

Figure S1. Binding of WS6 to diverse CoV spike proteins by ELISA compared to S2P6, related to Figures 1 and 2. ELISA binding curves of WS6 are shown in green and those of S2P6 in black.

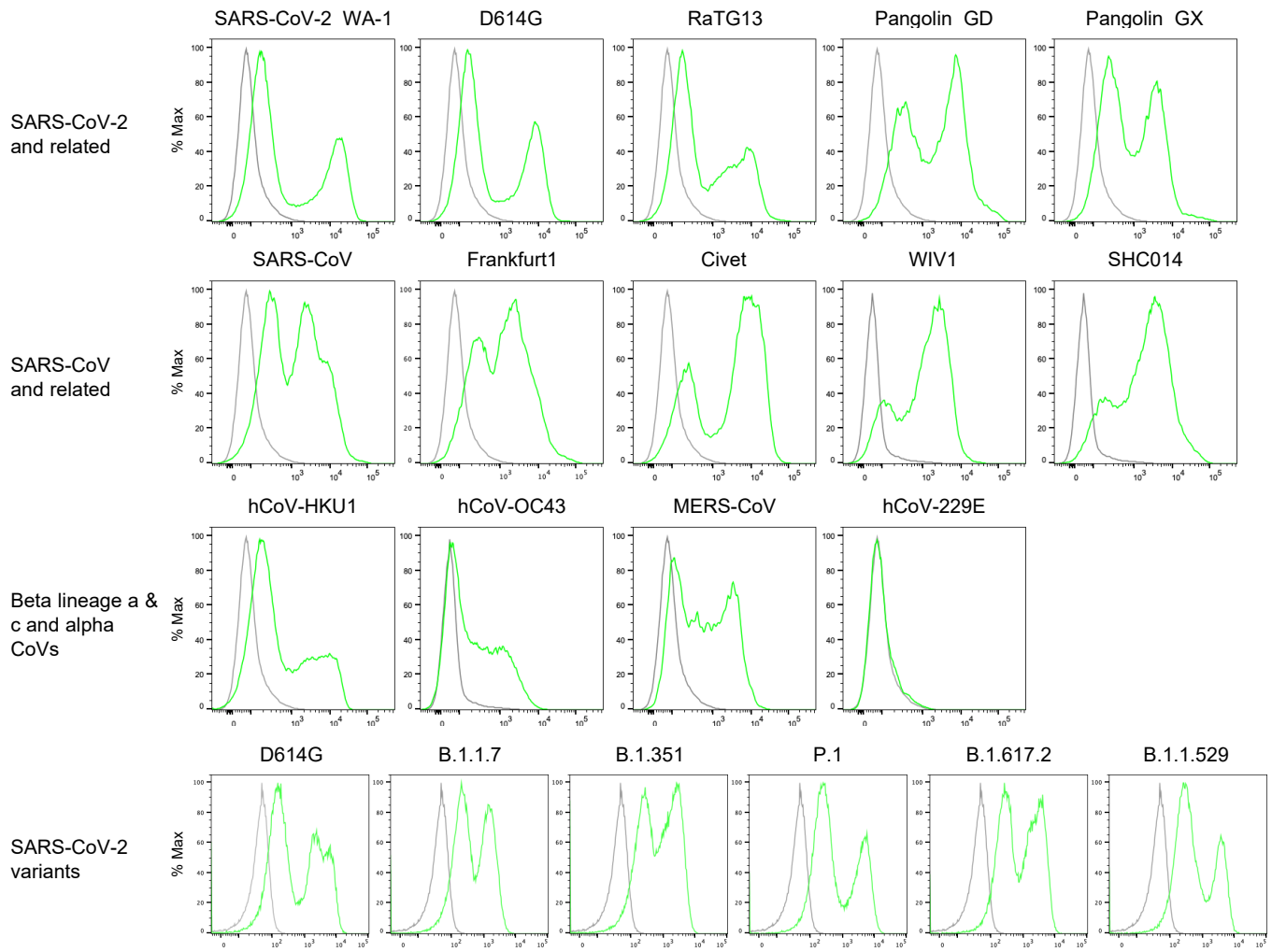


Figure S2. WS6 binding to cell-surface expressed coronavirus spikes, related to Figure 2.

Spike proteins were expressed on the surface of expi293 cells, and antibody binding was measured using flow cytometry. WS6 binding (green line) to cells transfected with the indicated coronavirus spike is compared to binding to untransfected cells (grey line).

