Cross-neutralizing antibodies bind a SARS-CoV-2 cryptic site and resist to circulating variants

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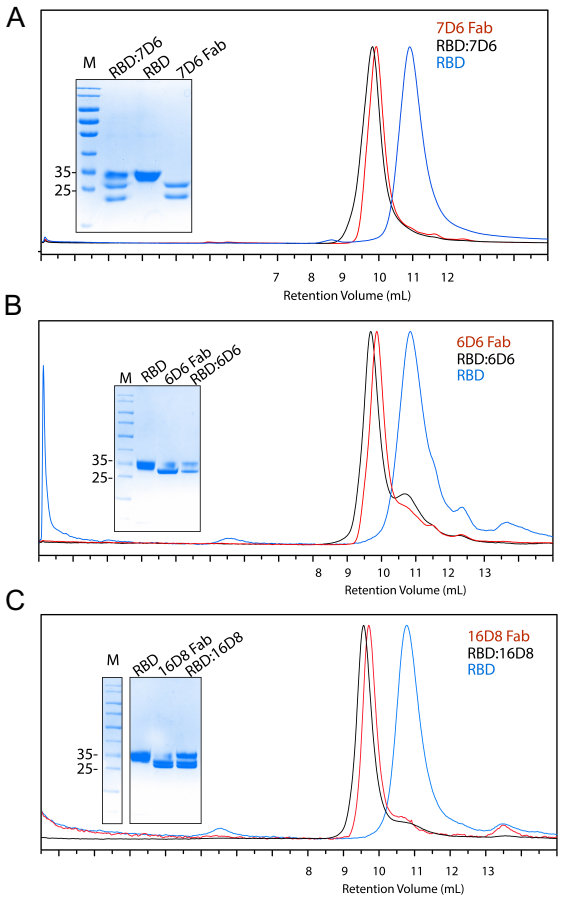

Fig. S1. HPLC profiles and SDS-PAGE analysis of RBD:7D6, RBD:6D6 and RBD:16D8 complexes.

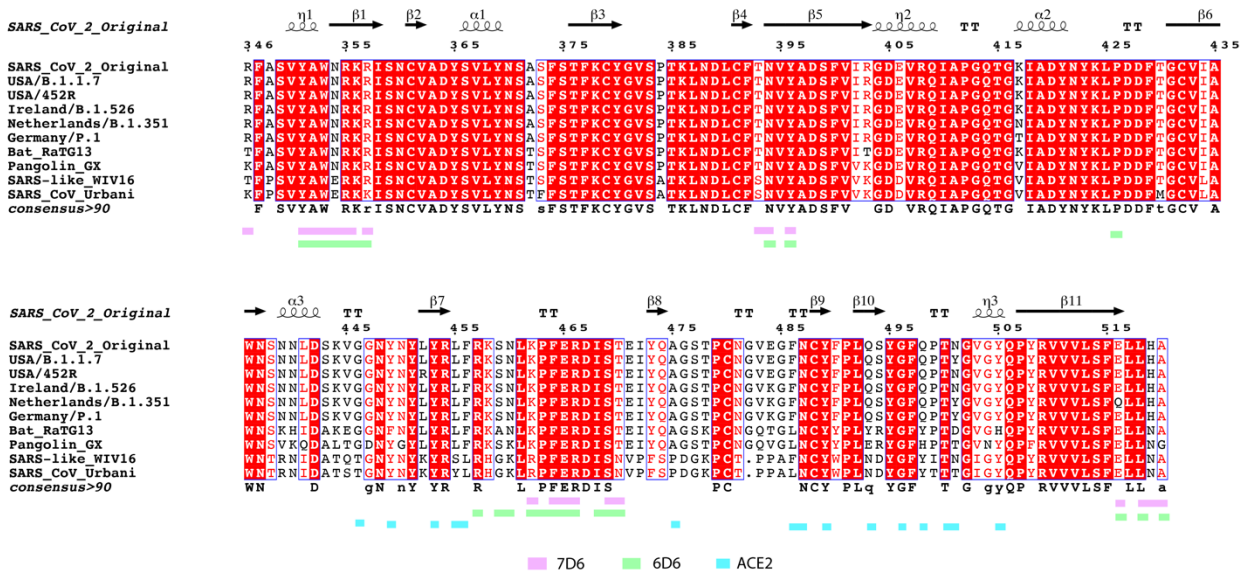

Fig. S2. Sequence alignment of the RBDs of multiple *Sarbecoviruses*. Secondary structure distribution is indicated according to the RBD structure in 7D6:RBD co-crystal structure (PDB no. 7EAM). The plot was prepared in the on-line server (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). The footprints of ACE2, 7D6, and 6D6 are marked in cyan, pink and green, respectively.

