

Figure S1. Effect of E484K substitution on C144/C051/C052 lineage neutralizing potency, related to Figure 1.

Neutralization of rVSV/SARS-CoV-2 2E1 or a plaque purified E484K mutant thereof, in 293T/ACE2cl.22 cells. Infected (%GFP+) cells relative to no antibody controls, mean and range of two independent experiments is plotted.

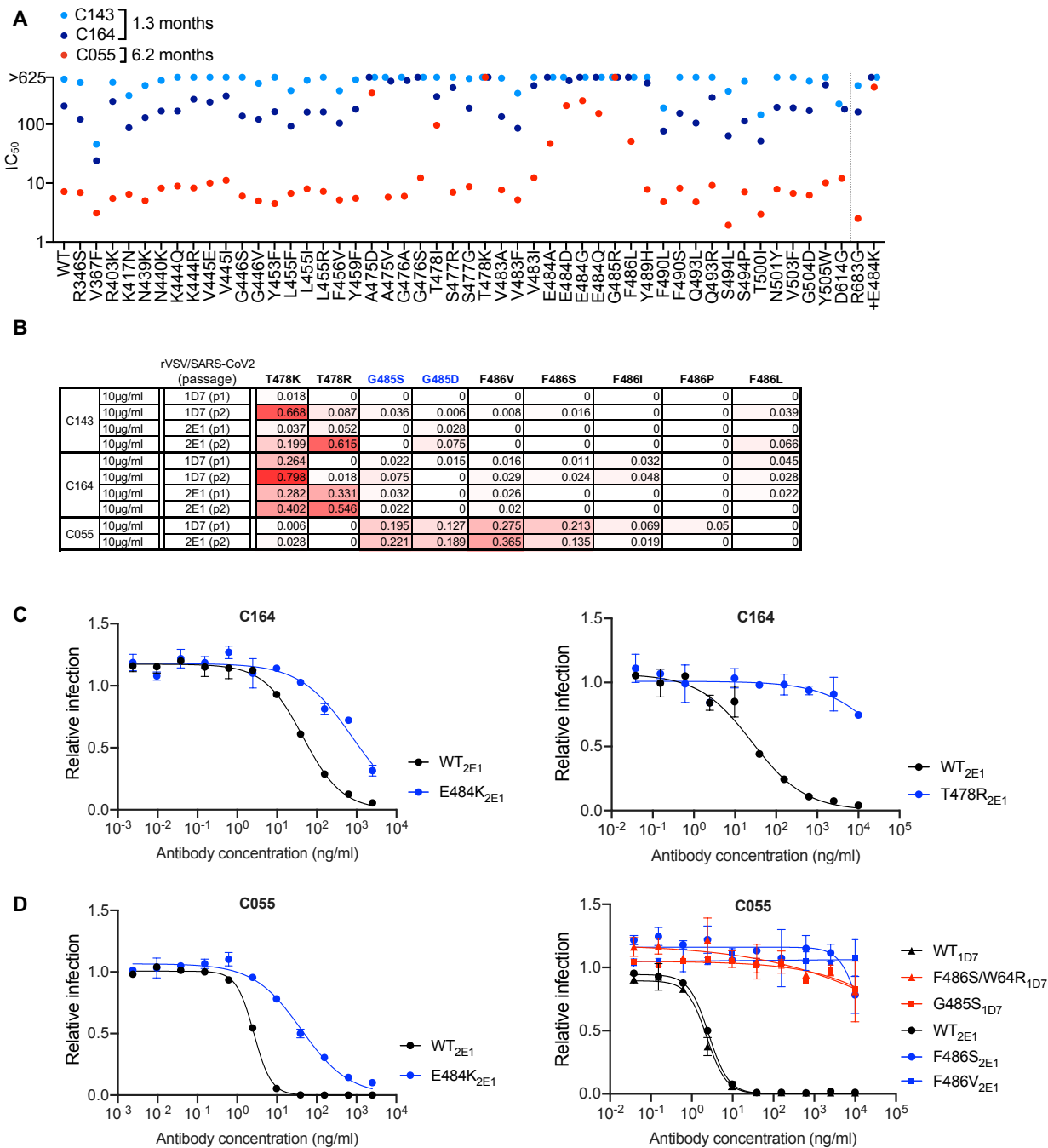


Figure S2. Effects of somatic mutation on potency and viral escape in the C143/C164/C055 class 2 antibody lineage, related to Figure 1.

(A) Neutralization potency (IC_{50}) of C143, C164 and C055 measured using HIV-1-based SARS-CoV-2 variant pseudotypes and HT1080/ACE2cl.14 cells. The E484K substitution was constructed in an R683G (furin cleavage site mutant) background to increase infectivity. Mean of two independent experiments.

