

Fig. S1. Identification of coldspot antibodies. (A) Cartoon diagram of the SARS-CoV-2 S with highlighted coldspot regions at the fusion peptide (FP, red) and near heptad repeat 2 (HR2, blue), as well as subdomain 1 (SD1, green). The thick horizontal lines underneath indicate the location of all coldspots, and the histograms show the frequency of aa changes throughout S at different time points: 31.12.2020 (671,006), 31.12.2021 (7,526,116) and 29.04.2022 (10,480,461 sequences from GISAID). (B) ELISA reactivity of plasma IgG antibodies to FP (top) and HR2 (middle) peptides or to SARS-CoV-2 RBD (bottom). SARS-CoV-2 ($n=67$) and common cold CoV ($n=6$) convalescent samples are compared to COVID-19 vaccinees (mRNA: BNT162b2 $n=11$, purple; mRNA-1273 $n=5$, pink;

inactivated virus: Sinovac n=2, dark green; Sinopharm n=24, light green; adenovirus: ChAdOx1-S n=19, orange; Ad26.COV2.S n=4, yellow), and to non-infected controls (n=25). Area Under the Curve (AUC); average of two experiments. The percentage of samples with antibody levels 4 standard deviations above controls is shown on top. (C) Gating strategy for sorting peptide-specific or SD1-enriched B cells by flow cytometry. (D) Identification of FP-peptide specific (top) and HR2-peptide specific (bottom) B cells by flow cytometry. Numbers indicate percentage of gated double-positive cells. (E) Clonal analysis of antibody sequences derived from peptide-specific B cells identified in (D). Pie charts show the total number of antibody sequences (center); the size of the slices is proportional to the number of clonally related sequences. White slices indicate antibody sequences that are not part of a clone.

