Supplemental information

N-terminal domain antigenic mapping

reveals a site of vulnerability for SARS-CoV-2

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Table S1. Summary table of NTD-specific mAbs. Related to Figure 1.

#	Donor	mAb	IgVH gene	HCDR3 length	VH % GL	IgVL gene	VL % GL	ELISA vs NTD (EC50 ng/ml)	Antigenic site	KD NTD (M)	NT IgG vs MLV-S2 pp (IC50 ng/ml)	Mx % NT	NT IgG vs live virus IC50 (ng/ml)	Mx % NT	NT Fab vs live virus IC50 (ng/ml)	Mx % NT
1		S2L11	2-70	21	98.63	K2-28	100	148.7	ii	7.25E-09	nn	nn				
2		S2L12	3-21	19	98.26	K3-15	97.49	8.943	iii	na	nn	nn				
3		S2L14	1-69	18	98.61	L4-69	98.64	507.4	iii	1.18E-08	nn	nn				
4		S2L20	3-30	15	97.92	K1-33	96.42	447.7	iv	3.05E-08	2982	98%				
5	L	S2L24	1-24	14	98.96	K1-27	99.28	602.3	i	1.17E-08	49.1	83.50%				
6		S2L26	1-24	14	97.22	L3-10	98.21	47.72	i	4.56E-09	19.41	77%				
7		**S2L28**	3-21	19	96.53	L2-14	97.57	34.26	i	1.20E-07	76.38	77%	26.2	98.20%	928	55.60%
8		S2L35	4-30	21	96.9	L1-51	98.25	22.1	iii	7.63E-09	nn	nn				
9		S2L38	3-23	17	97.22	K3-11	97.13	26.06	iii	1.62E-09	nn	nn				
10		S2L50	4-59	20	95.44	L3-25	98.92	63.72	i	5.96E-09	24.88	91%				
11	M	S2M24	4-61	20	97.25	K1-39	95.34	698.7	vi	6.98E-09	nn	nn				
12		**S2M28**	3-33	12	97.57	L3-25	97.85	31.66	i	6.89E-09	53.73	88%	5	98.50%	26.3	66%
13		S2X15	1-3	21	98.98	K3-11	96.14	12.3	iii	1.87E-09	nn	nn				
14		**S2X28**	3-30	18	97.92	L3-10	99.64	337.6	i	na	90	91%	9.1	99.10%	248.9	87.30%
15		S2X49	2-26	14	98.66	L1-40	98.98	18.5	iii	4.97E-09	nn	nn				
16		S2X51	3-21	20	98.95	K3-15	98.29	14.2	iii	3.24E-08	nn	nn				
17		S2X72	3-21	21	97.21	K3-15	98.93	19.4	iii	2.19E-08	nn	nn				
18		S2X90	4-4	13	97.61	L2-23	96.94	11.5	iii	3.35E-09	nn	nn				
19		S2X91	1-24	21	97.28	K2-24	97.64	60.5	i	3.39E-09	40.3	87%				
20		S2X93	3-33	20	97.97	K3-15	96.51	13.4	iii	6.71E-08	nn	nn				
21		S2X94	3-53	14	97.6	K3-15	98.59	8.7	iii	3.73E-08	nn	nn				
22		S2X98	3-21	24	97.61	K3-15	99.65	20	iii	6.14E-08	nn	nn				
23		S2X102	4-59	15	97.81	K3-20	96.81	24.3	iii	8.23E-09	nn	nn				
24		S2X105	4-59	17	98.29	K3-15	99.3	12.4	iii	1.39E-08	nn	nn				
25		S2X107	4-38	16	96.95	K1-39	96.83	69	i	4.08E-08	7.9	65%				
26		S2X124	3-30	22	98.98	K1-13	98.25	30	i	4.70E-08	8.9	68%				
27	X	S2X125	3-21	17	95.6	L1-40	97.57	24	iii	3.39E-09	nn	nn				
28		S2X158	1-24	16	96.25	L1-47	95.91	18.7	i	8.54E-09	32.2	96%				
29		S2X161	1-46	21	95.46	L3-25	99.64	16.4	i	8.42E-09	17	83%				
30		S2X165	4-61	20	96.65	L10-54	96.59	51.8	i	3.46E-08	61.07	98%				
31		S2X169	3-23	17	97.96	L1-51	98.64	10.9	iii	1.49E-08	nn	nn				
32		S2X170	3-21	20	96.96	K3-15	97.9	26.7	iii	1.62E-08	nn	nn				
33		S2X173	4-59	15	97.95	L3-21	97.21	52.8	v	1.22E-08	nn	nn				
34		S2X175	3-21	20	97.72	K3-15	96.167	16.8	iii	8.94E-09	nn	nn				
35		S2X176	2-70	10	98.65	K4-1	98.01	474.6	ii	1.08E-08	nn	nn				
36		S2X186	1-2	12	98.97	L2-23	98.62	18.3	iii	9.58E-09	nn	nn				
37		S2X303	2-5	17	95.88	L3-1	95.34	14.2	i	6.30E-09	79.1	85%				
38		S2X310	4-34	20	97.14	L2-23	96.53	8	iii	5.58E-09	nn	nn				
39		S2X316	3-48	18	99.66	K2-29	94.73	97.8	v	1.77E-08	nn	nn				
40		S2X320	3-33	17	96.53	K1-33	97.85	26.9	i	1.81E-08	147.9	42%				
41		**S2X333**	3-33	17	96.53	L3-21	97.49	7.6	i	2.89E-08	43.3	94%	3	98.70%	6.1	82.20%

Table S2. Cryo-EM data collection, refinement and validation statistics of NTD-specific neutralizing Fab fragments in complex with SARS-CoV-2 S. Related to Figure 2.