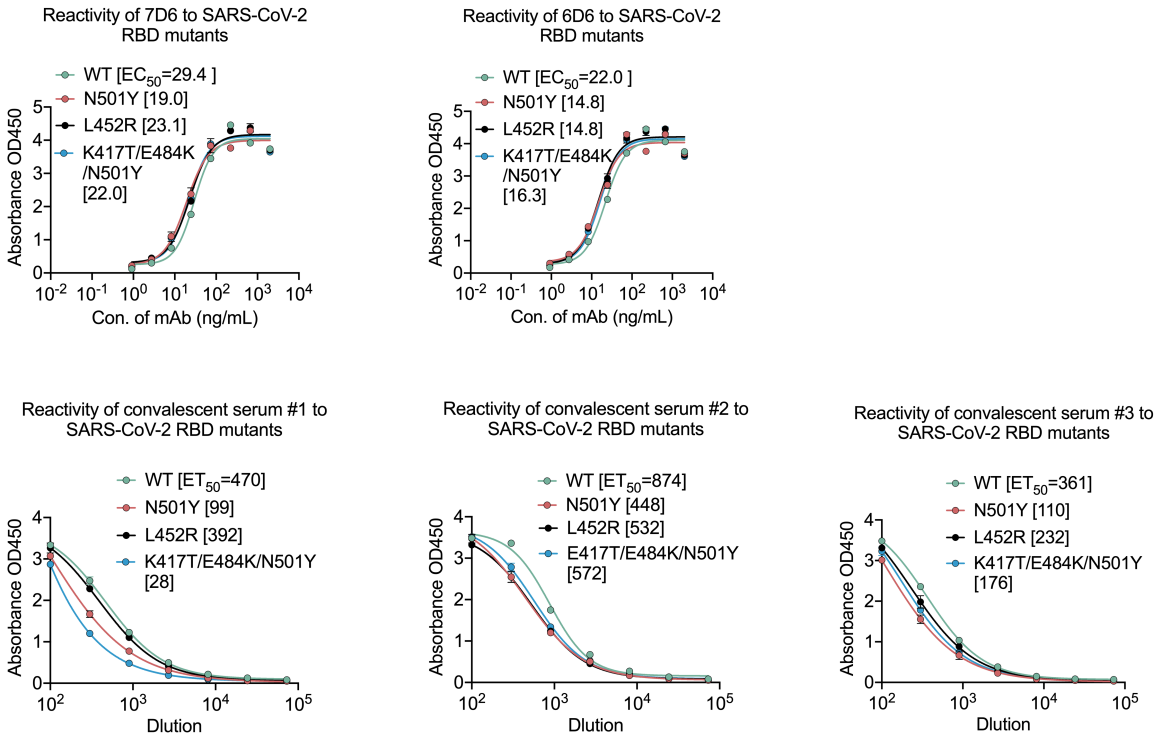

Fig. S3. Binding reactivities of 7D6, 6D6 and COVID-19 convalescent sera against the RBD mutants. COVID-19 convalescent sera exhibited lower reactivities to RBD mutants to various extents, the reactivities of serum #1 and #2 to mutants decreased by 2-20 folds compared to wild-type (WT) RBD.