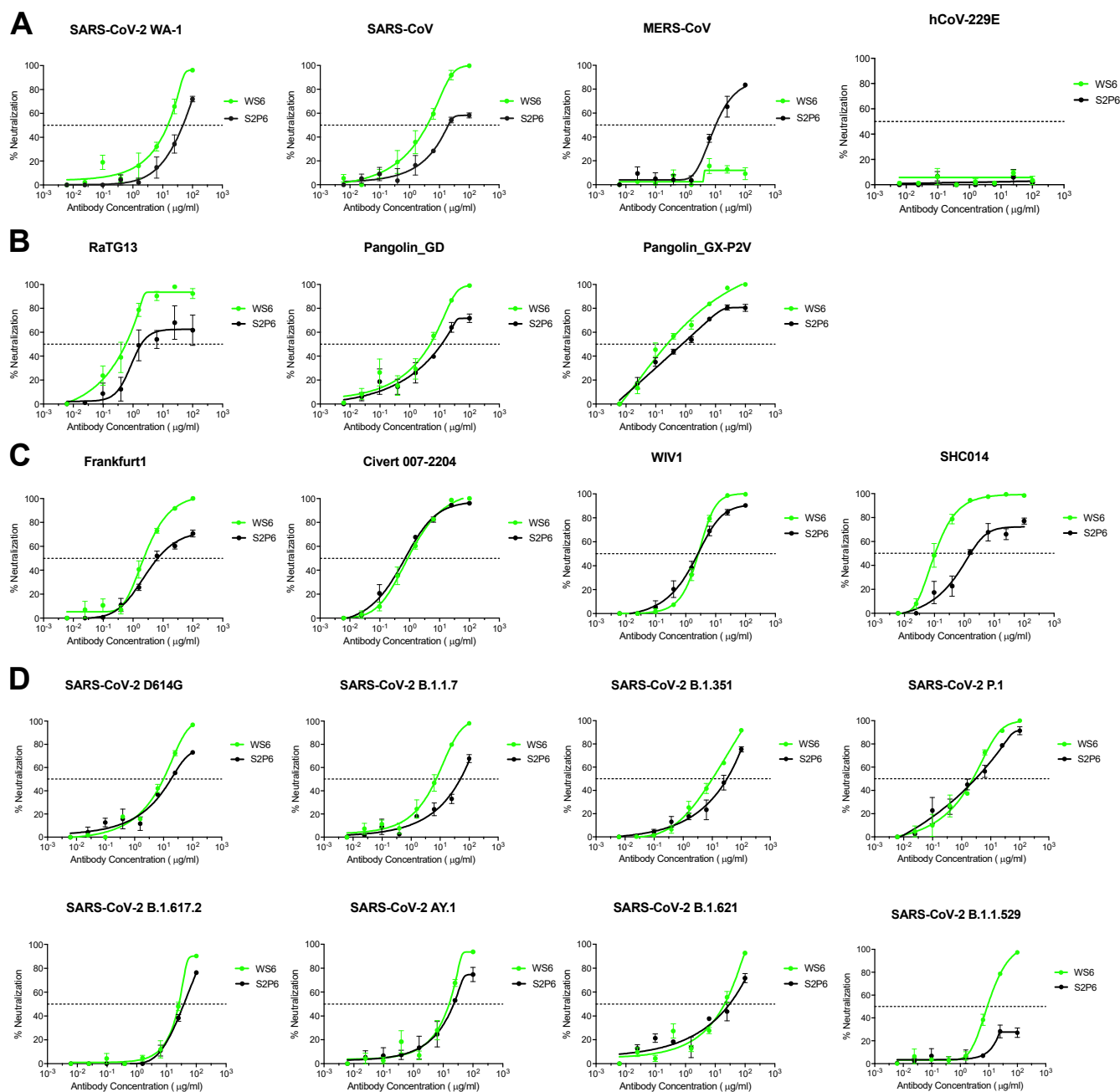
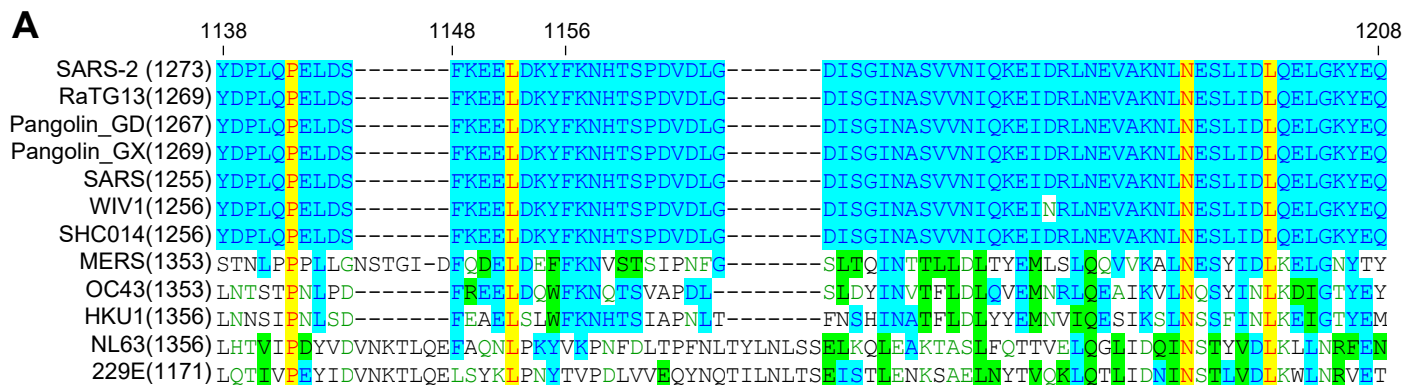