(B) Decimal fraction (color gradient; white = 0, red = 1) of Illumina sequence reads encoding the indicated RBD substitutions following rVSV/SARS-CoV-2 replication (1D7 and 2E1 virus

isolates) in the presence of the indicated amounts of antibodies for the indicated number of passages.

(C) C164 neutralization of rVSV/SARS-CoV-2 1D7, 2E1 or plaque purified mutants thereof, isolated following antibody selection, in 293T/ACE2cl.22 cells. Infected (%GFP+) cells relative to no antibody controls, mean and range of two independent experiments is plotted.

(D) As in C for antibody C055.

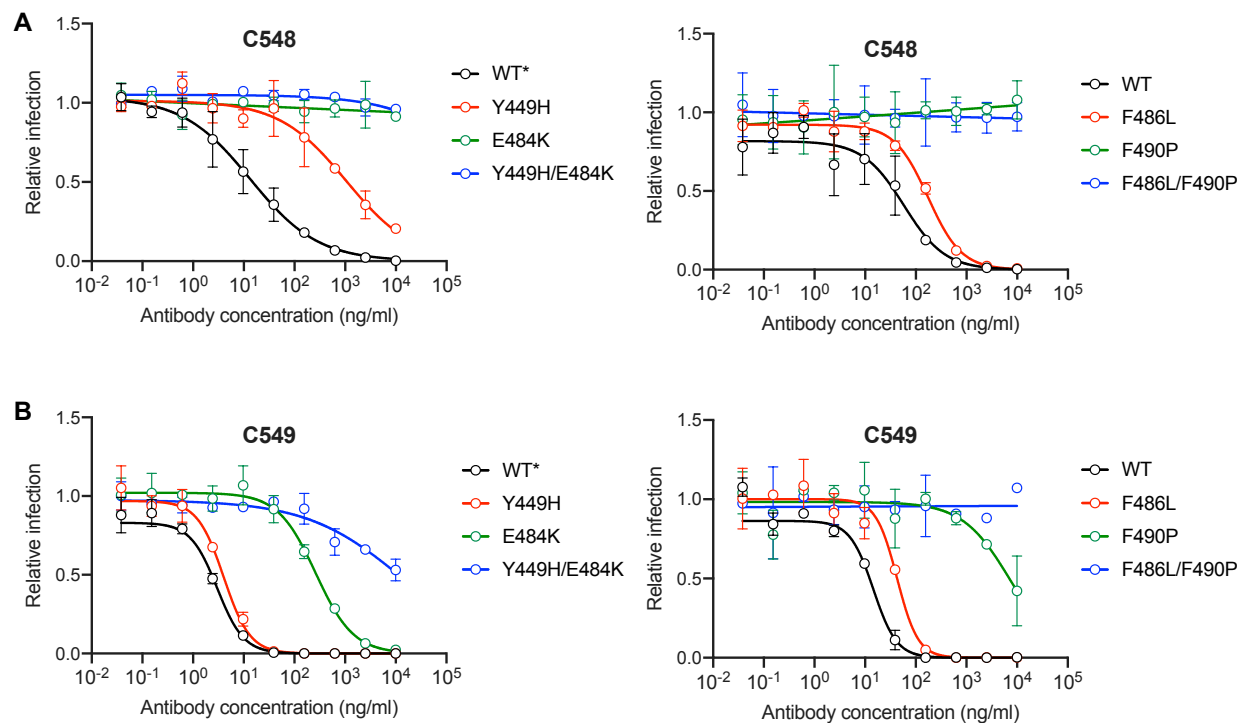


Figure S3. Effect of viral substitutions on neutralization by C548 and C549, related to Figure 1

(A) C548 neutralization of HIV-1-based SARS-CoV-2 pseudotypes harboring the indicated substitutions that were identified by selection experiments. Infection (NanoLuc luciferase activity) is normalized to that obtained in the absence of antibody, mean and range of two independent experiments is plotted.

(B) As in A for antibody C549.