B	Species	GenBank	FP peptide	HR2 peptide
ALPHA	SARS-CoV-2	Q869394.1	P S K R S F I E D L L F N K V T L A D A	E L D S F K E E L D K Y F K N H - - - - - T S P D
	Bat coronavirus CDPHE15	AGT1333.1	Y S T K S V E D I I L F D K V I T S G L	D F I D V N K T L E I I L S Q L O N - - - N T G P K
	Rousettus bat coronavirus HKU10	AU02104.1	- G - K S V E D I I L F D K V I T S G L	D Y I D V N K T L E D L L Q Q L P N - - - H T L P D
	BfR-AlphaCoV/HuB2013	AA62232.1	- S R G S V I E D L L F N K V I T S G L	E Y I D V N K T L E E L N S Y P N - - - Y T V P D
	CoV-229E	AK32181.1	A G R S S A I E D L L F S K I V I T S G L	E Y I D V N K T L E E L S Y K L P N - - - Y T V P D
	Lucheng Rn rat coronavirus	APU17645.1	G S R S T I E D L L F N K V I T T G L	A N P S F E E F A E L N K V N T - - - L E L E Q
	Mink coronavirus strain WD1127	AD18513.1	G S R S T I E D L L F N K V I T T G L	D Y I D N K T V Q D L L S Y K - P N W T V E E
	Miniploerius bat coronavirus HKU5	ACN32164.1	- G R S A I E D L L F S K I V I T S G L	D Y I D N K T L E F A A I I P N - - - R T E M Q
	Miniploerius bat coronavirus 1	ACN32171.1	- G R S A I E D L L F S K I V I T S G L	D Y I D N K T L E F A A I I P N - - - R T E M Q
	Phocaena diopatra coronavirus HKU1	AK42985.1	- G R S A I E D L L F S K I V I T S G L	D Y I D N K T L E F A A I I P N - - - R T E M Q
	BfV-AlphaCoV/SC2013	AK42985.1	- G R S A I E D L L F S K I V I T S G L	D Y I D N K T L E F A A I I P N - - - R T E M Q
	AlphaCoV/Bat-CoV/P.kuhlilfay/3398-102015	AZ518624.1	N K G S F I E D L L F D K V I T T G L	D F I D V N K T L Q D L D A L P K P N Y T L P N
	PEDEV	AK93656.1	Y Q K R S V I E D L L F N K V I T N G L	D Y I D V N K T L E I L A L P N - - - R T G P S
	Scotophilus bat coronavirus 512	ABG47078.1	P G A R S V I E D I I F D K V I T T G L	D Y I D V N K T L E I L A L P N - - - R T G P S
	Rhinolophus bat coronavirus HKU2 / SADS	ABG57208.1	F E S R S V I E D L L F S K I E I T T G L	S T F P I D E E F R E Y O N M S - - - S E L Q A
	CoV-NL63	AA538177.1	I A G R S A I E D L L F S K V I T S G L	D Y I D V N K T L E F A G N L P K - - - Y V P N
	NL63-related bat coronavirus	APD51475.1	S F R G S I E D L L F N K V I T S G L	D Y I D V N K T L E E A L S K L P N - - - Y T P D
	Common shrew coronavirus Tbet-2014	ATP96784.1	R G T R S I E D L L F D K I V I T T G P	E Y I D V N K T L E E A L S K L P N - - - Y T P D
	Wengcheng Sn shrew coronavirus	ASF90444.1	K S G R S V I E D L L F D K I V I T T G P	O L P N I G D D F A N L N V E N T S - - - K A L E E
	CoV-HuPn-2018	QVL91811.1	R K Y R S A I E D L L F D K V I T S G L	I E F P D G D F R G Q I K N V T I - - - L E L Q S
TGEV	CA91145.1	R K Y R S A I E D L L F D K V I T S G L	D Y I D I N Q T V Q D I L E N Y R - P N W T V P E	
CoV/Elmo02	AP27148.1	P G R R S A I E D L L F N K V I T S G L	D Y I D I N K T I S D M L E Q V S - P N T I P D	
FovV-KU2	AB47193.1	I G R S S A I E D L L F N K V I T S G L	D Y I D I N K T I A D M L E Q V N - P N T I P E	
BETA	CoV-OC43	AAT94352.1	A S R S R A I E D L L F D K V I S D V	N L P D F K E E L Q W F K N Q T - - - S V A P D
	Beta coronavirus HKU24	AJA91196.1	- - H R S A I E D I I F N K V I S D V	N L P D F K E E L Q W F K N Q S - - - S V A P D
	CoV-HKU1	AAT9580.1	- S R S L I E D L L F N K V I S D V	K L S D F E S L S H W F K N Q T - - - S I A P N
	Murine hepatitis virus	AAL03556.1	I R G R S A I E D L L F D K V I S D V	N P P D F K E E L Q W F K N Q T - - - S I A P N
	Rodent coronavirus RfMurf-CoV/2JL2014	ATP96744.1	- S G R S V I E D L L F N K V I A D V	T L P D F K E E L Q W F K N Q T - - - S I A P D
	Bat Hp-beta coronavirus/Zhejiang2013	AL94216.1	F G A R S A I E D L F D K I E T A D V	E L E F K O E L E E I R K N F S - - - T G L P N
	Beta coronavirus Ernaeus/VMC/DL02/012	AGX27196.1	N N Y R S A I E D L L F D K V I T S D P	S D V T F E D L E E F Y K N I T I - - - S E I P N
	MERS-CoV	AFS38836.1	R A R S A I E D L L F D K V I T A D P	T G I D Q D E L E F F K N V S - - - T S I P N
	Phiphiellus bat coronavirus HKU5	ABN10875.1	R A R S A I E D L L F D K V I T A D P	T G I D Q D E L E F F K N V S - - - T S I P N
	Tylopentes bat coronavirus HKU5	ABN10875.1	R A R S A I E D L L F D K V I T A D P	T G I D Q D E L E F F K N V S - - - T S I P N
Eidolon bat coronavirus/KY2008	ADX58466.1	N S Y R S A I S D L L F N K V S A D P	T L D V K E L E F F K N V S - - - S G S P N	
Rousettus bat coronavirus HKU9	ADCS9822.1	K Y R S A I S D L L Y N K V Y A D P	P N F I D E F F E K W Y K N Q S - - - S G I N I	
SARS-CoV	AAU10911.1	T T Y R S A F S D L L Y D K V R I T D P	S S F M E T E F F E K W Y K N Q S - - - T I F N N	
GAMMA	CoV-229E	AAP13441.1	P T K R S F I E D L L F N K V I T A D A	E L D S F K E E L D K Y F K N H - - - - - T S P D
	Goose coronavirus CB17	OCB5097.1	P G S R S F I E D L L F D K V E S V G L	S D F D F D A E F D K W N E T G - - - H D - - -
	Beluga whale coronavirus SW1	ABW67620.1	R O A R S T I E D I I F D K V I T V G L	P Y D F D K E F D K L E K W N - - - Y T - - -
	Avian coronavirus IBV-9203	ABW61427.1	N G R R S T I E D L L F D K V I T L G V	A L P M D D E F K D I F I E L N - - - T S K D A
	Avian coronavirus IBV-9203	AK093265.1	E R S R S F I E D L L F S W E S V G L	D P D F D E F D E S K W N A T K - - - H E - - -
	Duck coronavirus 2714	AK671724.1	P S G R S F I E D L L F D K V I V G V	E L P S F D D E F D N I Y T I E L N - - - T S K D L
	Wigeon coronavirus HKU20	AFD29234.1	K G G R S A I E D L L F T K V I T A G L	D F V D T N K T I E D I I S S I P N - - - N T A P S
	Bulbul coronavirus HKU11-934	SCGRSA	S G G R S A I E D L L F N K V I T N G L	D Y V D V N Q T V S D I I N S I P N - - - H Q T P T
	Common moorhen coronavirus HKU1	AFD29244.1	P R T F A I E D L L F N K V I T N G L	D Y V D V N T V S I I N S I P N - - - V T Y P D
	Porcine coronavirus HKU15	AFD29187.1	L G G R S A I E D L L F N K V I T S G L	D V I D V N Q T V S D I I D N L P N - - - A T P P Q
Munia coronavirus HKU13-3514	ACJ12062.1	I G E K S V I E D L L F N K V I T N G L	D Y V D V N Q T V S D I I G S L P N - - - P T P P Q	
White-eye coronavirus HKU16	AFD29201.1	G K D R S A I E D L L F D K V I T N G L	D Y V D V N K T V S D I I N G L P N - - - Y P P E	
Night heron coronavirus HKU19	AFD29226.1	P O K R S A I E D L L F S K V I T N G L	D Y V D I N S T I V E G I I N S L P L P - - - G K I P Q	
PDCoV/KRU14 / Hu_PDCoV	AP53198.1	L G R R S A I E D L L F N K V I T S G L	D Y I I D V N Q T V S D I I D N L P T I - - - A T P P Q	

