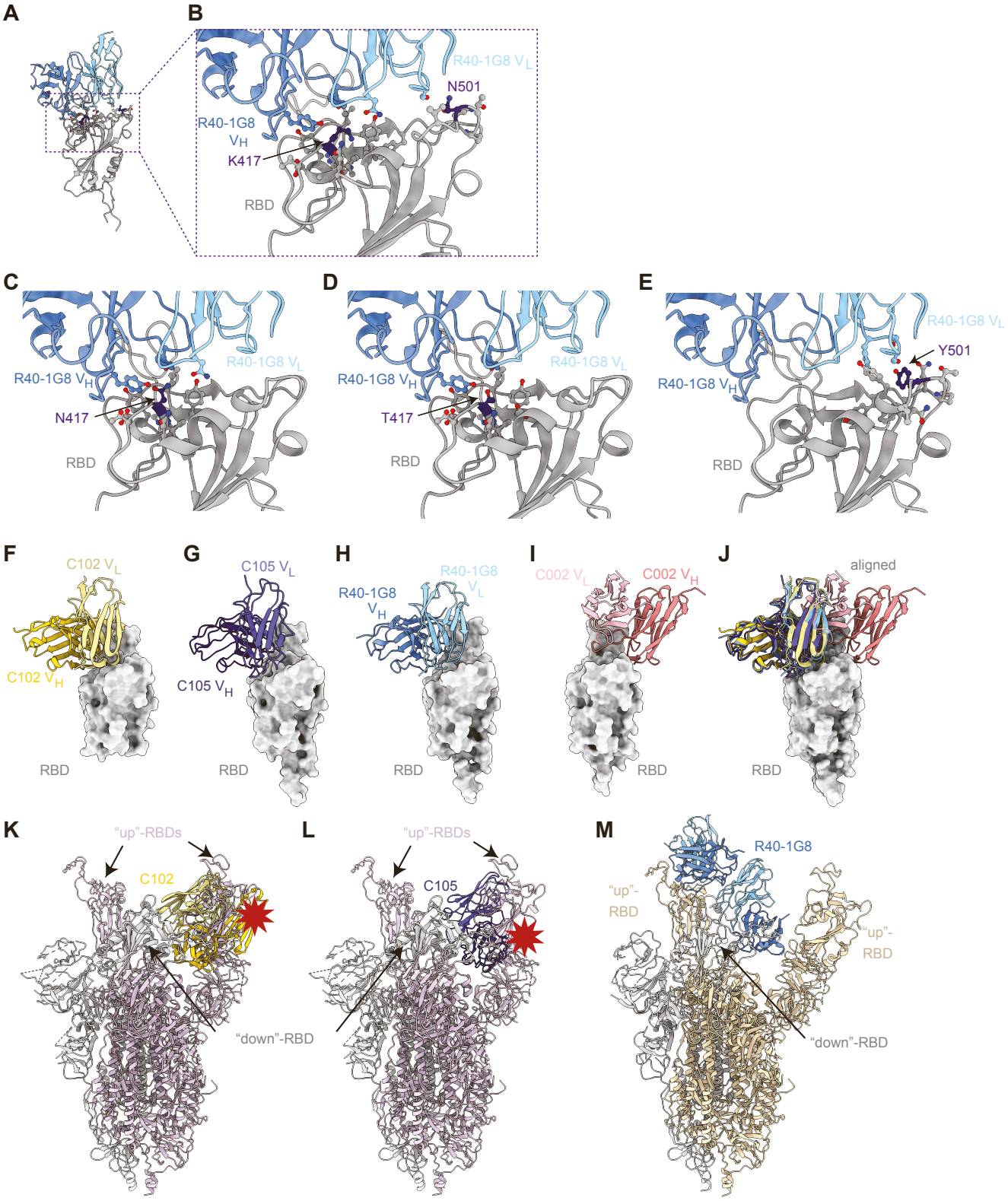

Figure S6: Predicted interactions of R40-1G8 Fab with two RBD residues that vary in SARS-CoV-2 VOC and structural comparisons of RBD class 1 antibodies, related to figure 6.
A, VH-VL domains of R40-1G8 Fab complexed with RBD. RBD positions K417 and 501 are highlighted in purple with sidechains in ball and stick representation. **B**, Close-up of R40-1G8 interactions with RBD residues near positions 417 and 501, the sites that are substituted in K417N/T and N501Y variants. Close-up views of R40-1G8 interactions with homology models of SARS-CoV-2 RBD including K417N (**C**), K417T (**D**), and N501Y (**E**) substitutions. Homology models were constructed using SWISS-Model, based on a high-resolution crystal structure of SARS-CoV-2 RBD (PDB 7EAM). Models of R40-1G8 Fab in complex with the RBD models were obtained by aligning the RBD portion of the R40-1G8 Fab-SARS-CoV-2 S 6P cryo-EM structure with the RBD homology models. Residues within 5 Å of RBD positions 417 and 501 are shown in ball and stick representation. **F-H**, Structures of the VH-VL domains of class 1 antibodies C102 (**F**) (PDB 7K8M), C105 (**G**) (PDB 6XCM), and R40-1G8 (**H**) (PDB 7SC1), demonstrating similar epitopes and binding poses. **I**, Structure of the VH-VL domains of the class 2 antibody C002 (PDB 7K8T) in complex with SARS-CoV-2 RBDs, demonstrating binding to a different RBD epitope. **J**, Overlay of C102, C105, R40-1G8 and C002 VH-VL domains bound to the SARS-CoV-2 RBD. **K-M**, Structural alignments on a 'down' RBD of an S trimer of the VH-VL domains for C102 (**K**) (PDB 7K8M) and C105 (**L**) (PDB 6XCM) showing that these Fabs would clash with a neighboring 'up' RBD, whereas the VH-VL domains of R40-1G8 (**M**) bound to a down RBD do not clash with neighboring 'up' RBDs.

Table S1: Demographics and the antibody response in study participants, related to Figure 1

A

Study ID	Age (years)	Gender	COVID-19 severity	Disease symptoms*	Pre-existing conditions**