	S + S2M11 + **S2X333**		S + S2M11 + **S2M28**		S + S2M11 + **S2L28**	
Data collection and processing						
Magnification (nominal)	130,000		130,000		130,000	
Voltage (kV)	300		300		300	
Electron exposure (e^-/Å^2)	70		70		70	
Defocus range (µm)	0.8-2.0		0.8-2.0		0.8-2.0	
Pixel size (Å)	0.896		1.05		1.05	
Processing Type	Global	Local	Global	Local	Global	Local
Symmetry imposed	C3	C1	C3	C1	C3	C1
Initial particle images (no.)	490,229	785,859	383,144	665,979	356,514	473,310
Final particle images (no.)	261,953	99,627	221,993	282,884	157,770	81,887
Map resolution (Å)	2.2	2.8	2.5	2.6	2.6	3.0
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143
Refinement						
Initial model used (PDB code)	7K43	6ZGE	7K43	6ZGE	7K43	6ZGE
Model resolution (Å)	2.4	3.3	2.7	3.1	2.7	3.3
FSC threshold	0.5	0.5	0.5	0.5	0.5	0.5
Map sharpening B factor (Å^2)	-56	-35	-69	-26	-70	-23
Model composition						
Nonhydrogen atoms	33,987	2,524	34,471	2,805	34,353	2,740
Protein residues	4,446	317	4,470	354	4,557	352
Glycan residues	69	5	69	6	78	4
Water	729					
B factors (Å^2)						
Protein	27.7	28.6	19.4	98.7	15.9	26.9
Glycans	45.1	31.9	20.9	119.6	34.4	28.5
R.m.s. deviations						
Bond angles (°)	1.2	1.00	0.01	0.98	1.2	0.96
Bond lengths (Å)	0.01	0.01	0.99	0.01	0.01	0.01
Validation						
MolProbity score	0.87	0.65	0.69	0.69	0.71	0.87
Clashscore	1.39	0.41	0.58	0.55	0.64	0.38
Rotamer outliers (%)	0.09	0.00	0.19	0.36	0.00	0.00
Ramachandran plot						
Favored (%)	98.2	98.7	98	98.8	98	96.43
Allowed (%)	1.8	1.3	2	1.2	2	3.57
EMRinger score	4.97	5.37	4.3	5.23	3.89	4.2
Data Availability						
EMDB	EMD-23579	EMD-23577	EMD-23582	EMD-23581	EMD-23580	EMD-23578
PDB	PDB 7LXY	PDB 7LXW	PDB 7LY2	PDB 7LY0	PDB 7LXZ	PDB 7LXX

Table S3. Data collection and refinement statistics. Related to Figure 2.

	SARS-CoV-2 NTD + S2M28 Fab
Data collection	
Facility	ALS
Beamline	5.0.2
Wavelength (Å)	0.97741
Space group	$P\,2_1\,2_1\,2_1$
Cell dimensions	
a, b, c (Å)	66.4 125.5 365.9
α, β, γ (°)	90, 90, 90
Resolution Range (Å)	50-3.0 (3.1-3.0)
R_{Sym} [†]	0.19 (2.16)
R_{pim} [†]	0.05 (0.60)
CC^* [†]	1.00 (0.91)
$I / \sigma I$	12.60 (1.39)
Completeness (%)	100 (100)
Redundancy	13.3 (13.5)
Refinement	
No. reflections	62,425
R_{work} / R_{free} [‡]	0.209 / 0.234
No. atoms	
Protein	11009
Ligand/ion	465
Water	0
B-factors	
Protein	91.4
Ligand/ion	150.2
R.m.s. deviations	
Bond lengths (Å)	0.04
Bond angles (°)	1.6
Ramachandran Statistics	
Favored	97.23
Allowed	100
Data availability	PDB 7LY3

Note: Values in parentheses correspond to the highest resolution shell.

[†], $R_{Sym} = \sum\sum| I - (I) | / \sum\sum I$, $R_{Pim} = \sum\sqrt{(1/(n-1)}\sum| I - (I) | / \sum\sum I$, and $CC^* = \sqrt{(2CC_{1/2}/(1+CC_{1/2}))}$ where $CC_{1/2}$ is the Pearson correlation coefficient of two half data sets as described elsewhere (Karplus and Diederichs, 2012)

[‡], $R_{work} = \sum | |F_{obs}| - k|F_{calc}| | / |F_{obs}|$ where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. R_{free} is the sum extended over a subset of reflections (5%) excluded from all stages of the refinement.

Table S4. Cryo-EM data collection, refinement and validation statistics of NTD-specific non neutralizing Fab fragments in complex with SARS-CoV-2 S. Related to Figure 2.

	2P-DS-S + S2X28	S + S2M11 + S2L20	S + S2M11 + S2X316	S + S2M11 + S2M24
Data collection				
Magnification (nominal)	130,000	36,000	130,000	130,000
Voltage (kV)	300	200	200	200
Electron exposure (e⁻/Å²)	60	60	60	60
Defocus range (μm)	0.8-2.0	0.8-2.0	0.8-2.0	0.8-2.0
Pixel size (Å)	1.16	1.16	1.16	1.16
Processing				
Symmetry imposed	C1	C3	C3	C3
Initial particle images (no.)	140,562	8,345	11,695	5,840
Final particle images (no.)	36,413	3,588	4,340	3,770
Map resolution (Å)	~4-5	6.3	6.0	6.5
FSC threshold	0.143	0.143	0.143	0.143
Map sharpening B factor (Å²)	-28	-264	-168	-255
Data Availability				
EMDB	EMD-23584	EMD-23586	EMD-23585	EMD-23583

Table S5. Percentage of S2X28 binding inhibition to NTD by plasma samples as measured in a blockade-of-binding ELISA. Related to Figure 3.