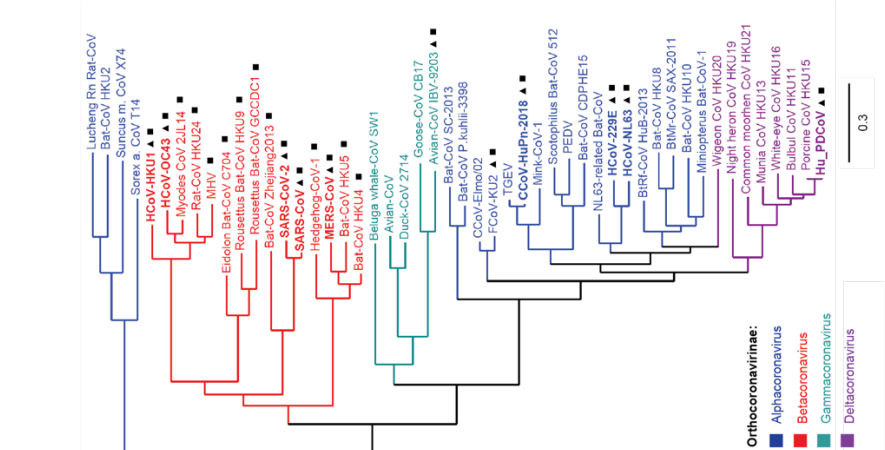


Fig. S2. Similarity of peptides at the FP and HR2 of Orthocoronavirinae. (A) Phylogenetic analysis of CoV species based on the aa sequence of S. (B) Alignment of SARS-CoV-2 FP and HR2 peptide sequences with those corresponding to related CoVs. In green identical residues; in yellow residues that are similar by side chain functionality.

Petides in this study: ▲ FP ■ HR2

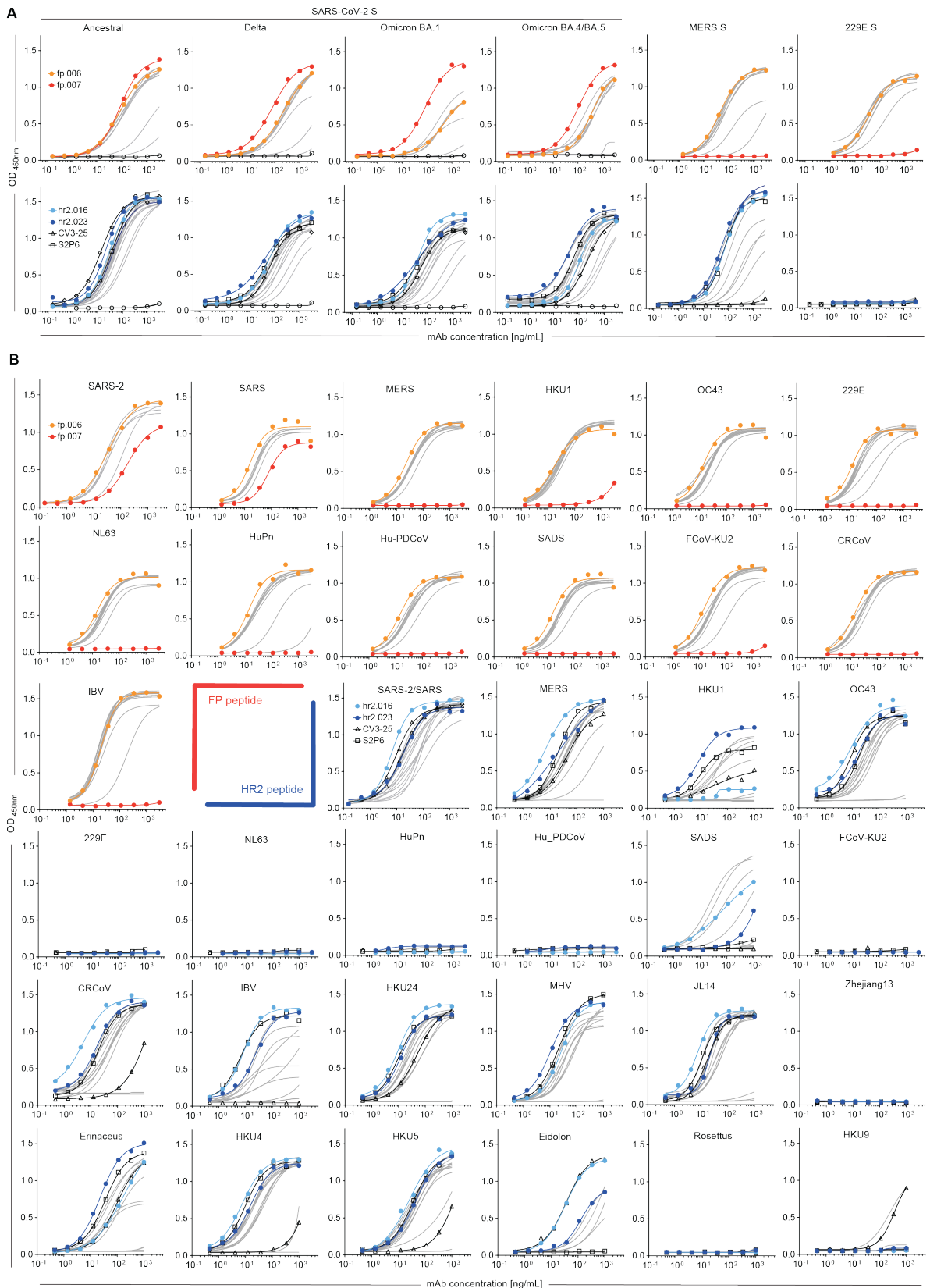


Fig. S3. Broadly crossreactive binding of FP and HR2 monoclonal antibodies. (A and B) Graphs show ELISAs measuring monoclonal antibodies reactivity to S (A) and to coldspot peptides (B) of coronaviruses. Mean of two independent experiments.