Figure S3. Neutralization of WS6 against diverse coronaviruses, related to Figure 2.

Neutralization curves are showing using CoV spike pseudotyped lentivirus to test neutralization capacity of WS6 compared to S2P6. Neutralization was tested on HEK293-TMPRSS2-ACE2 stable cells for SARS-CoV-2, SARS-CoV and related CoVs and Huh7.5 cells for MERS-CoV. (A) WS6 neutralizes SARS-CoV-2, SARS-CoV, but not MERS-CoV or hCoV-229E. (B) WS6 neutralizes SARS-CoV-2 related coronaviruses. (C) WS6 neutralizes SARS-CoV related coronaviruses. (D) WS6 neutralizes SARS-CoV-2 variants.

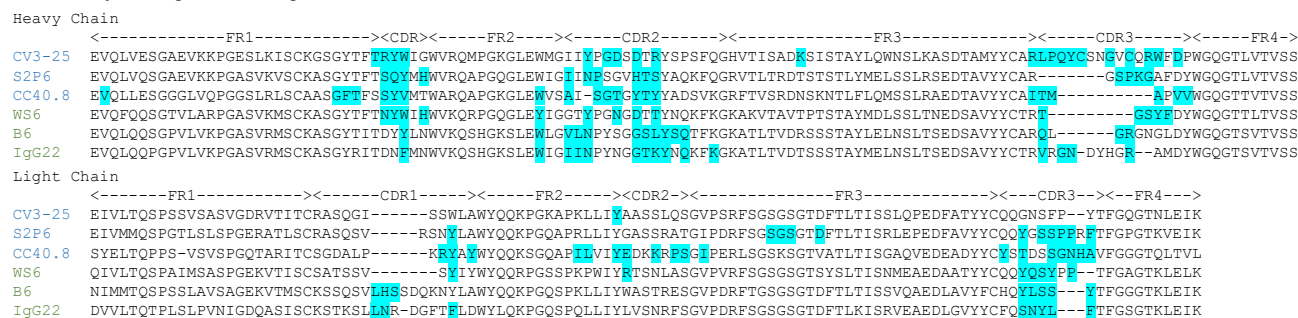


B Germline gene usage of antibodies targeting S2-helical region

Antibody	VH	VH Identity (%)	CDRH3	VL	VL Identity (%)	CDRL3	Ref
CV3-25	IGHV5-51	97.6	CARLPQYCSNGVCQRWFDPW	VK1-12	97.5	CQQGNSFPYTF	(Li et al., 2022)
S2P6	VH1-46	96.5	CARGSPKGAFDYW	VK3-20	97.5	CQQYGSSPPRFTF	(Pinto et al., 2021)
CC40.8	VH3-23	93.8	CAITMAPVWW	VL3-10	96.2	CYSTDSSGNHAVF	(Song et al., 2021)
WS6	VH1-5	92.9	CTRTRGSY-FDYW	VK4-61	97.9	CQQYQSYPTTF	This study
B6	VH1-19	-	CARQLGRGNGLDYW	VK8-27	-	CHQYLSSYTF	(Sauer et al., 2021)
IgG22	VH1-19	95.2	CTRVRGNDYHGRAMDYW	VK1-99	98.6	CFQSNYLFTF	(Hsieh et al., 2021)