Status	Sample code	% S2X28 inhibition	SD%
Hospitalized	P5-OLS	69.38	3.12
	P16-OLS	25.73	2.68
	P6-OLS	75.39	5.03
	P7-OLS	70.12	0.86
Symptomatic	P6-DVL	17.63	1.84
	P2-CLM	20.49	0.73
	P5-DVL	25.61	1.26
Asymptomatic	AS-4-FR	39.13	3.24
	AS-1-AL	21.30	4.70
Convalescent-Vx1	CLM-A-001	87.63	1.75
	CLM-A-002	60.26	0.14
	CLM-A-003	75.25	0.49
	CLM-A-004	56.06	12.65
	CLM-A-005	57.14	1.41
Vx1	CLM-B-002	9.97	2.51
	CLM-B-003	3.79	1.26
	CLM-B-004	3.55	0.17
	CLM-B-005	3.86	0.10
	CLM-B-006	9.95	0.85

Note: Values collected at 1:6 plasma dilution.
Convalescent-Vx1= plasma collected 14 days after administration of the first dose of mRNA-based vaccine from individuals previously exposed to SARS-CoV-2; Vx1 = plasma collected 14 days after administration of the first dose of mRNA-based vaccine from individual with no history of SARS-CoV-2 infections.

Data S1. Extended data sets

Related to Figure 1

Binding of the 41 isolated NTD mAbs to immobilized SARS-CoV-2 S ectodomain trimer, NTD (domain A) or RBD analyzed by ELISA. (Pages 2-3)

SARS-CoV-2 S pseudovirus neutralization. (Pages 4-5)

Related to Figure 3

Epitope binning of the 41 NTD-specific mAbs isolated using the SARS-CoV-2 S ectodomain trimer or the isolated NTD. (Pages 6-9)

**Related to Figure 4**

NTD mAb binding to a panel of sarbecovirus S glycoproteins analyzed by flow cytometry (MFI values). (Page 10)

Related to Figures 4 and 5

Binding of the S2X333, S2M28, S2L28, S2X28, S2L20 or 4A8 mAbs to immobilized NTD mutants analyzed by ELISA. (Page 11)

Related to Figure 5

S2L28, S2M28, S2X28, and S2X333 escape clone sequencing results (VSV SARS-CoV-2 S experiments). (Page 12)

Binding of the 41 isolated NTD mAbs to immobilized SARS-CoV-2 S ectodomain trimer, NTD (domain A) or RBD analyzed by ELISA. Related to Figure 1.