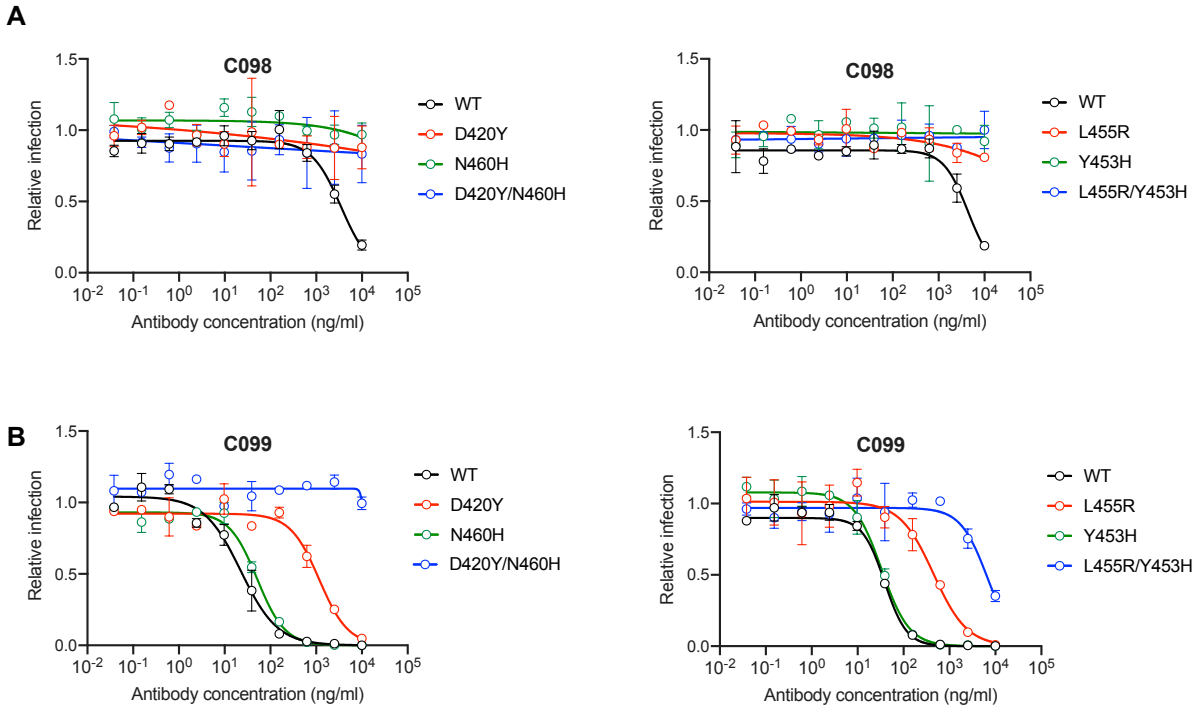


Figure S4. Effect of viral substitutions on neutralization by C098 and C099, related to Figure 2

(A) C098 neutralization of HIV-1-based SARS-CoV-2 pseudotypes harboring the indicated substitutions that were identified by selection experiments. Infection (NanoLuc luciferase activity) is normalized to that obtained in the absence of antibody, mean and range of two independent experiments is plotted.

(B) As in A for antibody C099.