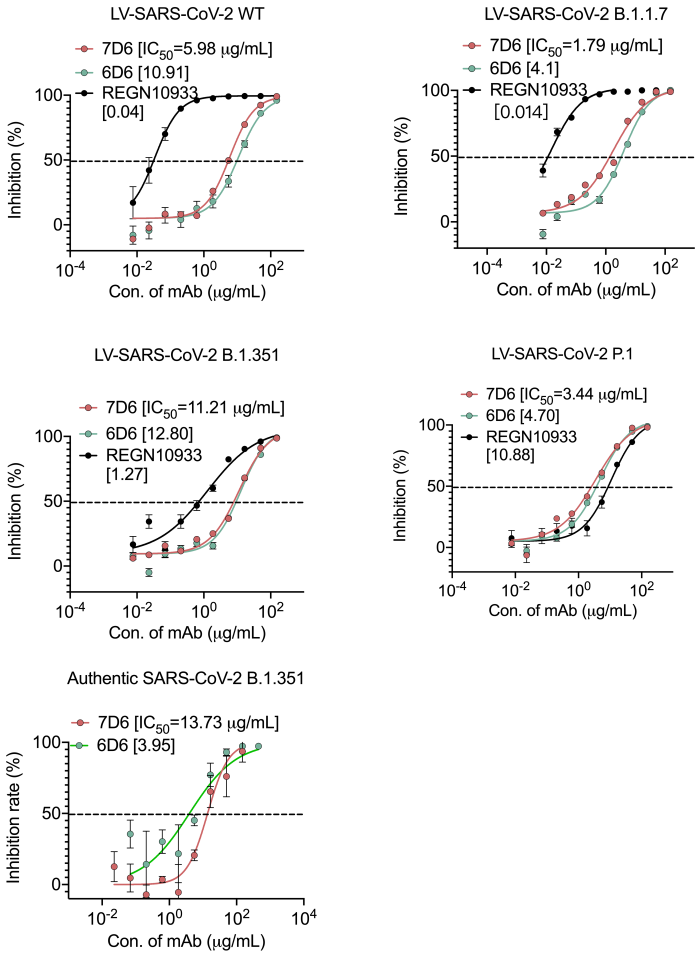

Fig. S4. Neutralization activities of 7D6, 6D6 against pseudotyped LVs and authentic virus of the major SARS-CoV-2 variant(s).

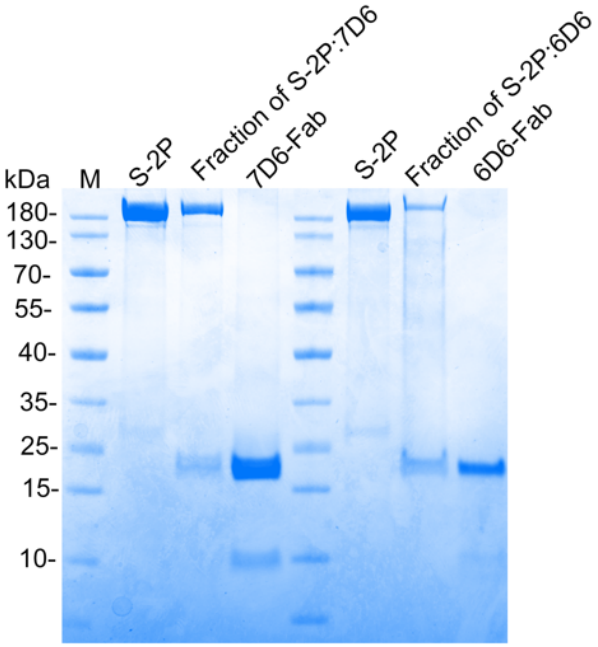

Fig. S5. SDS-PAGE analysis of the fractions harvested from the gel filtration chromatography of the mixture of S-2P and 7D6 or 6D6 Fab. The fractions are the peaks indicated by arrows in Figure 6.

Supplementary Table

Table S1 Binding activities and neutralizing titers of SARS-CoV-2 antibodies.

	EC50 (ng/mL)					IC50 (μg/mL)		
mAb	**SARS-CoV-2 S-2P binding**	**SARS-CoV S-2P binding**	SARS-CoV-2 S1 binding	RBD binding	S2 binding	**SARS-CoV-2 LV**	**SARS-CoV LV**	SARS-CoV-2 VSV
7D6	**11.28**	**12.86**	12.22	7.42	>10,000	**2.56**	**10.11**	0.04
5A12	**23.81**	**11.13**	>10,000	>10,000	15.00	**>150**	**>150**	0.76
13F1	**21.05**	**27.00**	>10,000	>10,000	87.31	**>150**	**ND**	>150
12F7	**137.00**	**343.00**	>10,000	>10,000	81.39	**>150**	**ND**	>150
12F9	**30.68**	**286.8**	>10,000	>10,000	40.36	**>150**	**ND**	>150
6D6	**21.78**	**120.00**	39.32	68.02	>10,000	**8.91**	**1.67**	0.21
16D8	**19.96**	**44.06**	44.57	111.7	>10,000	**3.52**	**1.2**	0.26
19F10	**24.62**	**39.59**	44.25	33.97	>10,000	**1.06**	**2.67**	1.08
17F10	**35.20**	**26.29**	57.53	43.5	>10,000	**1.58**	**0.44**	ND
4A5	**42.40**	**87.80**	46.16	90.3	>10,000	**0.62**	**57.56**	ND
10D1	**29.35**	**25.84**	49.66	88.77	>10,000	**2.94**	**18.12**	ND
20H2	**45.57**	**32.97**	>10,000	>10,000	45.57	**>150**	**>150**	>150
8G5	**28.82**	**44.61**	46.16	84.25	>10,000	**>150**	**>150**	ND
5E7	**318.50**	**670.20**	581.80	217.60	>10,000	**>150**	**>150**	>150
1B11	**80.42**	**17.48**	72.93	>10,000	>10,000	**>150**	**>150**	>150

*ND denoted not detected.