S2X161

OD 405 nm / ng/ml

	EC50
Spike	23.68
RBD	145058
Dom A	16.41

S2X165

OD 405 nm / ng/ml

	EC50
Spike	65.89
RBD	1212703
Dom A	51.77

S2X169

OD 405 nm / ng/ml

	EC50
Spike	30.61
RBD	185472
Dom A	10.87

S2X170

OD 405 nm / ng/ml

	EC50
Spike	26.51
RBD	315801
Dom A	26.75

S2X173

OD 405 nm / ng/ml

	EC50
Spike	22.81
RBD	1081081
Dom A	52.76

S2X175

OD 405 nm / ng/ml

	EC50
Spike	18.69
RBD	1705061
Dom A	16.83

S2X176

OD 405 nm / ng/ml

	EC50
Spike	188.3
RBD	10754895
Dom A	474.6

S2X186

OD 405 nm / ng/ml

	EC50
Spike	38.38
RBD	293385
Dom A	18.29

S2X303

OD 405 nm / ng/ml

	EC50
Spike	48.42
RBD	11971824
Dom A	14.16

S2X310

OD 405 nm / ng/ml

	EC50
Spike	26.89
RBD	17703864
Dom A	8.000

S2X316

OD 405 nm / ng/ml

	EC50
Spike	64.43
RBD	265868
Dom A	97.83

S2X320

OD 405 nm / ng/ml

	EC50
Spike	29.83
RBD	15427
Dom A	13.46

S2X333

OD 405 nm / ng/ml

	EC50
Spike	25.97
RBD	88280155
Dom A	7.644

SARS-CoV-2 S pseudovirus neutralization. Related to Figure 1.
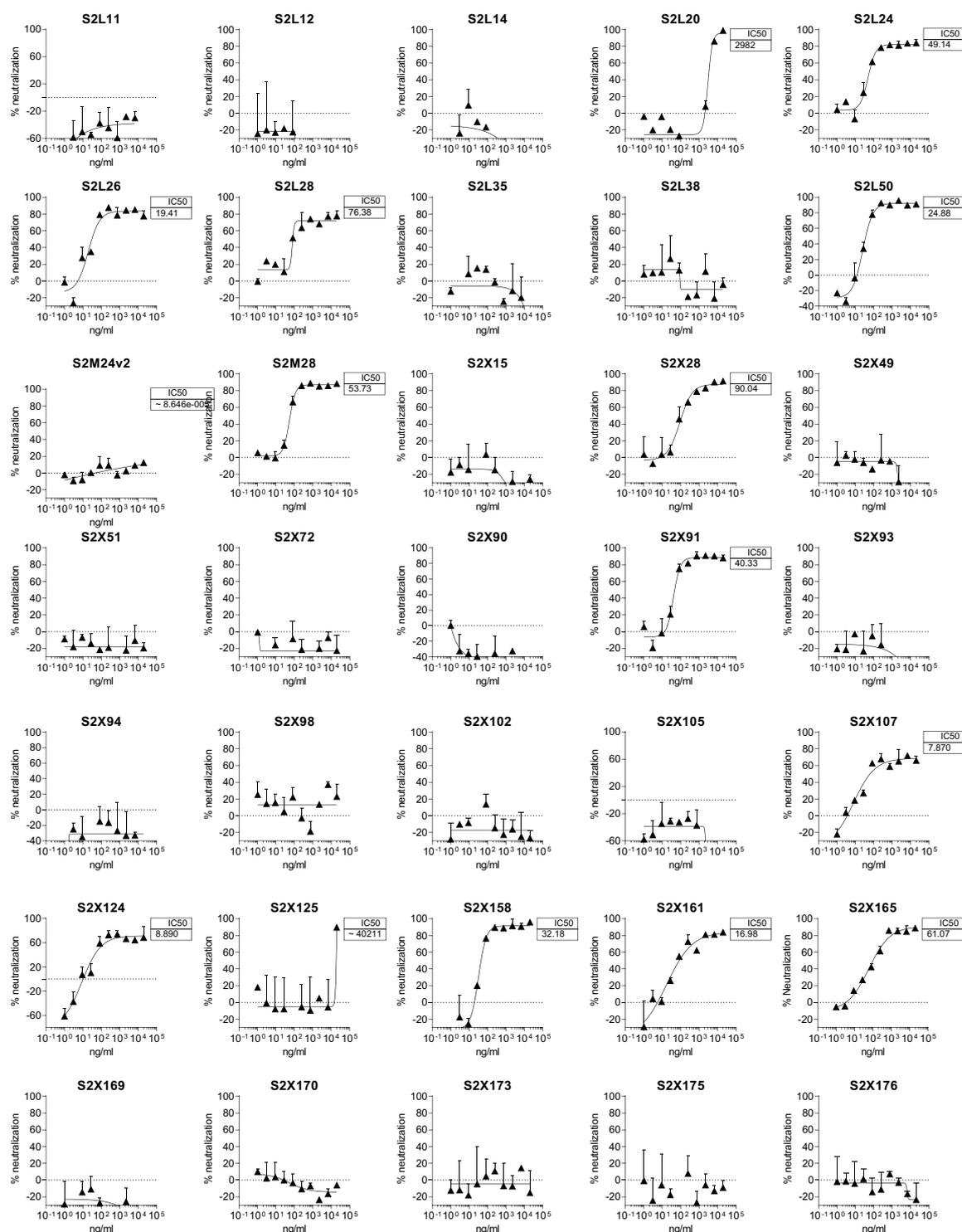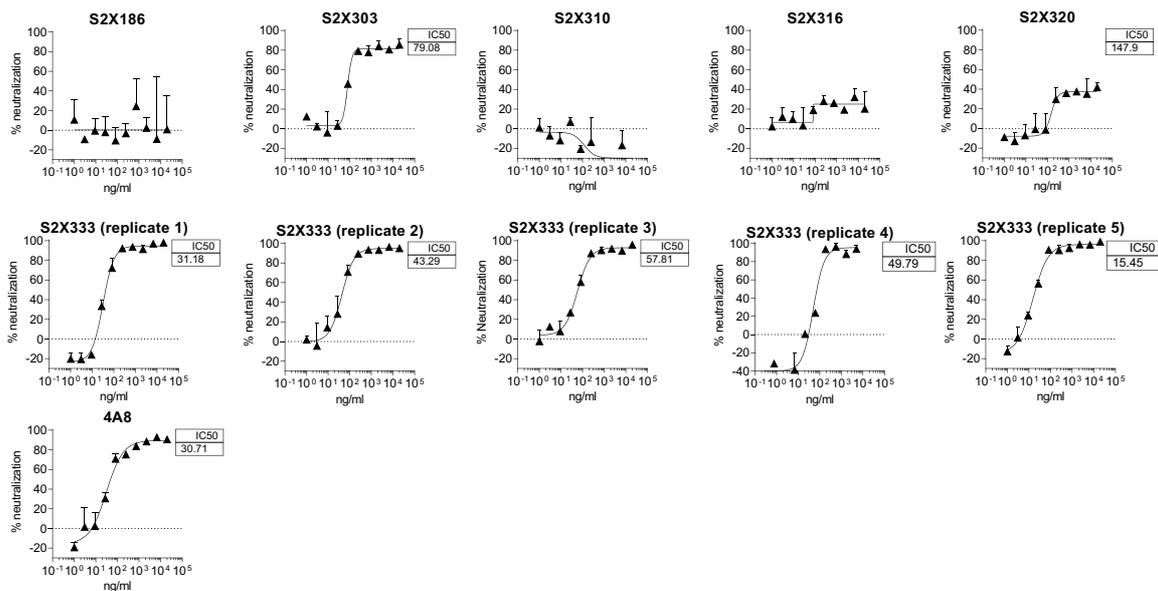