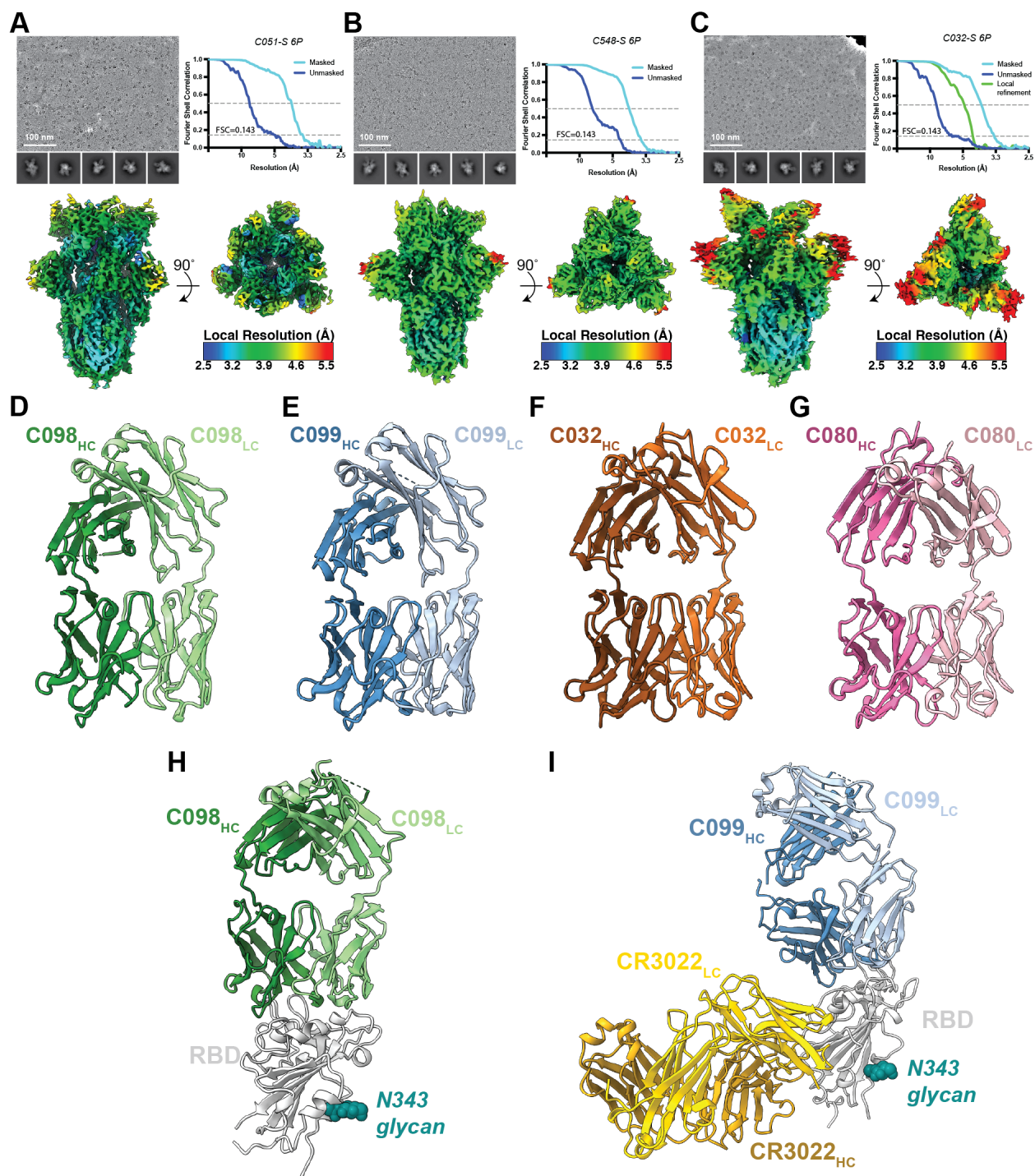


Figure S5 Cryo-EM data processing and X-ray structures, related to Figures 6,7
 (A-C) Representative micrograph, 2D class averages, FSC plots calculated using the gold-standard FSC criteria, and local resolution maps rendered in cryoSparr v2.15 for the cryo-EM structures of (A) C051-S, (B) C032-S, and (C) C548-S complexes.

(D-G) Cartoon representations of crystal structures of (D) C098, (E) C099, (F) C032, and (G) C080 Fabs.

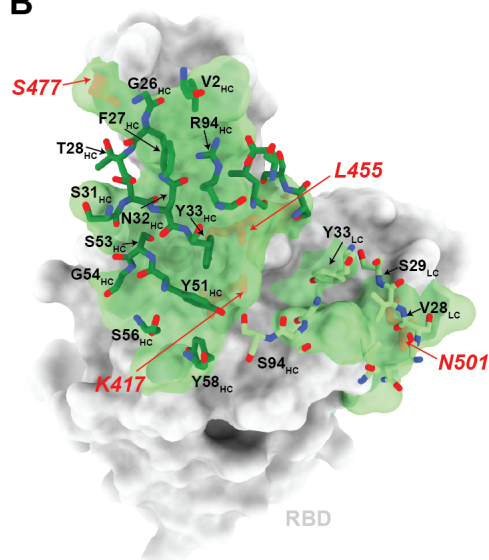
(H) Cartoon representation of C098 Fab – SARS-CoV-2 RBD crystal structure.

(I) Cartoon representation of C099-CR3022 – SARS-CoV-2 RBD crystal structure.

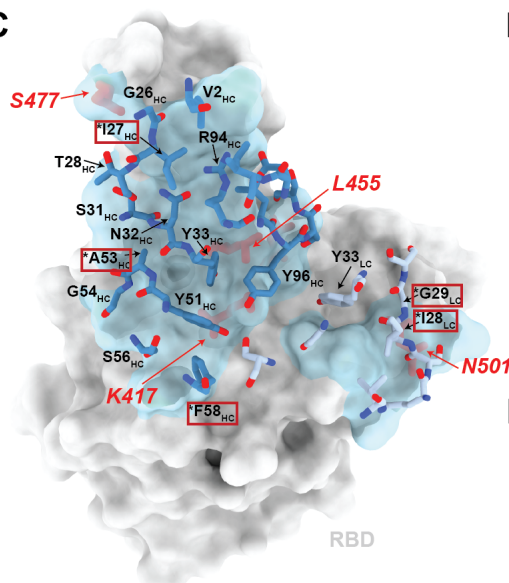
A

<u>C098/C099 Heavy Chain</u>		CDRH1	CDRH2	CDRH3
IGHV3-53	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKLEWVSVIYSGGSIYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAR			
C098
C099
<u>C098/C099 Light Chain</u> <th>CDRL1</th> <th>CDRL2</th> <th>CDRL3</th>		CDRL1	CDRL2	CDRL3
IGLV2-20	EIVLTQSPGTLSLSPGERATLSCRASQVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSS			
C098
C099