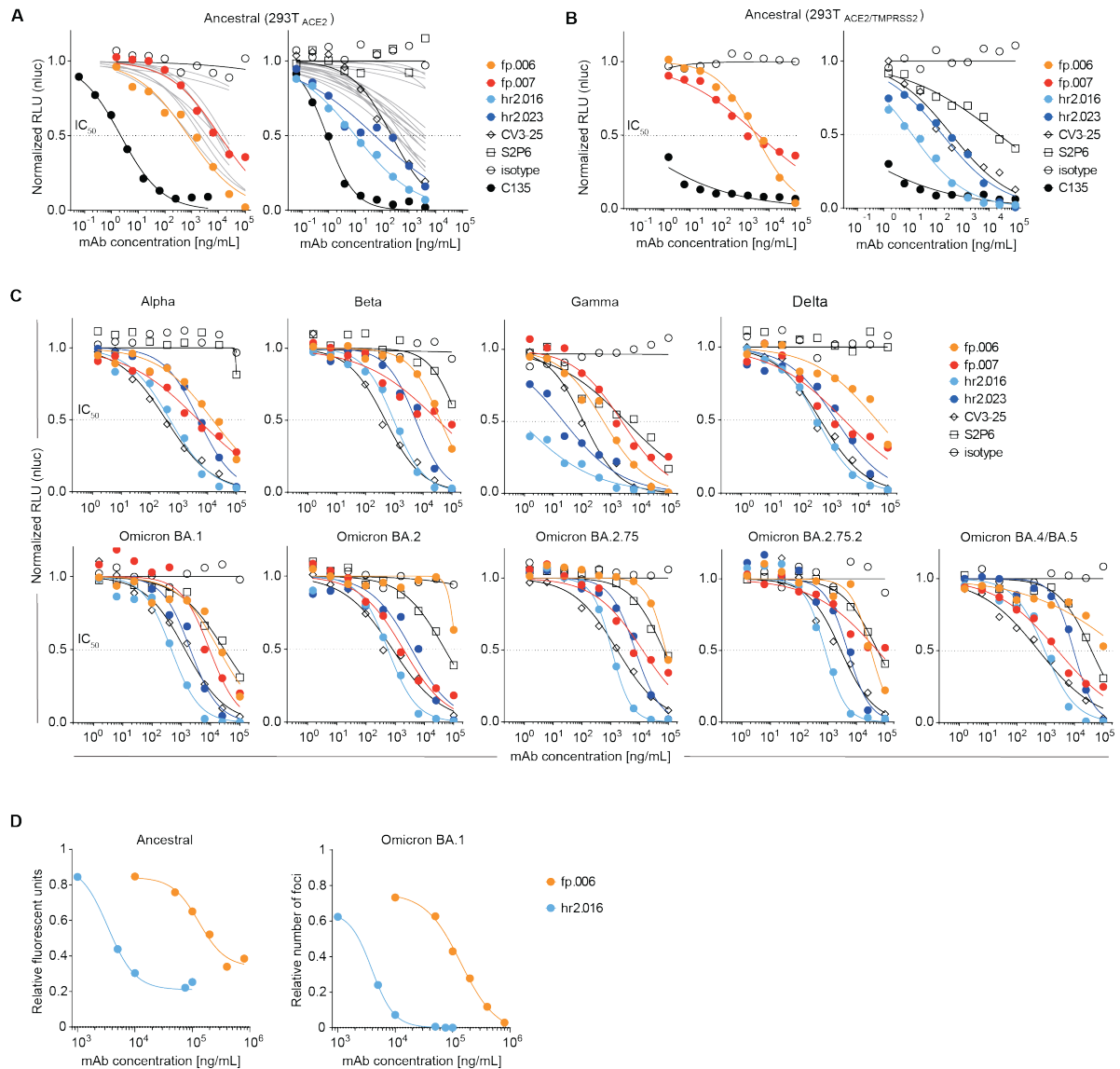


Fig. S4. Broad neutralization by FP and HR2 monoclonal antibodies. (A and B) Neutralization of ancestral SARS-CoV-2 pseudovirus. Graphs show normalized relative luminescence values in cell lysates of 293T_{ACE2} (A) or 293T_{ACE2/TMPRSS2} (B) cells 48 hours after infection with nanoluc-expressing SARS-CoV-2 pseudovirus in the presence of increasing concentrations of monoclonal antibodies to the FP (top) and HR2 (bottom). Isotype control and antibodies C135 (4), CV3-25 and S2P6 were assayed alongside for comparison; additional antibodies in grey (data file S2). Mean of at least two independent experiments. (C) Same as in (A), but for SARS-CoV-2 pseudoviruses corresponding to VOC. (D) Neutralization of SARS-CoV-2 ancestral and Omicron BA.1 by Focus Reduction Neutralization Test (FRNT).