Epitope binning of the 41 NTD-specific mAbs isolated using the SARS-CoV-2 S ectodomain trimer or the isolated NTD. Related to Figure 3.
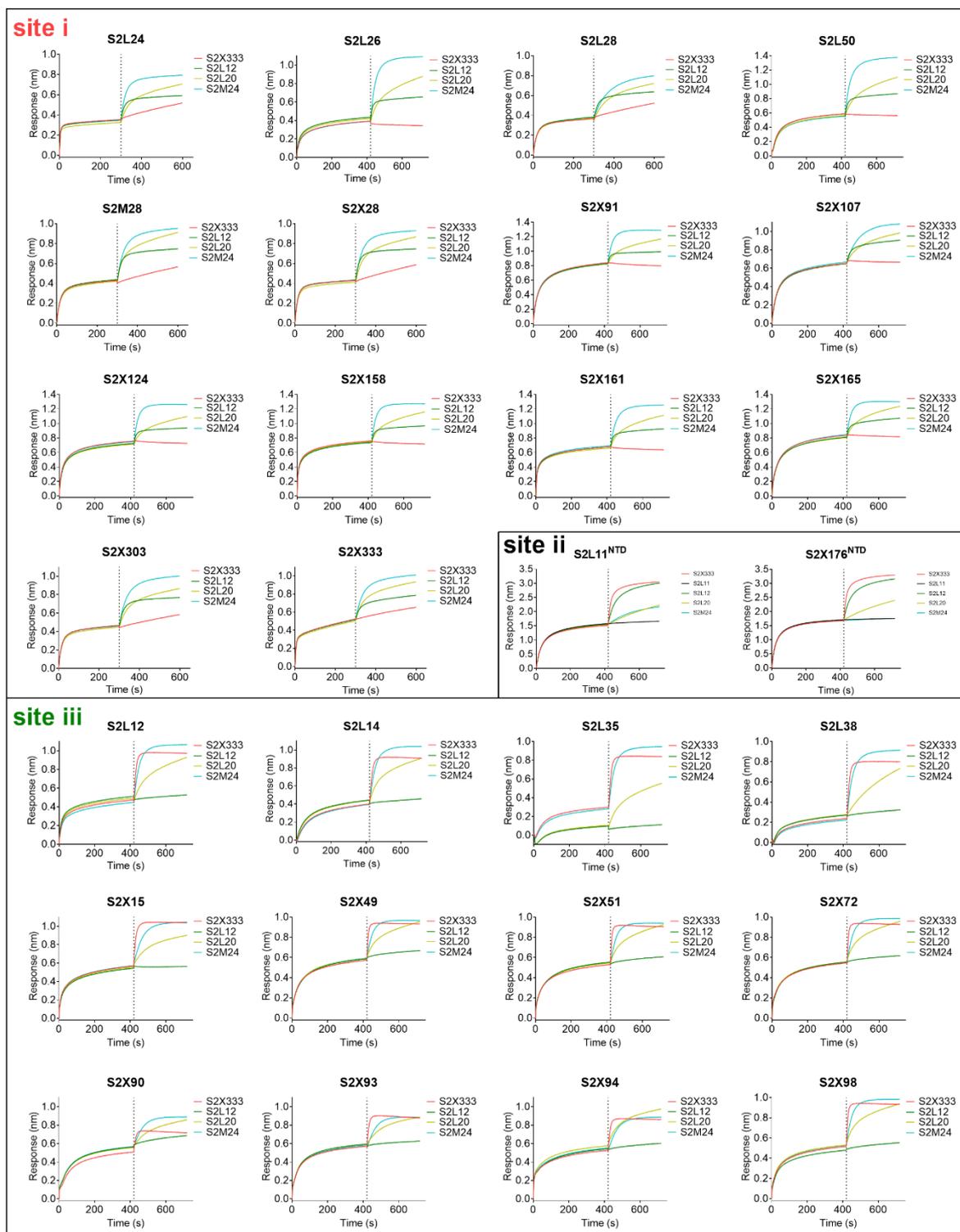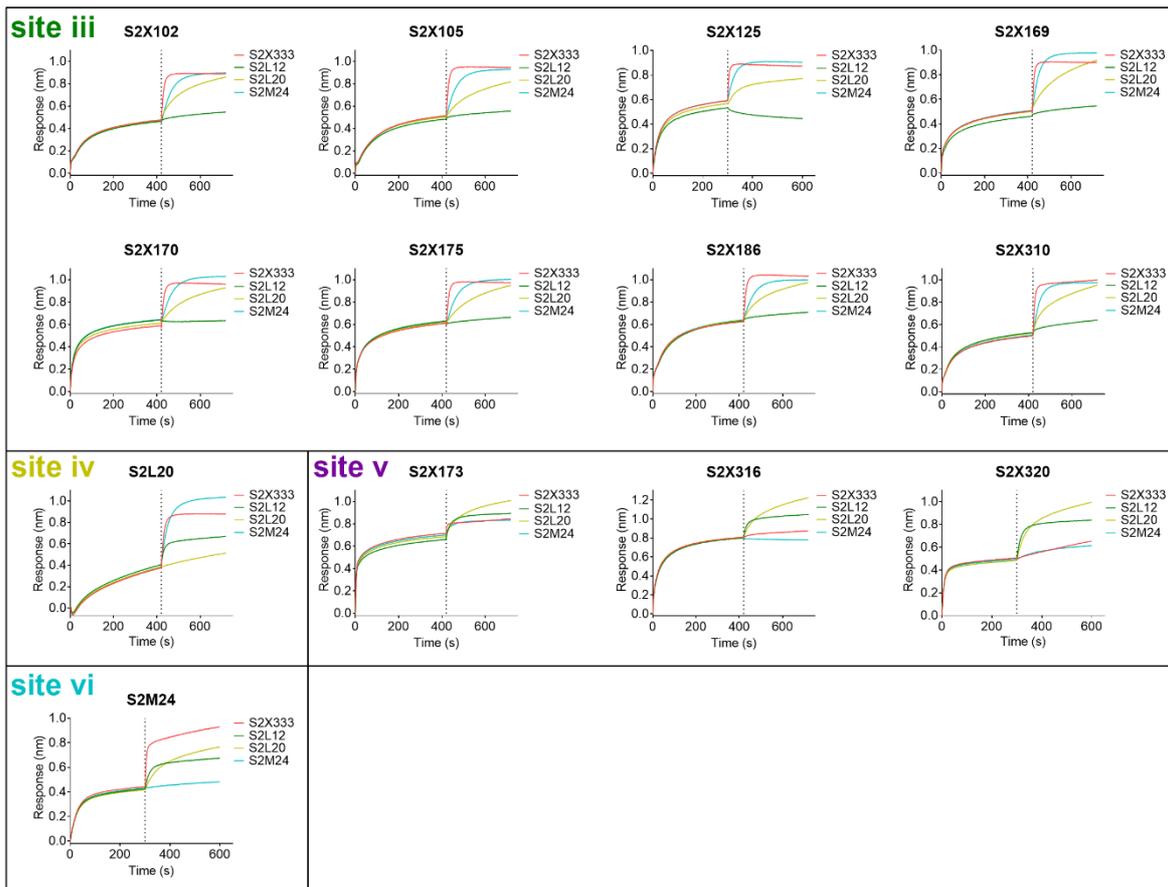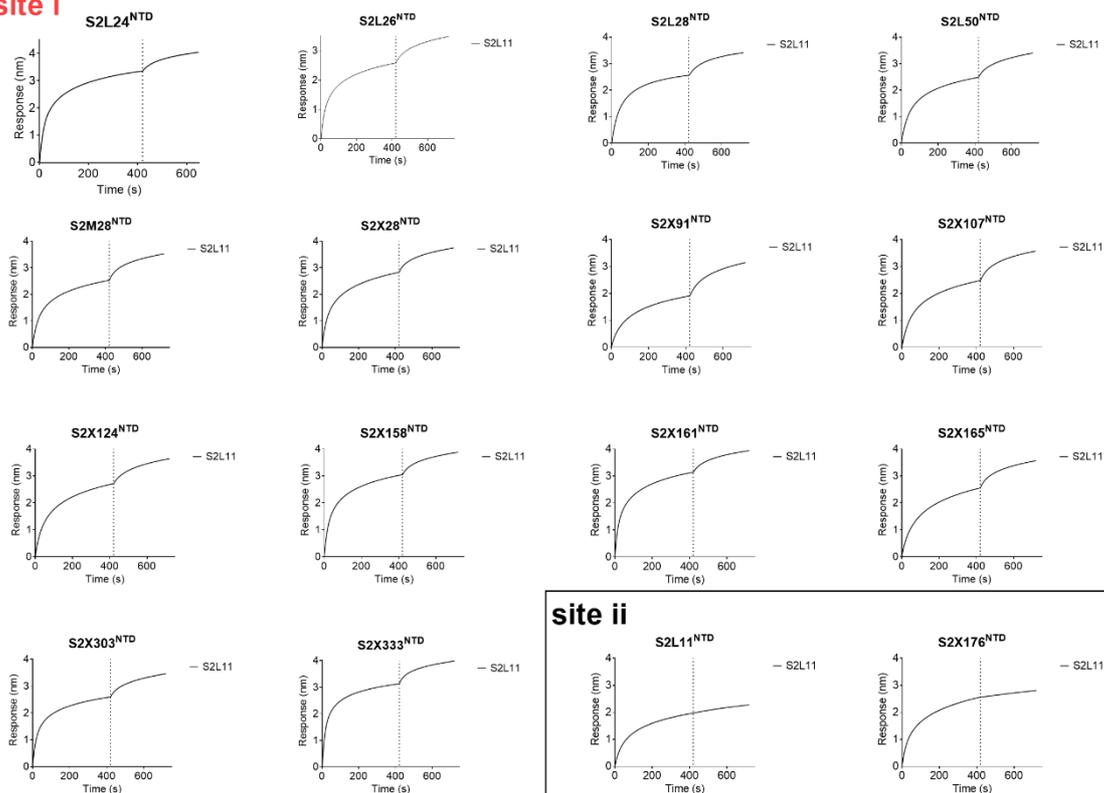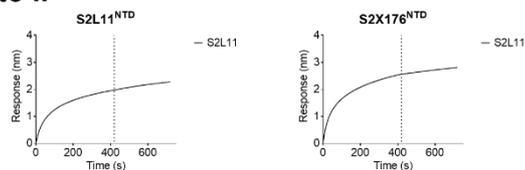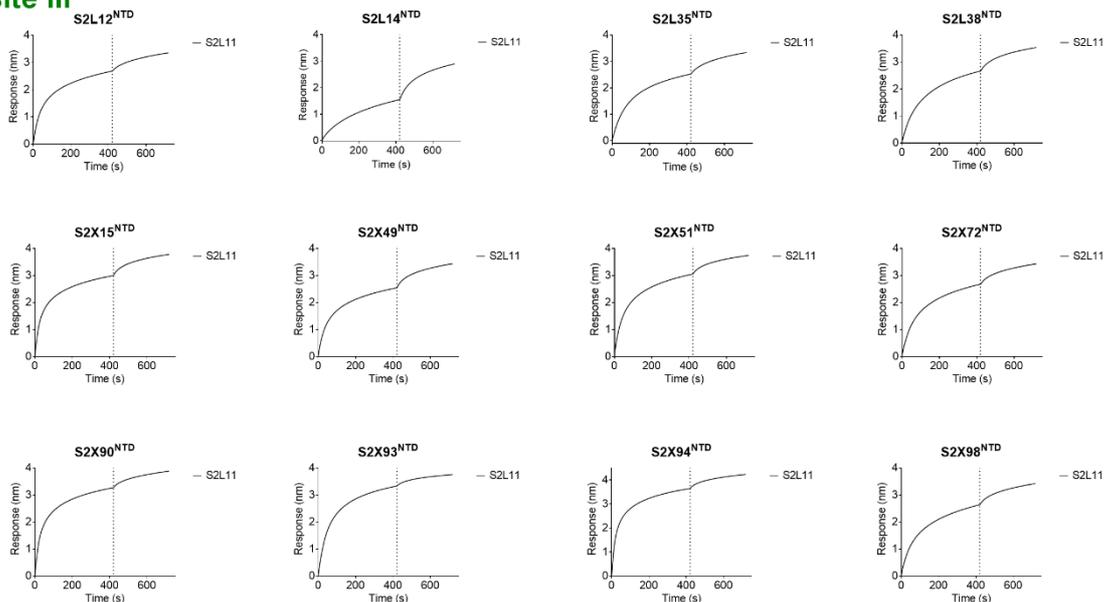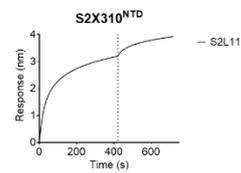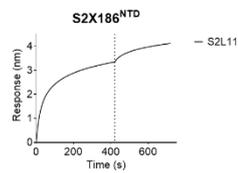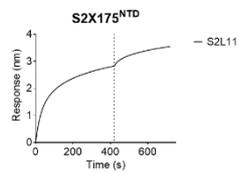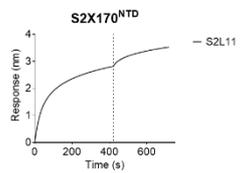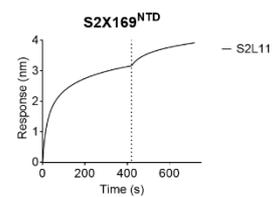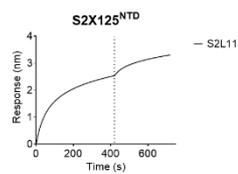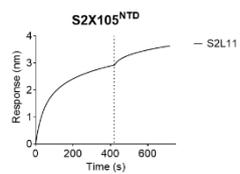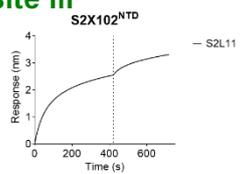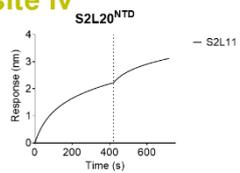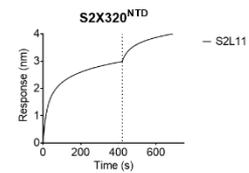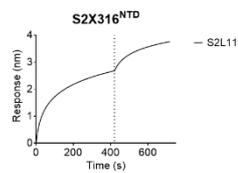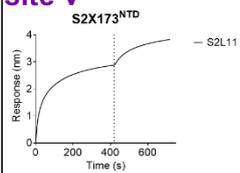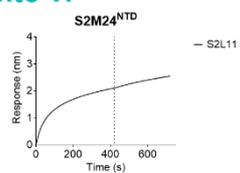
NTD mAb binding to a panel of sarbecovirus S glycoproteins analyzed by flow cytometry (MFI values). Related to Figure 4.