Table S2 Kinetic constants for binding activities of antibodies to SARS-CoV-2 or SARS-CoV proteins.

mAb	Proteins	ka ($M^{-1}S^{-1}\times10^{4}$)	kd ($s^{-1}\times10^{-5}$)	K_D (nM)
7D6	SARS-CoV-2 S-2P	1.52	8.81	5.89
	SARS-CoV S-2P	4.78	24.10	5.04
	SARS-CoV-2 RBD	18.00	0.11	<0.01
	SARS-CoV RBD	5.85	0.17	0.03
6D6	SARS-CoV-2 S-2P	12.30	0.42	0.03
	SARS-CoV S-2P	3.06	5.56	2.14
	SARS-CoV-2 RBD	11.50	0.15	0.01
	SARS-CoV RBD	1.70	0.63	0.37
16D8	SARS-CoV-2 S-2P	3.55	0.18	0.04
	SARS-CoV S-2P	65.60	9.14	13.90
	SARS-CoV-2 RBD	3.93	0.02	<0.01
	SARS-CoV RBD	3.15	0.27	0.08

Table S3 Data collection and refinement statistics for 7D6:RBD and 6D6:RBD complexes.

	7D6:RBD	**6D6:RBD**
Data collection		
Cell parameters (Å, º)	a=37.4, b=88.0, c=102.7 α=89.9, β=87.1, γ=89.9	a=58.2, b=85.3, c=160.7 α=90, β=90, γ=90
Space group	P1	$P2_12_12_1$
Resolution range [a] (Å)	29.32-1.40 (1.44-1.40)	30.08-1.91 (1.96-1.91)
Wavelength (Å)	0.97918	0.97918
Observed hkl (I>σ)	827,533	419,267
Unique hkl	244,264	62,975
Redundancy	3.4 (2.7)	6.7 (6.5)
Completeness (%)	94.7 (89.5)	99.8 (99.7)
Overall (I/σI)	8.8 (0.8)	13.7 (0.9)
R_{sym} [b] (%)	6.1 (124.7)	6.4 (216.7)
R_{pim} [c] (%)	3.9 (93.6)	2.7 (91.3)
CC1/2	0.996 (0.331)	0.999 (0.402)
Refinement		
Resolution range (Å)	21.68-1.40	28.67-1.91
Number of Reflections	244,139	62,867
R_{factor} [d]	17.2	18.9
R_{free} [e]	18.2	22.4
RMSD bond lengths (Å)	0.009	0.014
RMSD bond angles (°)	1.35	1.32
No. atoms	11,084	5,036
Protein	9,836	4,853
Glycan	84	14
Water	1,164	169
Wilson B-factor (Å^2)	22.7	42.9
Average B-factors (Å^2)	38.2	57.7
Protein	37.0	57.7
Glycan	104.3	113.4
Water	43.2	53.5
Ramachandran Plot		
Favored and allowed region (%)	97.0	97.9
Generously allowed regions (%)	2.6	1.9
Disallowed regions (%)	0.4	0.2

[a] Numbers in parentheses refer to the highest resolution shell.

[b] $R_{sym} = \Sigma h \Sigma i|I_I(h) - <I(h)| / \Sigma h \Sigma i\ I_I(h)$

[c] R_{pim} = ΣhSQRT(1/(N-1)) Σi|I(ih) - <I(h)| / Σh Σi I (ih)

[d] R_{factor} = Σhkl||Fobs| − k|Fcalc||/Σhkl|Fobs|.

[e] R_{free} is calculated using the same equation as that for R factor but 10.0% of reflections were chosen randomly and omitted from the refinement

Table S4 Conservation of 7D6 and 6D6 epitopes.