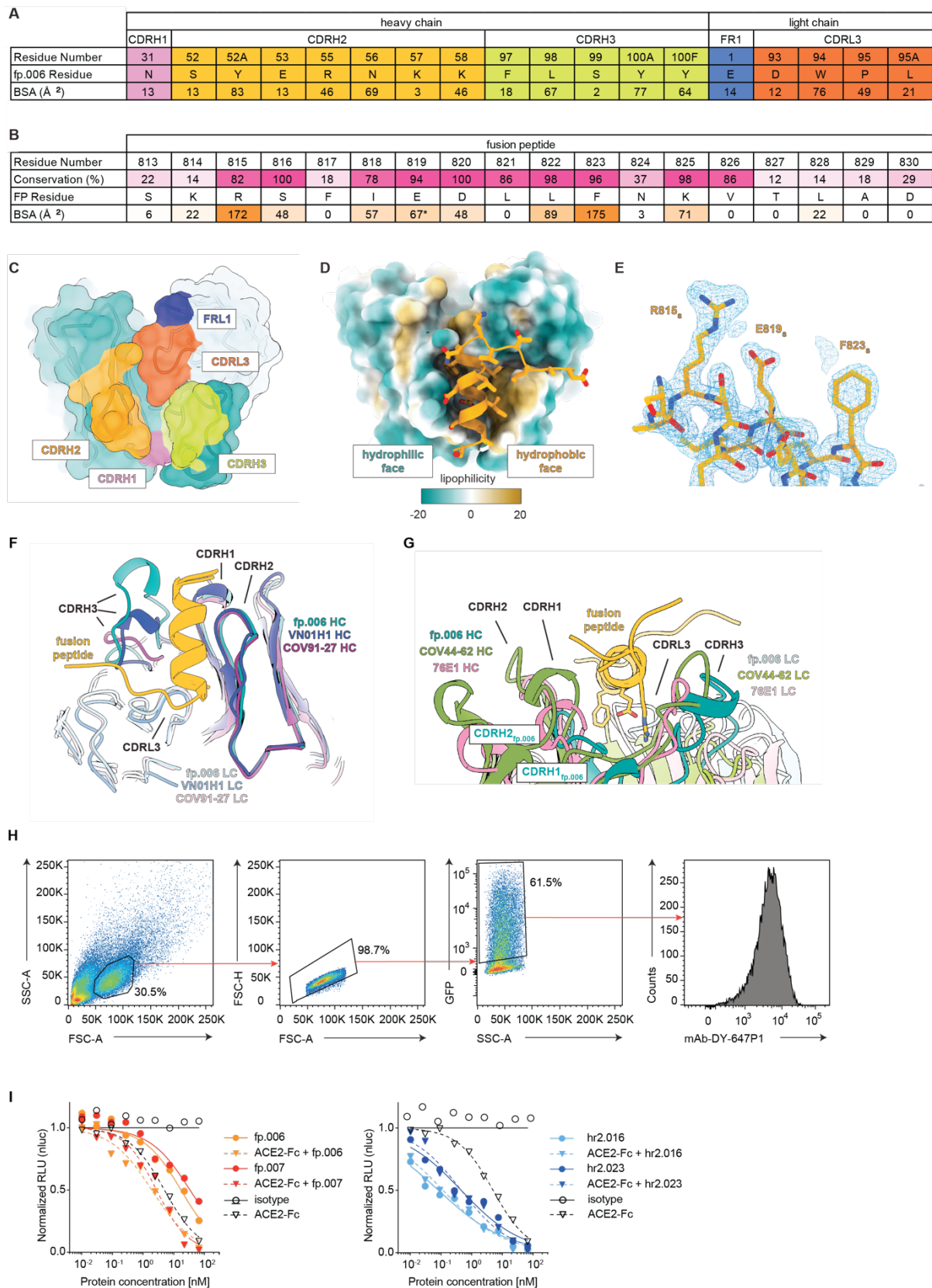


Fig. S5. Structure of fp.006 in complex with peptide antigen and effects of ACE2 engagement. (A) Contributions of fp.006 heavy and light chain paratope residues to the binding of the fusion peptide. Residue number, identity, and buried surface area (BSA) are shown, and colored as in panel (C). **(B)**

*Per residue contributions of the FP to the fp.006 epitope. Conservation calculated from the 49 CoV sequences listed in fig. S2B and BSA calculations per FP residue are listed. *Indicates that the BSA calculation does not include contacts mediated by water molecules. (C) Surface representation of the fp.006 paratope with interacting residues colored according to their CDR loop identity. Non-interacting heavy chain and light chain residues are colored teal and light teal, respectively. (D) Surface representation of the fp.006 paratope with per residue lipophilicity calculations shown. More hydrophobic patches are colored orange and more hydrophilic patches are colored teal, as calculated by ChimeraX. (E) Representative electron density for a portion of the FP contoured at 1.7σ . (F) Overlay of complex structures between the SARS-CoV-2 fusion peptide and fp.006 (this study; PDB 8D47), VN01H1 (PDB 7SKZ), and COV91-27 (PDB 8D6Z) illustrates a convergent binding mode independent of V gene usage. Structures are aligned on their fusion peptide chains and colored as shown. (G) Overlay of complex structures between the SARS-CoV-2 fusion peptide and fp.006 (this study; PDB 8D47), COV44-62 (PDB 8D36), and 76E1 (PDB 7X9E) Fabs, illustrating that anti-FP mAbs of different poses bind to the same face of the amphipathic fusion peptide α -helix, which includes highly-conserved residues, which are shown as sticks. (H) Gating strategy for measuring the binding of monoclonal antibodies to the SARS-CoV-2 S expressed by 293T cells by flow cytometry. (I) Normalized relative luminescence values in cell lysates of 293T_{ACE2} cells after infection with ancestral SARS-CoV-2 pseudovirus in the presence of increasing concentrations of FP or HR2 antibodies alone or as a cocktail with soluble ACE2. Isotype control in black. For the combination of fp.006+ACE2, $P=0.0017$ over fp.006 and $P=0.0047$ over ACE2; for the combination of fp.007+ACE2, $P=0.0072$ over fp.007 and $P=0.2$ over ACE2 ($n=4$; Welch's t-test, two-tailed).*