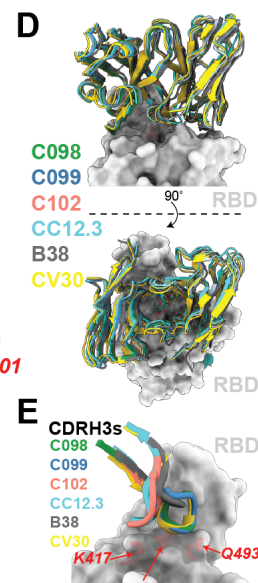
B



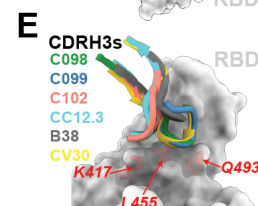
C



D



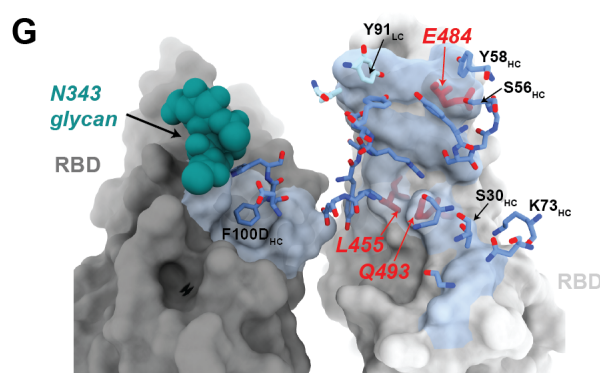
E



F

<u>C144/C051 Heavy Chain</u>		CDRH1	CDRH2	CDRH3
IGHV3-53	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMSWVRQAPGKLEWVSVIYSGGSIYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAR			
C144
C051
<u>C144/C051 Light Chain</u>		CDRL1	CDRL2	CDRL3
IGLV2-14	QSALTQPASVSGSPGQSIIFISCTGTSDDVGGYNYVSWYQQHPGKAPKLMIVDSNRPSGVNRFSGSKSGNTASLTISGLQAEDEADYICSSYTSST			
C144
C051

G



H

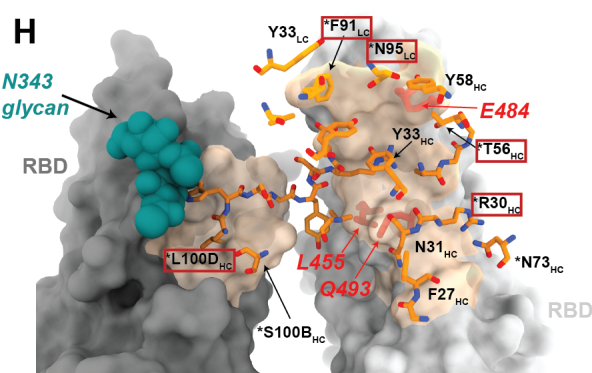


Figure S6. Class 1 and 2 antibody sequence alignments and interactions with RBD, related to Figure 6.

(A) Sequence alignment between heavy and light chains of C098 and C099 relative to inferred germline sequences. Paratope residues highlighted in red.

(B) C098 epitope (light green surface on RBD with paratope sidechains from C098 highlighted as sticks).

- (C) C099 epitope (light cyan surface on RBD with paratope sidechains from C099 highlighted as sticks). Somatic hypermutations found in C099 are highlighted with a red box.
- (D) Overlay of V_H-V_L domains of class 1 Fabs bound to RBD (C098, green – this study; C099, blue – this study; C102, salmon – PDB 7K8M; CC12.3, cyan – PDB 6XC4; B38, gray – PDB 7BZ5; CV30, yellow – PDB 6XE1).
- (E) Overlay of CDRH3 loops of class 1 Fabs described in panel D at the RBD interface.
- (F) Sequence alignment between heavy and light chains of C144 and C051 relative to inferred germline sequences. Paratope residues highlighted in red.
- (G) C144 epitope (light blue surface on RBD with paratope sidechains from C144 highlighted as sticks).
- (H) C051 epitope (light orange surface on RBD with paratope sidechains from C051 highlighted as sticks).

A

C548/C549 Heavy Chain

	CDRH1	CDRH2	CDRH3
IGHV1-69	QVQLVQSGAEVKKPGSSVKVCKASGGTFSYAIISWVRQAPGQGLEWMGGIIPFGTANYAQKFKGRVTITADESTSTAYMELSSLRSEDYVYCA		
C548RREA Y GERDY Y Y Y Y Y GMDVWGQGTIVTVSS
C549TS.....F...P.....I...T.....G...F...S.....P.....FF.....P.....