Blue: human antibody
Green: mouse antibody

C Heavy and light chain alignment



D Frequency of antibodies targeting S2-helical region

		HC	LC	Class	Average Class
		Frequency	Frequency	Frequency	Frequency
CV3-25	HIP1	8.11E-10	5.46E-03	2.65E-12	1.90E-12
	HIP2	4.58E-10	4.11E-03	1.13E-12	
	HIP3	6.39E-10	5.02E-03	1.92E-12	
S2P6	HIP1	6.54E-09	2.82E-04	1.10E-12	1.71E-12
	HIP2	5.81E-09	3.01E-04	1.05E-12	
	HIP3	1.37E-08	3.63E-04	2.99E-12	
CC40.8	HIP1	4.09E-14	5.67E-07	9.27E-21	2.52E-20
	HIP2	5.58E-14	1.68E-06	3.76E-20	
	HIP3	6.57E-14	1.10E-06	2.88E-20	
WS6	Mouse1	3.57E-08	2.90E-05	9.83E-13	5.00E-13
	Mouse4	2.60E-08	1.56E-06	3.84E-14	
	Mouse5	7.11E-08	7.07E-06	4.78E-13	
B6/IgG22	Mouse1	1.38E-04	2.92E-04	3.83E-08	3.72E-08
	Mouse4	1.39E-04	3.67E-04	4.84E-08	
	Mouse5	2.38E-04	1.10E-04	2.48E-08	

Figure S4. Sequences of conserved S2 stem region and antibodies that target this region, related to Figure 5.

(A) S2-stem sequences of diverse coronaviruses. Aqua highlight for amino acids conserved on SARS/SARS2. Yellow/red font shown full conserve. Green highlight shown amino acids with similar physicochemical property. (B) Identified human and mouse antibodies targeted on coronavirus spike S2-Helix epitope. (C) Alignments of heavy and light chain sequences. Residues that contact the helix epitope are highlight in cyan. (D) The frequencies of antibody targeting SP2 helical region calculated by software OLGA. See Methods for the signatures used to calculate frequency.