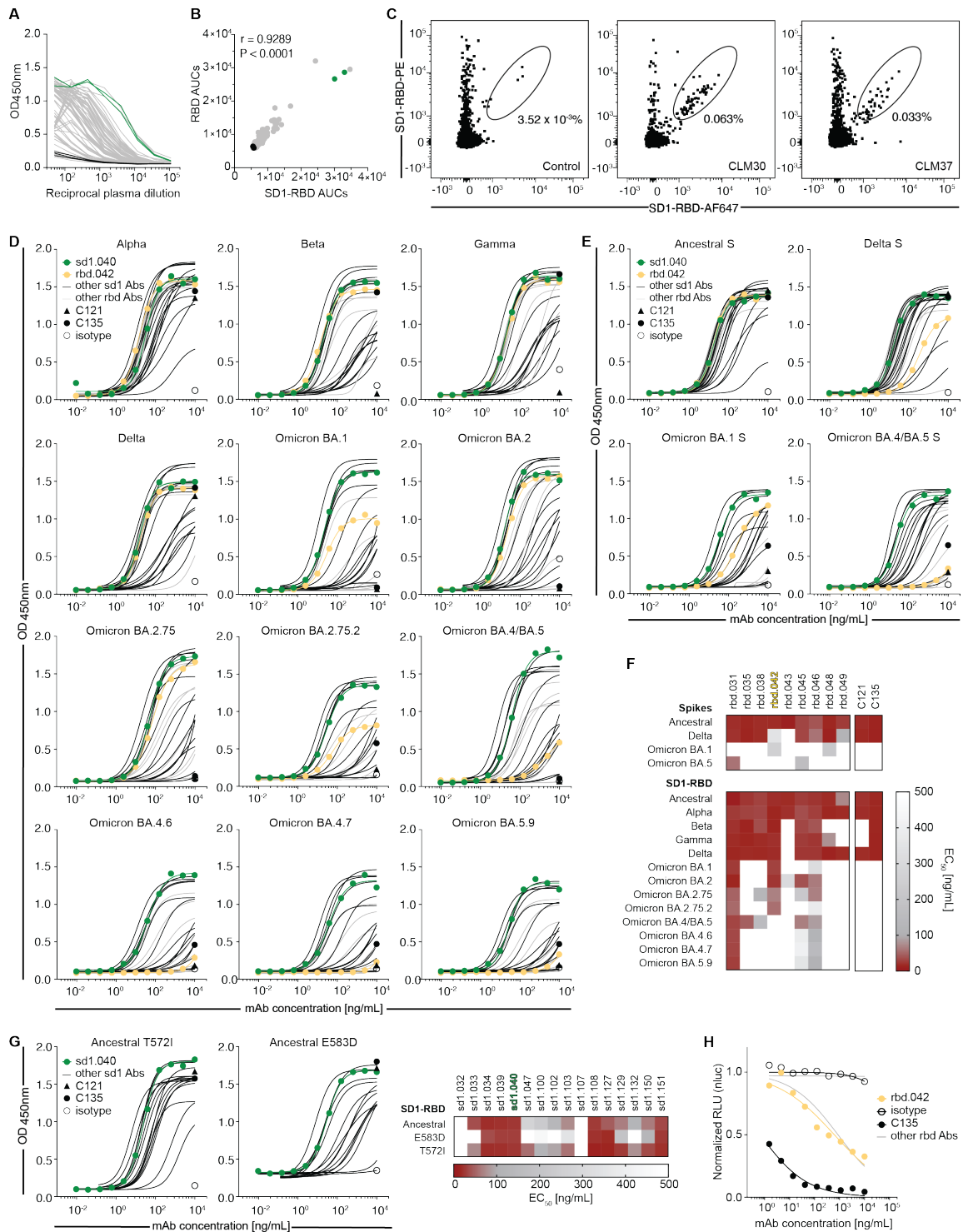


Fig. S6. Identification of SD1 and RBD antibodies. (A) Graph shows ELISAs measuring plasma IgG reactivity to RBD. Negative controls in black. Mean of two independent experiments. (B) Correlation of plasma IgG reactivities to SD1-RBD and RBD. Pearson correlation. (C) Identification of SD1-enriched B cells by flow cytometry. (D and E) Graphs show ELISAs measuring monoclonal antibodies reactivity to SD1-RBD (D) and S (E) corresponding to SARS-CoV-2 VOC. Mean of two independent experiments. (F) Heatmaps with the binding (EC_{50}) of RBD monoclonal antibodies to S (top) or SD1-RBD (bottom) proteins corresponding to SARS-CoV-2 VOC. Two experiments. (G) Graphs and summary heatmap for SD1 antibodies binding to SD1-RBD mutant proteins T572I and E583D. (H) Neutralization of ancestral SARS-CoV-2 pseudovirus by antibodies to the RBD that are broadly crossreactive to VOC.