C548/C549 Light Chain

	CDRL1	CDRL2	CDRL3
IGLV9-49	QVLTLPQPPSASASLGASVLTICTLSSGYSNYKVDWYQQRPGKPRFVMRVTGGIVGSKGDGIPDRFVSLGSLNRYLTIKNIQEEDSDYHCGADHGSGSNFV		
C548	.SA.....Q...W...GVFGGGTKLTV
C549	.SA.....F.....D...Y.....I...P.....F.....S...N...G...E...GT	

C032/C080 Heavy Chain

	CDRH1	CDRH2	CDRH3
IGHV5-51	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKLEWGMIIYPGDSITRYSPSPFQGVITISADKSISTAYLQWSSLKASDTAMYCAR		
C032GVAVD W YFDLWGRGLTVTVSS
C080N...A.....I...A...L.....R.....S.V.....	

C032/C080 Light Chain

	CDRL1	CDRL2	CDRL3
IGLV1-40	QSVLTLPQPPSVSGAPGQRVITISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNRNRPSPGVPDRFSGSKSGTSASLAITGLQAEDEADYQCYSYSSLS		
C032A...LYVFGTGTQVTVL
C080A.....F...Y.....N.....SG.V...D.....

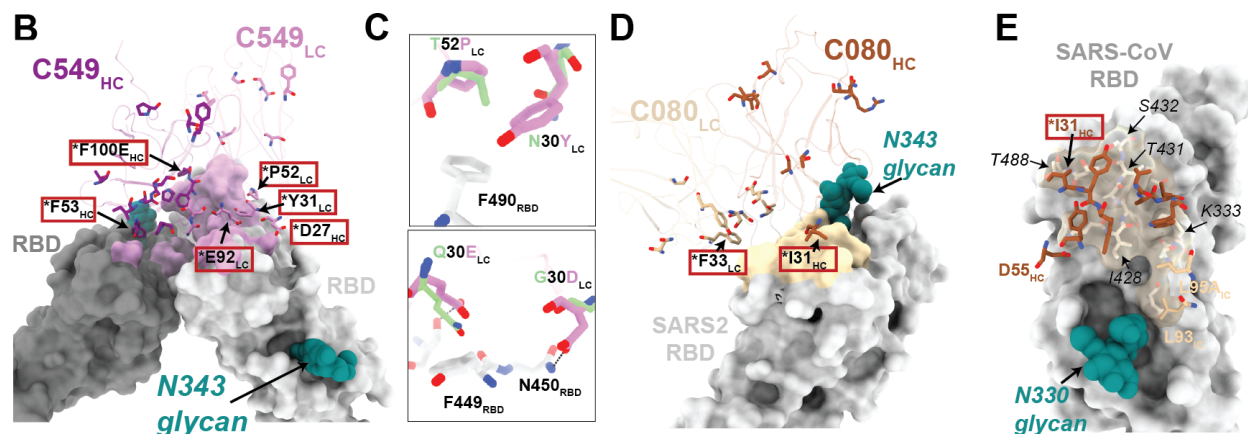


Figure S7. Class 2 and 3 antibody sequence alignments and homology models, related to Figure 7.

(A) Sequence alignment between heavy and light chains of C548/C549 and C032/C080 antibody pairs relative to inferred germline sequences. Paratope residues highlighted in red.

(B) Homology model of C549-RBD complex. Antibody somatic mutations are shown as sticks. Residues changed by somatic hypermutation at the predicted RBD interface are indicated by an asterisk and enclosed in a red box.

(C) Predicted interactions between RBD (light gray) and C549 homology model LC residues (violet). C548 residues (light green) are shown.

(D) Homology model of the C080-RBD complex. Antibody somatic mutations are shown as sticks. Residues changed by somatic hypermutation at the predicted RBD interface are indicated by an asterisk and enclosed in a red box.

(E) Homology model of the C080-SARS-CoV RBD complex. Predicted RBD epitope and Fab paratope are shown as colored surface and sticks, respectively. Residues changed by somatic hypermutation at the predicted RBD interface are indicated by an asterisk and enclosed in a red box. Sequence differences in SARS-RBD relative to SARS-CoV-2 RBD are indicated with italics.