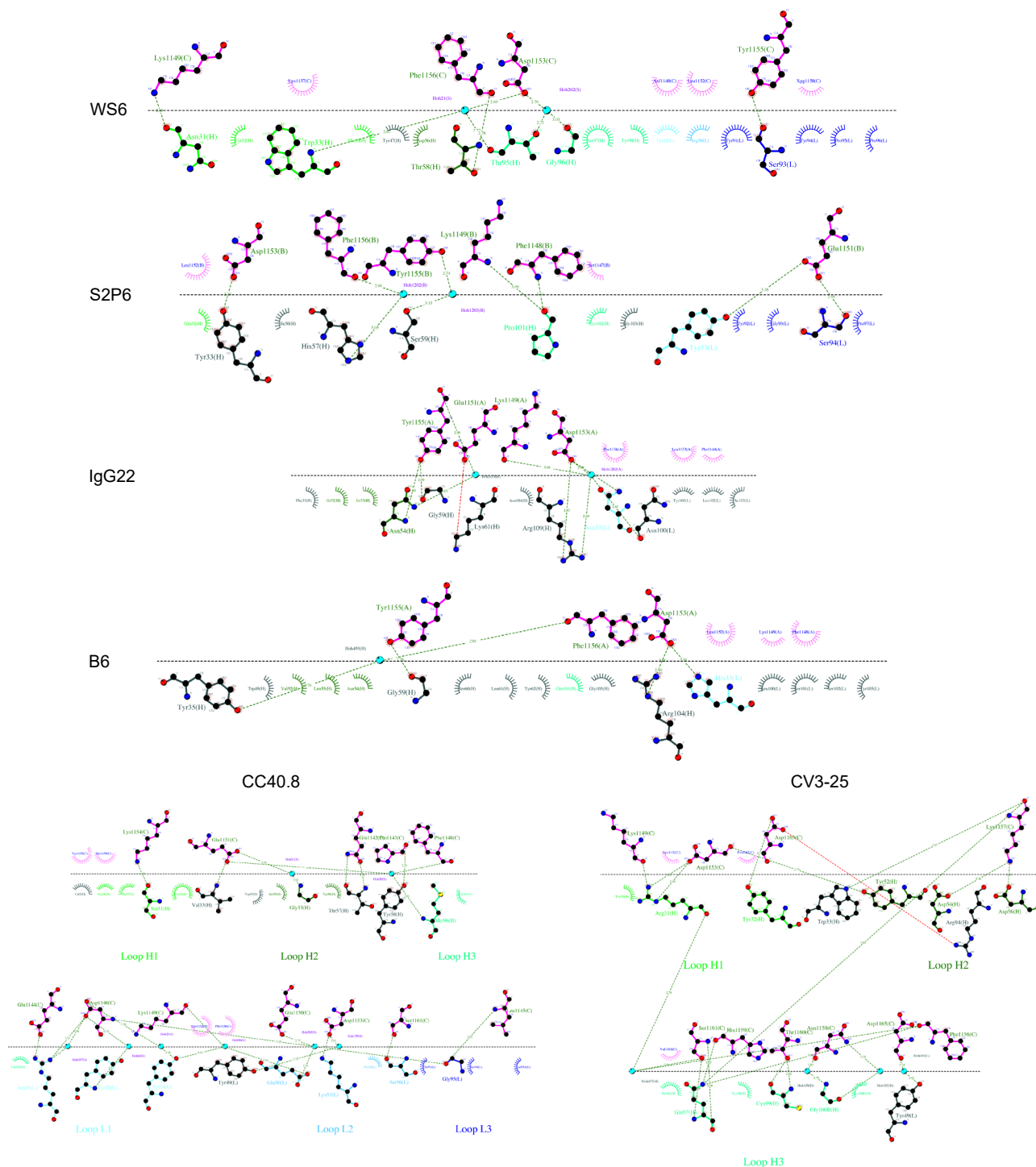


Figure S5. Detailed interactions between antibodies and S2 peptide residues, related to Figure 5.

Hydrophilic and hydrophobic interaction between antibody and S2 residues were plotted with LigPlot⁺ (Laskowski R A, Swindells M B (2011). LigPlot⁺: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.*, 51, 2778-2786. [PubMed id: 21919503]). Interface is depicted with a horizontal line between antibody and peptide residues. Hydrophilic interactions are shown as lines between atoms with distance labeled. Hydrophobic interactions are shown as eyelash symbols.

Table S1. Pseudovirus neutralization of WS6 of diverse coronaviruses, related to Figures 1 and 2.

Pseudoviruses	WS6		S2P6		ug/ml
	IC50	IC80	IC50	IC80	
WA-1*	4.28	24.01	16.12	76.39	0.1-1
D614G	9.88	34.86	17.55	>100	1-10
B.1.1.7	6.31	26.81	49.25	>100	10-100
B.1.351	9.83	53.74	32.02	>100	>100
P.1	2.46	10.45	3.06	28.39	
B.1.617.2	26.52	69.85	38.90	>100	
Delta+	15.59	32.37	22.46	>100	
B.1.621	20.26	63.82	31.05	>100	
B.1.529	3.43	35.76	>100	>100	
RaTG13	0.52	1.79	1.74	>100	
Pangolin_GD	4.91	18.34	11.71	>100	
Pangolin_GX	0.24	3.89	0.76	27.22	
SARS	1.93	9.57	24.03	>100	
Frankfurt 1	2.27	8.63	6.72	>100	
Civet 007-2004	0.84	4.67	0.68	4.32	
WIV1	2.65	6.41	2.44	15.87	
SHC014	0.11	0.39	1.57	>100	
MERS-CoV	>100	>100	10.59	>100	
hCoV-229E	>100	>100	>100	>100	

* The titers shown here reflect neutralization tested on HEK293-TMPRSS2-ACE2 stable cells and differ from those in Figure 1D, which were tested on 293T-ACE2 cells.

Table S2. Crystal diffraction data and structure refinement statistics, related to Figure 3.

Wavelength (Å)	1.0000
Resolution range (Å)	40.23 - 2.02 (2.092 - 2.02)
Space group	C 1 2 1
Unit cell (a, b, c, α , β , γ)	155.1, 64.5, 138.0, 90.0, 117.3, 90.0
Total reflections	262622
Unique reflections*	75855 (6642)
Multiplicity	3.5 (2.9)
Completeness (%)	94.81 (81.69)
Mean I/sigma(I)	10.5 (1.1)
Wilson B-factor	27.88
R-merge	0.101 (0.943)
R-pim	0.083(0.513)
CC1/2	0.995 (0.765)
Reflections used in refinement	75540 (6475)
Reflections used for R-free	1989 (170)
R-work	0.1718 (0.2427)
R-free	0.2133 (0.2848)
Number of non-hydrogen atoms	7587
macromolecules	6696
ligands	176
solvent	735
Protein residues	871
RMS(bonds, Å)	0.008
RMS(angles, °)	0.91
Ramachandran favored (%)	97.78
Ramachandran allowed (%)	2.22
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.92
Clashscore	4.58
Average B-factor (Å ²)	32.90
macromolecules	31.84
ligands	49.90
solvent	38.92
* Statistics for the highest-resolution shell are shown in parentheses.	