SARS-CoV-2 positions	Conservation	SARS-CoV and Other SARS-related	Conservation	Conservation in sarbecovirus	Average conservation *	Average conservation of 7D6/6D6 site
346R		K/R	85.0%	92.5%		
351Y[#]		Y	100%	100%		
352A		A	100%	100%		
353W		W	100%	100%		
354N		N	10.3%	55%		
355R		R	100%	100%		
356K		K	74.7%	87.3%		
357R		R/K	100%	100%		
393T		T	33.3%	66.6%		
394N		N	74.7%	87.3%		
396Y		Y	100%	100%		
426P		P	100%	100%		
457R		R	96.6%	98.3%		
459S	99.9%	S	17.3%	58.6%	88.9%	91.5%
460N		N	4.6%	52.3%		
462K		R/K	97.7%	98.8%		
463P		P	100%	100%		
464F		F	95.4%	97.7%		
465E		E	98.9%	99.4%		
466R		R	100%	100%		
468I		I	73.6%	87.0%		
469S		S	98.9%	99.4%		
470T		T	10.3%	55.1%		
471E		E	15.0%	57.4%		
516E		E	100%	100%		
518L		L	100%	100%		
519N		N	98.9%	99.4%		
520A	99.8%	A	94.3%	97.1%		
n	415,516		85			

***** Conservation of 7D6 and 6D6 epitope in *Sarbecovirus* include clade 1, 2, 3. The epitopes of 7D6 and 6D6 in SARS-CoV-2 (N=415,516) were calculated according to GIDAID on 5 March 2021.

[#] Number in bold denoted the 7D6/6D6 sites.

Table S5 Cross-reactive or cross-neutralizing antibodies against SARS-CoV-2 and SARS-CoV.

Antibodies	Source	Reactivity	PDB no. for immune complex	Resolution (Å)	Antibody footprint on SARS-CoV-2 RBD	Identity	Reference
EY6A	Human	Cross-reactive	6ZCZ	2.64	L369, Y370, N371, F375, S376, T377, F378, K379, C380, Y381, G382, V383, S384, P385, T386, K387, N389, D390, L391, F393, A411, P412, G413, Q414, D427, D428, F429, T430, F515, F517,	28/30 (93%)	(*1*)
S304	Human	Cross-reactive	7JW0	4.30	Y369, N370, F377, K378, C379, Y380, G381, V382, S383, P384, T385, K386, N388, L390, F392, P412, G413, Q414, P426, D427, D428, F429, T430, F515, L517	23/25 (92%)	(*2*)
CR3022	Human	Cross-neutralizing	6W41	3.10	Y369, N370, S371, A372, P374, S375, T376, P377, K378, C379, Y380, G381, V382, S383, P384, T385, K386, D389, L390, P392, D427, D428, P429, T430, P515, E516, L517, H519	24/28(86%)	(*3*)
H014	Humanized	Cross-neutralizing	7CAI	3.49	Y369, A372, S373, F374, S375, T376, F377, K378, C379, Y380, S383, T385, K386, D405, V407, R408, A411, P412, G413, N439, V503	17/21(81%)	(*4*)
S309	Human	Cross-neutralizing	6WPT	3.70	T333, N334, L335, P337, G339, E340, V341, N343, A344, T345, R346, E354, K356, R357, I358, S359, N360, C361, N440, L441, K443	17/22 (77%)	(*5*)
VHH-72	Camelid	Cross-neutralizing	6WAQ	2.20	L368, Y369, N370, S371, A372, S373, F374, S375, T376, F377, K378, C379, V382, P384, T385, G404, D405, V407, R408, W436, N437, N439, V503, G504, Y508	22/27 (81%)	(*6*)
COVA1-16	Human	Cross-neutralizing	7JMW	2.89	L368, Y369, S371，F374, S375，T376，F377，K378，C379，Y380, G381，V382, S383, P384, T385, R408，Q409, P412，G413，Q414, T415, G416, D427, D428，F429	18/25（72%）	(*7*)
7D6	Mouse	Cross-neutralizing	7EAM	1.40	R346, Y351, A352, W353, N354, R355, R357, T393, N394, Y396, K462, P464, E465, R466, S469, T470, E471, E516, L518, H519, A520	16/21(76%)	This study
6D6	Mouse	Cross-neutralizing	7EAN	1.92	Y351, A352, W353, N354, R355, K356, R357, N394, Y396, P426, R457, S459, N460, K462, P463, P464, E465, R466, I468, S469, T470, E471, E516, L518, A520	20/25(80%)	This study

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