Table S1 Clonally related antibody lineages in this study

mAb ID	Heavy				Light		
	IGHV	IGHD	IGHJ	CDR3	IGLV	IGLJ	CDRL3
Germline	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	AR-----YDFWSG-----YFDY	IGLV2-14*01	IGLJ1*01	SSYTSST-V
C144 (1.3 months)	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	..EGEVEG.N....YSRDR.....	IGLV2-14*01	IGLJ1*01R.
C051 (6.2 months)	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	..EGDVEG.H.S....YSRDR.....	IGLV2-14*01	IGLJ1*01NNN.R.
C052 (6.2 months)	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	..EGDVEG.....YSRDR.....	IGLV2-14*01	IGLJ1*01	N....NN.R.
C053 (6.2 months)	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	..EGDVEG.....YSRDR.....	IGLV2-14*01	IGLJ1*01AR.
C054 (6.2 months)	IGHV3-53*01	IGHD3-3*01	IGHJ4*02	..EGDVEGFS.L....YSRDR.....	IGLV2-14*01	IGLJ1*01	..F...N.R.
Germline	IGHV1-69*01	IGHD3-10*01	IGHJ6*02	----AYG---YYYYGMDV	IGLV9-49*01	IGLJ3*02	GADHGSGSNFV-V
C548 (1.3 months)	IGHV1-69*01	IGHD3-10*01	IGHJ6*02	ARRE...PRD.....	IGLV9-49*01	IGLJ3*02	...Q.....G.
C549 (6.2 months)	IGHV1-69*01	IGHD3-10*01	IGHJ6*02	ARREP..PRD...FF....	IGLV9-49*01	IGLJ3*02	...E...GT..G.
Germline	IGHV5-51*01	IGHD6-19*01	IGHJ2*01	AR--AVDWYFDL	IGLV1-40*01	IGLJ1*01	QSYDSSL--YV
C032 (1.3 months)	IGHV5-51*01	IGHD6-19*01	IGHJ2*01	..GV.....	IGLV1-40*01	IGLJ1*01AL..
C080 (6.2 months)	IGHV5-51*01	IGHD6-19*01	IGHJ2*01	..GV.....	IGLV1-40*01	IGLJ1*01	..SG.V..DL..
Germline	IGHV3-53*01	IGHD6-19*01	IGHJ3*02	AR--YSSG--DI	IGKV3-20*01	IGKJ1*01	QQYGSST-T
C098 (1.3 months)	IGHV3-53*01	IGHD6-19*01	IGHJ3*02	..DL....GT..	IGKV3-20*01	IGKJ1*01G.
C099 (6.2 months)	IGHV3-53*01	IGHD6-19*01	IGHJ3*02	..DL....GT..	IGKV3-20*01	IGKJ1*01G.
Germline	IGHV3-66*01	IGHD4-23*01	IGHJ3*02	AR-----VAFDI	IGLV2-23*03	IGLJ3*02	CSYAGSSTFV
C143 (1.3 months)	IGHV3-66*01	IGHD4-23*01	IGHJ3*02	..DSSEVRDHPGHPGRSVG....	IGLV2-23*03	IGLJ3*02A....
C164 (1.3 months)	IGHV3-66*01	IGHD4-23*01	IGHJ3*02	..DSSEVRDHPGHPGRSVG....	IGLV2-23*02	IGLJ3*02A....
C055 (6.2 months)	IGHV3-66*01	IGHD4-23*01	IGHJ3*02	..DSSEVRDHPGHPGRSVG....	IGLV2-23*01	IGLJ3*02H....
Germline	IGHV4-4*02	IGHD5-18*01	IGHJ4*02	AR--DTAM---YFDY	IGLV2-14*01	IGLJ3*02	SSYTSSTL-
C132 (1.3 months)	IGHV4-4*02	IGHD5-18*01	IGHJ4*02	..GG....GPE....	IGLV2-14*01	IGLJ3*02L
C512 (6.2 months)	IGHV4-4*02	IGHD5-18*01	IGHJ4*02	..KGG.R..GPE...S	IGLV2-14*01	IGLJ3*02	..FA.....L

Table S2. X-ray data collection and refinement statistics.