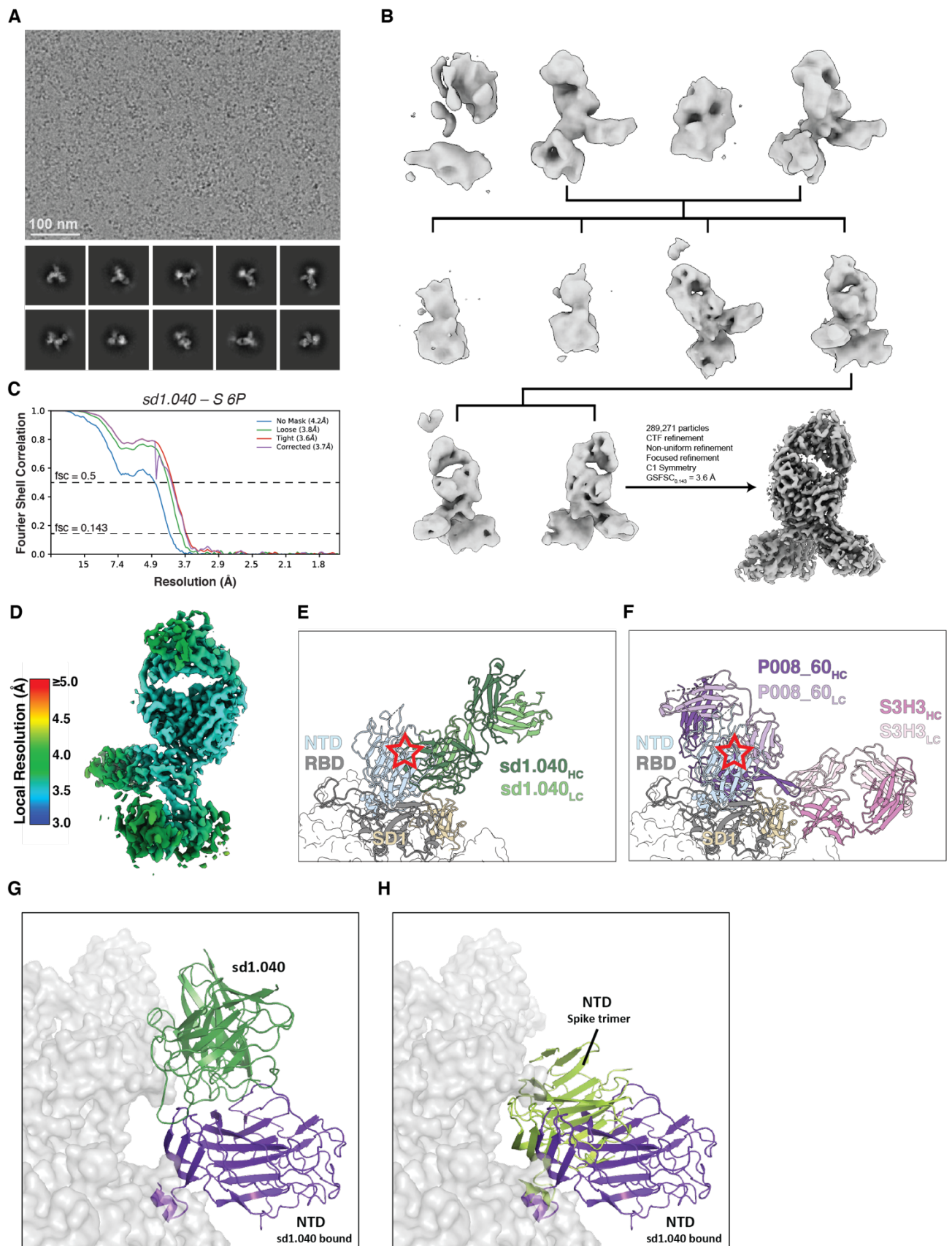


Fig. S7. Structure of sd1.040 in complex with S. (A) Representative micrograph and 2D class averages selected from the sd1.040-S dataset. (B) 3D classification workflow and final refinement strategy. (C) Gold-standard FSC plot for the final cryo-EM density map. (D) Local resolution estimates calculated

in cryoSPARC v3.1. **(E)** Close-up view of the sd1.040 Fab – S complex superimposed onto a prefusion S trimer (PDB 6VXX). Potential clashes with the NTD (cyan) on the neighboring S1 protomer are denoted by a red star. **(F)** Close-up views of the P008_60 Fab (PDB 7ZBU) and S3H3 Fab (PDB 7WD9) superposed onto a prefusion trimeric Spike. Antibody P008_60 shows prominent clashes with the adjacent NTD (red star), while the binding orientation of S3H3 does not clash with neighboring protomers. **(G and H)** According to docking simulations, sd1.040 binds also to the NTD of the adjacent protomer. In **(G)**, NTD moves from the position observed in the experimental structure of the free S to make room for antibody binding (sd1.040 in dark green bound to NTD in purple). In **(H)**, comparison between NTD bound to sd1.040 in simulations (purple) and NTD in the context of free spike trimer (lime).