R40	55	Male	Mild disease	1,2,3,5,6,7,8	None reported
R121	45	Male	Mild disease	1,2	None reported
R200	32	Female	Mild disease	1,4,7,8,9	None reported
R207	50	Female	Mild disease	1,2,3,4,5,6,7,8,9	None reported
R259	54	Female	Mild disease	2,8,9	None reported
R339	57	Male	Hospitalized	1,2,4,5,8,9	Heart attack
R410	55	Male	Mild disease	2,5,6,8,9	None reported
R568	60	Female	Hospitalized	1,3,5,7,8	Hypertension
R616	41	Male	Mild disease	1,5,6,8,9	Thrombocytopenia
R849	47	Female	Mild disease	1,2,3,4,6,8,9	Asthma

* 1 = Fever, 2= Cough, 3= Sore throat, 4= Rhinitis, 5= Muscle and body ache, 6= Headache, 7= Diarrhea, 8= Change in taste , 9 = Change in olfaction
** Conditions that are risk factors for COVID-19

B

Study ID	Weeks since disease onset	Neutralization IC_{50} (μg/ml)			ELISA Area under curve | EC50 in μg/ml															
		IgG SARS-2	IgG SARS-1	IgA SARS-2	SARS-2 Trimer		SARS-2 RBD		SARS-2 NTD		SARS-2 S1		SARS-2 S2		SARS-1 Trimer		MERS Trimer		HKU1 Trimer		OC43 Trimer	
R40	4.1	14.2	22.4	13.4	576	6.4	437	24.8	427	53.8	576	6.0	309	78.2	360	19.8	113	154.5	350	65.5	363	8.0
R121	4.6	14.1	47.9	9.3	478	3.7	344	125.4	403	103.1	425	13.3	311	151.9	291	16.3	255	8.4	193	403.9	463	2.9
R200	6.4	9.3	391.7	87.8	546	6.6	436	21.6	386	126.9	510	9.9	340	40.2	303	44.7	0	>500	260	147.5	384	29.2
R207	5.0	12.3	55.0	93.6	585	7.1	411	35.6	396	166.5	539	8.8	422	58.0	325	28.8	226	44.1	391	32.8	408	8.0
R259	5.4	8.7	61.8	48.0	585	4.3	405	23.4	38	>500	539	6.9	599	4.2	350	18.6	149	104.5	344	48.9	349	11.9
R339	6.1	14.7	40.4	110.5	615	3.6	382	43.1	331	204.9	474	8.8	595	4.5	517	3.5	357	3.6	280	161.1	417	2.7
R410	6.1	19.8	33.7	105.5	577	2.5	433	16.4	568	12.1	618	4.0	313	84.2	317	40.0	160	109.7	257	241.0	420	22.4
R568	8.6	0.7	12.1	3.0	618	3.6	487	29.1	533	32.0	615	5.8	587	17.1	511	4.0	202	44.4	353	41.9	359	1.1
R616	7.7	1.8	33.1	11.5	577	6.6	473	31.0	385	115.6	583	10.0	406	46.7	362	43.3	202	58.4	345	70.3	429	14.0
R849	9.6	31.0	5.1	80.9	450	27.0	263	235.5	178	156.3	326	64.4	455	26.0	279	25.1	184	13.3	339	69.2	454	4.4