PDB ID	C098 Fab	C099 Fab	C032 Fab	C080 Fab	C098 Fab	C099 Fab
	SARS-CoV-2 RBD	CR3022 Fab SARS-CoV-2 RBD				
Data collection^{a,b}						
Space group	C2	P4 ₁ 2 ₁ 2	C2	P2 ₁ 2 ₁ 2 ₁	P6 ₃ 22	P2 ₁ 2 ₁ 2 ₁
Cell Dimensions						
<i>a, b, c</i> (Å)	191.3, 87.8, 56.8	110, 110, 228.6	133.9, 61.7, 69.2	58.6, 67.4, 130.6	88.1, 88.1, 216.2	44.1, 92.6, 115.2
α, β, γ (°)	90, 99.8, 90	90, 90, 90	90, 97.3, 90	90, 90, 90	90, 90, 120	90, 90, 90
Resolution (Å)	40.7-2.0 (2.1-2.0)	39.6-2.6 (2.7-2.6)	36.0-2.1 (2.13-2.06)	36.6-1.9 (2.0-1.9)	43.2-1.4 (1.5-1.4)	43.0-1.3 (1.31-1.26)
R _{merge} (%)	9.7 (79.4)	25.2 (405)	7.7 (95.0)	16.73 (175.9)	7.9 (185.9)	9.6 (152.4)
R _{pim} (%)	6.5 (53.6)	7.4 (121.9)	5.2 (66.0)	8.1 (85.9)	2.0 (46.2)	4.3 (70.4)
CC _{1/2} (%)	99.5 (57.7)	99.5 (35.1)	99.2 (52.1)	99.2 (39.9)	99.9 (67.2)	99.7 (48.5)
<I/σI>	6.8 (1.3)	8.9 (1.1)	5.9 (1.1)	7.9 (2.0)	17.4 (1.8)	8.3 (1.0)
Completeness (%)	95.7 (97.0)	100 (100)	94.3 (95.5)	98.8 (97.4)	99.4 (99.1)	90.5 (97.7)
Redundancy	3.0 (3.0)	12.9 (12.6)	2.9 (2.8)	5.2 (5.2)	16.5 (16.8)	5.6 (5.5)
Wilson <i>B</i> -factor	30.4	58.6	40.9	24.2	18.9	13.2
Refinement and Validation						
Resolution (Å)	40.7-2.0 (2.1-2.0)	39.6-2.6 (2.66-2.6)	36.0-2.1 (2.13-2.06)	36.6-1.9 (2.0-1.9)	43.2-1.4 (1.5-1.4)	43.0-1.3 (1.31-1.26)
Unique Reflections	57,268 (5,762)	43,864 (2,600)	32,875 (3,295)	41,224 (4,044)	92,932 (9,042)	115,943 (12,409)
Number of atoms						
Protein	4750	8,109	3244	3266	3352	3266
Ligand	14	14	-	-	-	-
Waters	480	94	132	371	336	625
R _{work} /R _{free} (%)	17.9/20.8	18.9/23.7	20.0/22.7	18.6/22.3	18.6/20.5	18.0/20.1
R.m.s. deviations						
Bond lengths (Å)	0.009	0.009	0.008	0.012	0.01	0.011
Bond angles (°)	1.1	1.1	1.1	1.1	1.2	1.2
Poor rotamers (%)	0	0.82	0	0.55	1.0	1.10
Ramachandran plot						
Favored (%)	97.4	95.9	96.5	97.9	98.6	98.1
Allowed (%)	2.1	3.8	3.5	2.1	1.4	1.7
Disallowed (%)	0.5	0.3	0	0	0	0.2
Average <i>B</i> -factor (Å)	40.6	59.7	55.4	31.2	31.0	19.9

^aFor each structure reported, data were derived from a single crystal.

^bNumbers in parentheses correspond to the highest resolution shell

Table S3. cryo-EM data collection and refinement statistics.

	C032	C051	C548
	SARS-CoV-2 S6P	SARS-CoV-2 S 6P	SARS-CoV-2 S 6P
PDB			
EMD			
Data collection conditions			
Microscope	Talos Arctica	Talos Arctica	Talos Arctica
Camera	Gatan K3 Summit	Gatan K3 Summit	Gatan K3 Summit
Magnification	45,000x	45,000x	45,000x
Voltage (kV)	200	200	200
Recording mode	counting	counting	counting
Dose rate (e ⁻ /pixel/s)	13.5	13.5	13.5
Electron dose (e ⁻ /Å ²)	60	60	60
Defocus range (μm)	0.7 – 2.0	0.7 – 2.0	0.7 – 2.0
Pixel size (Å)	0.8689	0.8689	0.8689
Micrographs collected	3,480	3,402	1959
Micrographs used	2,683	2,927	1687
Total extracted particles	844,544	390,630	554,852
Refined particles	192,286	134,506	94,255
Symmetry imposed	C1	C3	C3
Nominal Map Resolution (Å)			
FSC 0.143 (unmasked/masked)	5.4/3.4	4.9/3.5	4.5/3.5
FSC 0.143 local (unmasked/masked)	6.7/4.4	5.8/4.1	N/A
Refinement and Validation			
Initial model used	6XKL	7K90	7K43
Number of atoms			
Protein	25,866	28,401	28136
Ligand	56	462	574
MapCC (global/local)	0.70/0.69	0.80/0.77	0.77/0.76
Map sharpening B-factor	69.3	64.5	110.1
R.m.s. deviations			
Bond lengths (Å)	0.004	0.008	0.003
Bond angles (°)	0.64	1.1	0.52
MolProbity score	2.18	2.29	2.48
Clashscore (all atom)	16.3	16.4	18.26
Poor rotamers (%)	0	0.2	0.38
Ramachandran plot			
Favored (%)	92.4	93.8	97
Allowed (%)	7.5	6.9	2.7
Disallowed (%)	0	0.3	0.3