| Strain										mAb							
		S2L20	S2L24	S2L26	S2L28	S2L50	S2M28	S2X28	S2X91	S2X107	S2X124	S2X158	S2X161	S2X165	S2X303	S2X320	S2X333
	SARS-CoV2	424	772	661	715	559	955	670	887	1313	814	724	760	893	1091	1077	921
	RaTG13	280	830	684	766	611	882	807	753	1069	740	722	767	844	849	1109	892
	Pangolin-GX	931	352	144	71.7	44.7	404	53	473	102	1310	43.3	36.4	66.5	386	386	386
	Pangolin-GD	32.6	34.8	35.3	133	40.8	191	69.5	37.8	77.9	31.6	25.9	28.5	37.9	61.1	77.1	64.6
	ZC45	23.8	22.3	22	21.5	20.3	44.1	43.3	28.3	73.4	26.3	24.7	25	41.4	44.6	45.5	38
	ZXC21	25.2	24.6	22.4	22.3	21	49.4	48.2	30.4	76.4	24.8	25.2	26.7	43.7	45	50	39.3
	SARS-CoV1	39.9	31.4	28.5	63.3	27.2	125	40.2	24.9	50.4	21.3	21.1	23.5	37.8	32.5	46.3	32.1
	YN2013	29.8	25.6	25.8	24.9	21.2	29.9	27.9	20.7	52.2	18.7	17	19.2	39.6	29.3	36.1	30.4
	BtK72	28.2	26.3	27.4	26.7	25.4	30.6	30.3	18.6	51.5	18.6	17.6	18.3	36.5	30.6	36.8	26.5
	BM48-31	32.6	29.9	29.1	30	25.6	31.8	30.7	19.8	55.1	18.6	17.5	18.8	37.2	29	35.8	27.7
	RmYN02	24.4	23.3	23.2	23.2	19.2	49.5	33.4	29.8	80.1	27.2	24.3	25.3	49.2	44.1	46	40.1