Table S3. WS6-peptide binding interface analysis, related to Figure 3.

The crystal structure of WS6 in complex with the stem-helix peptide was analyzed by PISA (https://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). ASA, accessible surface area in Å²; BSA, buried surface area in Å²; ΔiG, solvation free energy gain upon formation of the interface in kcal/M. Bars of BSA indicates buried area percentage, one bar per 10%. Atoms with superscript "H" are involved in interface hydrogen bonds.

WS6					Peptide			
	ASA	BSA	ΔiG	CDR-BSA		ASA	BSA	ΔiG
Heavy chain interface								
H:Asn31 [O] ^H [CB]	87.76	17.02	-0.18	CDR H1 126.61	C:Phe1148 [C] [O] [CB]	380.59	39.54	0.00
H:Tyr32 [CA] [CD2] [CE2] [CZ] [OH]	71.35	19.09	0.29		C:LYS1149 [N] [CA] [CB] [CG] [CD] [CE] [NZ] ^H	182.72	103.04	0.41
H:Trp33 [N] [CB] [CG] [CD1] [CD2] [CE2] [CE3] [NE1] ^H [CZ2] [CZ3] [CH2]	81.33	75.56	0.67		C:Leu1152 [CB] [CG] [CD2]	100.74	55.93	0.89
H:His35 [CE1]	14.94	14.94	0.24		C:Asp1153 [CA] [O] ^H [CB] [CG] [OD1] [OD2]	68.05	41.86	-0.16
H:Tyr47 [CD1] [CD2] [CE1] [CE2] [CZ] [OH]	74.50	14.78	0.13	CDR H2 87.62	C:PHE1156 [CA] [C] [O] ^H [CB] [CG] [CD1] [CD2] [CE1] [CE2] [CZ]	152.52	105.56	1.16
H:Tyr52 [CE1] [CZ] [OH]	59.05	7.43	0.10		C:Lys1157 [N] [CA] [C] [O] [CB] [CG] [CD] [CE]	210.66	57.36	0.58
H:Asn55 [ND2]	101.01	2.18	-0.02					
H:Asp57 [CB] [CG] [OD1] [OD2]	73.89	29.15	-0.17					
H:Thr59 [CB] [CG2] [OG1] ^H	78.40	34.08	0.28					
H:Thr99 [O] [CB] [CG2] [OG1]	27.78	22.48	0.13	CDR H3 145.3				
H:Gly100 [CA] [C] [O]	44.61	42.18	-0.15					
H:Ser101 [N] [CA] [C] [O] [CB] [OG]	107.81	68.21	0.93					
H:Tyr102 [CA]	151.61	4.68	0.07					
H:Phe103 [CZ]	82.75	7.75	0.12					
Light chain interface								
L:Tyr31 [CE2] [CE2] [OH]	106.29	23.26	0.00	CDR L1 23.26	C:Phe1148 [CB] [CG] [CD1] [CD2] [CE1] [CE2] [CZ]	380.59	75.6	0.00
L:Arg49 [CD] [NE] [CZ] [NH1] [NH2]	112.09	54.24	-0.42	CDR L2 54.24	C:Lys1149 [N]	182.72	0.15	-0.00
L:Tyr90 [CA] [O] [CB] [CG] [CD1] [CD2] [CE1] [CE2] [CZ] [OH]	122.46	72.86	0.63	CDR L3 151.58	C:Glu1151 [OE1]	146.85	6.74	-0.13
L:Gln91 [O]	91.56	1.71	-0.02		C:Leu1152 [CD1] [CD2]	100.74	39.55	0.63
L:Ser92 [CA] [C] [O] ^H	52.85	4.77	0.03		C:Tyr1155 [O] [CD2] [CE1] [CE2] [CZ] [OH] ^H	164.25	112.17	0.36
L:Tyr93 [N] [CA] [CB] [CD2] [CE2] [CZ] [OH]	196.27	54.06	0.80		C:Phe1156 [CA] [O] [CD1] [CE1] [CZ]	152.52	38.32	0.48
L:Pro95 [CG] [CD]	57.14	18.18	0.29					