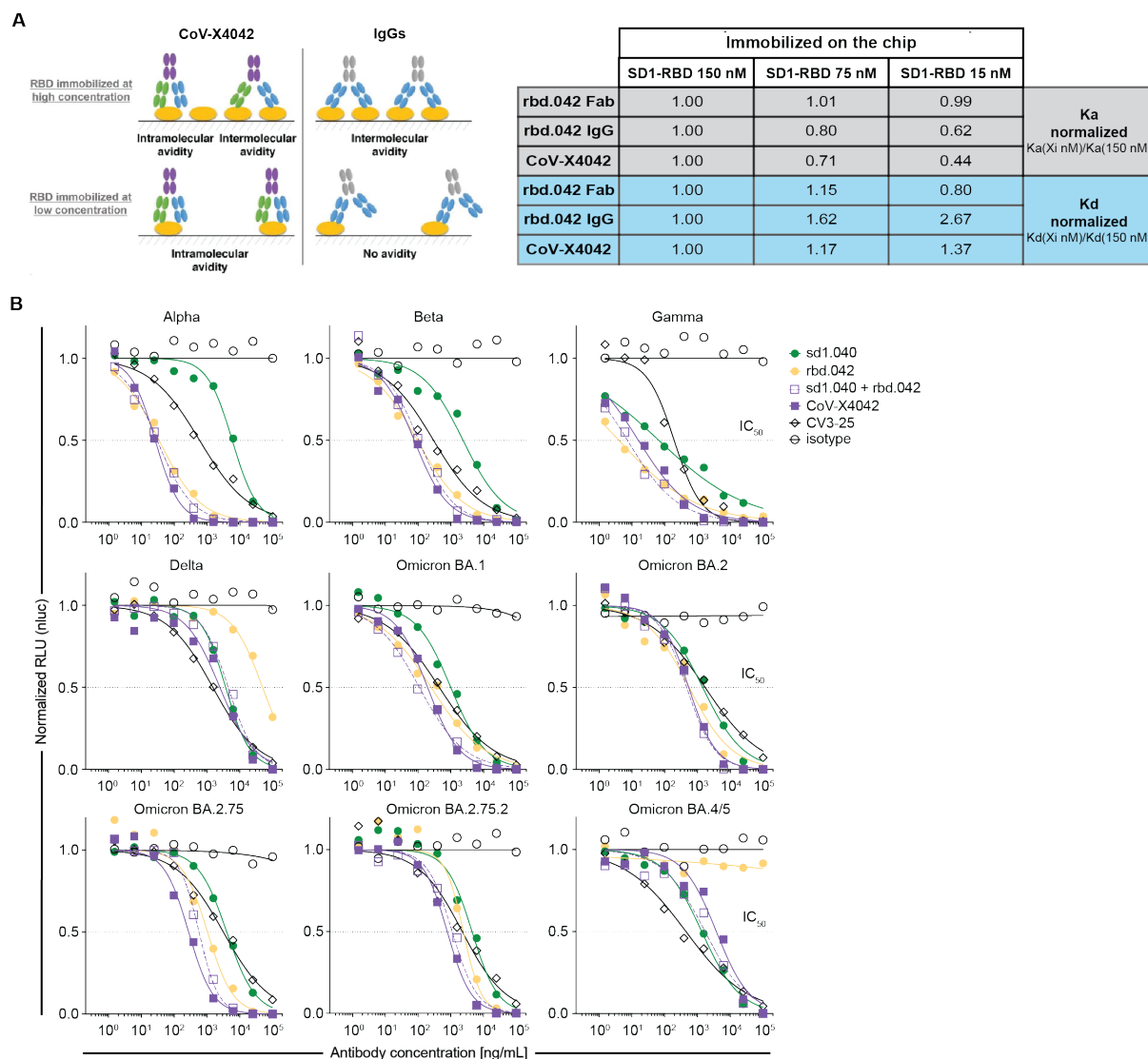


Fig. S8. Binding and neutralizing properties of CoV-X4042. (A) Both arms of CoV-X4042 bind simultaneously to the same SD1-RBD molecule. Left, diagram showing that at high SD1-RBD concentrations, monoclonal antibodies have avidity effects owing to intermolecular binding (which results in a slower k_d), but this is not the case at low SD1-RBD concentrations, because bivalent binding to a single SD1-RBD is impossible. By contrast, the bispecific antibody has avidity at both high and low concentrations, since bivalent binding to its two epitopes on a single SD1-RBD is possible. k_a is not affected by avidity. Right, table summarizing the SPR results used to determine k_a and k_d of IgG, Fab and the bispecific antibody at different concentrations of immobilized SD1-RBD, which confirm experimentally that CoV-X4042 engages bivalently on a single SD1-RBD. (B) Neutralization of VOC by CoV-X4042 and parental monoclonal antibodies.

Table S1. Crystallographic data collection and refinement statistics

PDB ID	fp.006-fusion peptide 8D47
Data collection^{a,b}	
Space group	P 1 2 ₁ 1
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	90.0, 73.1, 90.1
α , β , γ (°)	90.0, 109.0, 90.0
Resolution (Å)	37.9 - 2.0 (2.04 - 2.00)
<i>R</i> _{merge}	0.117 (0.996)
<i>I</i> / σ <i>I</i>	6.1 (1.5)
CC _{1/2} (%)	0.988 (0.372)
Completeness (%)	97.9 (98.8)
Redundancy	3.0 (3.0)
Refinement	
Resolution (Å)	37.9 - 2.0
No. reflections	73148 (4545)
<i>R</i> _{work} / <i>R</i> _{free}	.1836/.2378
No. atoms	7723
Protein	6926
Ligand/ion	45
Water	752
<i>B</i> -factors (mean)	
Protein	30.7
Ligand/ion	41.7
Water	36.5
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.840

^aData were derived from a single crystal

^bValues in paratheses describe the highest resolution shell

Table S2. Cryo-EM data collection, refinement and validation statistics

	sd1.040 – S 2P (EMDB-27177) (PDB 8D48)
Data collection and processing	
Magnification	105,000x
Voltage (kV)	300
Electron exposure (e-/Å ²)	60
Defocus range (μm)	1.0 - 2.5
Pixel size (Å)	0.8521
Symmetry imposed	C1
Initial particle images (no.)	9,894
Final particle images (no.)	9,469
Map resolution (Å)	
FSC threshold (0.143)	3.7
Map resolution range (Å)	3.7 – 4.2
Refinement	
Initial model used (PDB code)	6VXX, 5AVE, 7D0D
Model resolution (Å)	
FSC threshold (0.143)	3.6
Model resolution range (Å)	3.5 – 3.8
Map sharpening <i>B</i> factor (Å ²)	114
Model composition	
Non-hydrogen atoms	5,545
Protein residues	714
Ligands	2
<i>B</i> factors (Å ²)	
Protein	44
Ligand	132
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.8
Validation	
MolProbity score	2.0
Clashscore	11.7
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	92.8
Allowed (%)	7.2
Disallowed (%)	0