C

Study ID	Age (years)	Gender	Disease severity	Neutralization group	IgG IC50 (mg/ml) SARS-2
R102	54	Male	Mild symptoms	High	99.3
R301	56	Fem	Mild symptoms	High	99.2
R501	45	Male	Mild symptoms	High	80.0
R561	44	Male	Mild symptoms	High	38.4
R702	43	Fem	Hospitalized	High	79.7
R759	52	Male	Mild symptoms	High	47.9
R851	53	Male	Mild symptoms	High	88.3
R10	54	Male	Asymptomatic	Average	206.0
R649	56	Fem	Mild symptoms	Average	318.8
R674	50	Fem	Mild symptoms	Average	248.1
R675	32	Male	Mild symptoms	Average	278.9
R679	47	Male	Mild symptoms	Average	229.2
R709	47	Male	Mild symptoms	Average	394.4
R803	29	Male	Mild symptoms	Average	493.0
R369	31	Fem	Mild symptoms	Low	703.9
R452	39	Male	Mild symptoms	Low	555.6
R456	36	Male	Mild symptoms	Low	696.8
R457	25	Fem	Mild symptoms	Low	679.4
R680	54	Fem	Mild symptoms	Low	740.0
R753	59	Fem	Mild symptoms	Low	562.8
R807	45	Male	Mild symptoms	Low	601.9

* T cell response compared to elite neutralizers R40, R121, R339, R410, R568, R616, R849

Table S2: Features of IGHV3-53 NAbs from SARS-CoV-2 elite neutralizers, related to Figure 4

Antibody	IGHV	CDRH3 a.a. length	CDRH3 Sequence	% Breadth	K417E escape	E484K escape	IGKV	Average** IC_{50} (µg/ml)
R207-2F11	IGHV3-53	11	ARDLVYRGMDV	100	No	No	IGKV1-33	0.0043
R40-1G8	IGHV3-53	11	ARDLYVFGMDV	100	No	No	IGKV1-9	0.0047
R568-2G5	IGHV3-53	11	ARDLYYYGMDV	100	No	No	IGKV1-9	0.0055
R568-2B11	IGHV3-53	11	TRDLVYYGMDV	100	No	No	IGKV1-9	0.0072
R207-2G4	IGHV3-53	11	ARDLVAYGMDV	100	No	No	IGKV1-9	0.0078
R40-1C8	IGHV3-53	11	VRDLVDYGMDV	100	No	No	IGKV1-9	0.0097
R568-2B9	IGHV3-53	11	ARDLVHYGMDV	100	No	No	IGKV1-9	0.0102
R568-1B3	IGHV3-53	11	ARDLVAYGMDV	100	No	No	IGKV1-9	0.0115
R568-2E1	IGHV3-53	11	ARDLIVYGMDV	100	No	No	IGKV1-9	0.0200
R207-2A6	IGHV3-53	11	ARDYGDYYFDY	96	Yes	No	IGKV3-15	0.0329
R207-2C2	IGHV3-53	12	ARGEGWDLPFDY	91	Yes	No	IGLV2-8	0.0096
R207-1C4	IGHV3-53	11	ARDRYVLGMDV	91	Partial*	No	IGKV1-9	1.1306
R568-1E8	IGHV3-53	11	ARDLDYYGMDV	83	Yes	No	IGKV1-9	0.1571
R616-1G4	IGHV3-53	15	ARDKRIPYYFYGMDV	70	No	Partial*	IGLV2-14	1.0849
C102	IGHV3-53	11	ARDYGDYYFDY	91	Yes	No	IGKV3-20	0.1146

* Partial escape when fold change in IC_{50} of greater than 10-fold observed
** Average IC_{50} based on neutralization profile against variants tested in Figure 4

Table S3: Cryo-EM data collection, refinement and validation statistics, related to Figure 6

	SARS-CoV-2 S 6P + R40-1G8 Fab
Data Collection and processing	
Microscope	Titan Krios at Caltech
Camera	Gatan K3
Magnification	x105,000
Voltage (keV)	300
Exposure (e/Å^2)	60
Pixel size (Å)	0.832
Defocus Range (μm)	- 1.0 to -3.0
Initial Particle Image (no.)	841,017
Final Particle Image (no.)	178,957
Symmetry Imposed	C3
Map Resolution (Å)	3.17
FSC Threshold	0.143
Map Resolution Range (Å)	3.1 - 3.4
Refinement	
Initial Model Used	PDB ID: 7K8T
Model Resolution (Å)	3.40
FSC Threshold	0.143
Model composition	
non-hydrogen atoms	29,415
protein residues	3,705
ligands	45
Average B-factors (Å^2)	
protein	142
ligands	135
R.m.s. deviations	
Bond length (Å)	0.006
Bond angles (°)	0.604
Validation	
MolProbity score	1.79
Clashscore	10.0
Rotamer outliers	0.06
Ramachandran plot	
Ramachandran favored (%)	96.02
Ramachandran allowed (%)	3.98
Ramachandran outliers (%)	0
PDB ID	7SC1