Binding of the S2X333, S2M28, S2L28, S2X28, S2L20 or 4A8 mAbs to immobilized NTD mutants analyzed by ELISA. Related to Figures 4 and 5.
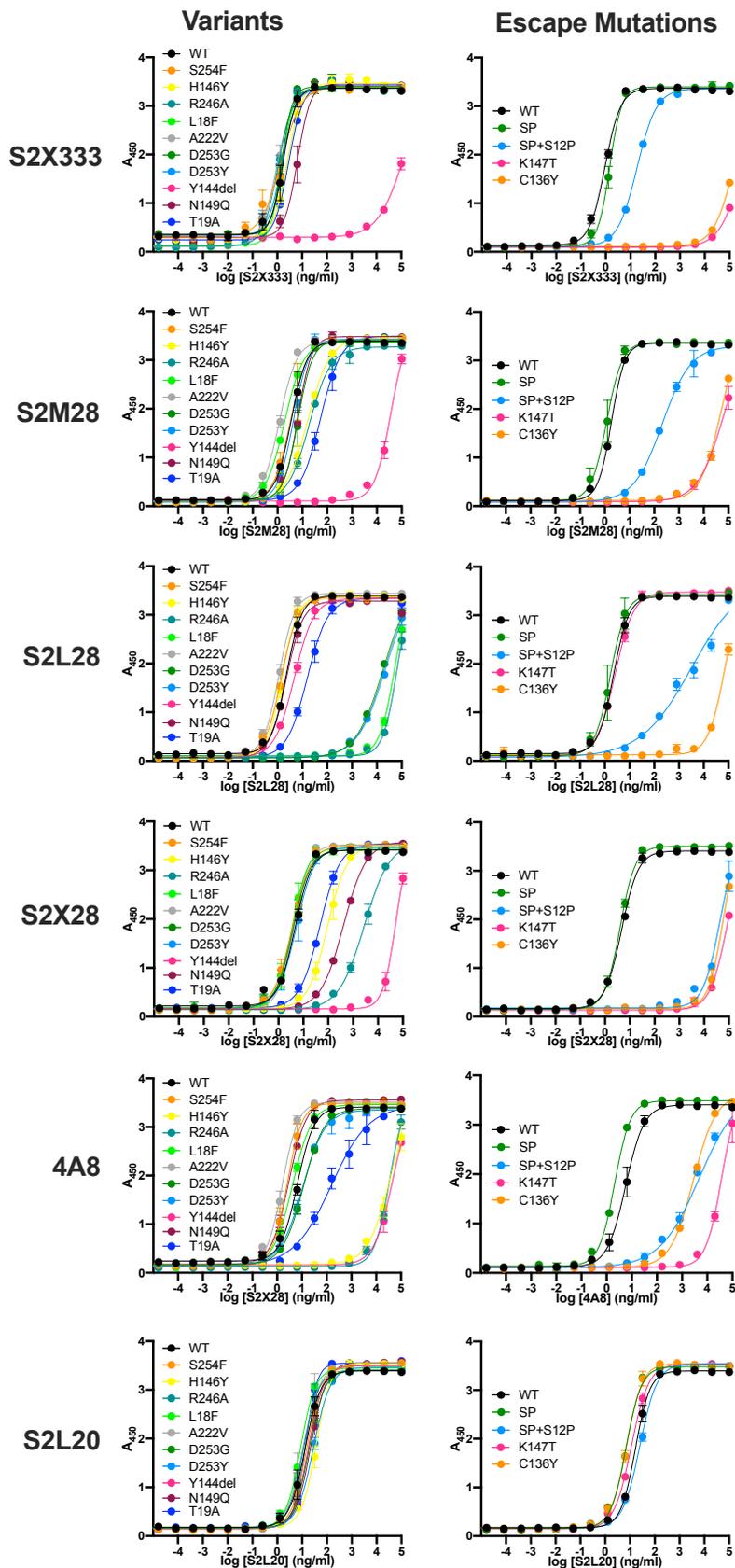
S2L28, S2M28, S2X28, and S2X333 escape clone sequencing results (VSV SARS-CoV-2 S experiments). Related to Figure 5.

S2L28		
AA mutation	NT mutation	counts
P9S	C25T	3
L18P	TC53C	3
T22P	A63C	1
K77E	A229G	2
D80N	G238A	2
P139S	C415T	1
F140S	T419C	1
S247R	A739C	13
D253E	T759G	1
D253G	A758G	1
S255F	C764T	2
G257S	G769A	1
W258R	T772A	1

S2M28		
AA mutation	NT mutation	counts
P9Q	C26T	2
P9S	C25T	3
S12P	T34C	1
C15F	G44T	1
C15R	T43C	1
L18P	T53C	10
Y28C	A83G	1
G142D	G425A	1
K147Q	A439C	1
K147T	A440C	2
R246G	A736G	4
P251L	C752T	1
G252C	G754T	1

S2X28		
AA mutation	NT mutation	counts
P9S	C25T	1
S12P	T34C	3
C15W	T45G	1
L18P	T53C	6
C136G	T406G	1
P139Q	C416A	1
F140S	T419C	1
L141S	T422C	2
G142C	G424T	1
G142D	G425A	2
Y144C	T430A	1
Y144N	A431G	1
K147E	A439G	1
K147Q	A439C	3
R158G	A472G	1
L244S	T731C	5
R246G	A736G	7

S2X333		
AA mutation	NT mutation	counts
P9L	C26T	1
S12P	T34C	2
C15S	T43C	2
C15Y	G44A	2
L18P	T53C	3
C136S	T406A	1
C136Y	G407A	3
F140C	T419G	1
G142D	G425A	1
K147E	A439G	1
K147T	A440C	1