

Supplemental information

Therapeutic antibodies, targeting the SARS-CoV-2

spike N-terminal domain, protect

lethally infected K18-hACE2 mice

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Table S1. *In vitro* neutralization of SARS-CoV-2 by NTD-specific mAbs

mAb	IC₅₀ (µg/ml)
BLN1	0.008
BLN2	0.25
BLN3	0.10
BLN4	9
BLN5	0.26
BLN6	5.4
BLN7	0.07
BLN8	54.9
BLN9	0.39
BLN10	0.13
BLN12	0.008
BLN14	0.03

SARS-CoV-2 *in vitro* neutralization potency of the selected anti-NTD mAbs, was evaluated by plaque reduction neutralization test (PRNT). Half-maximum inhibitory concentration (IC₅₀) values are presented. Relates to Figure 1.

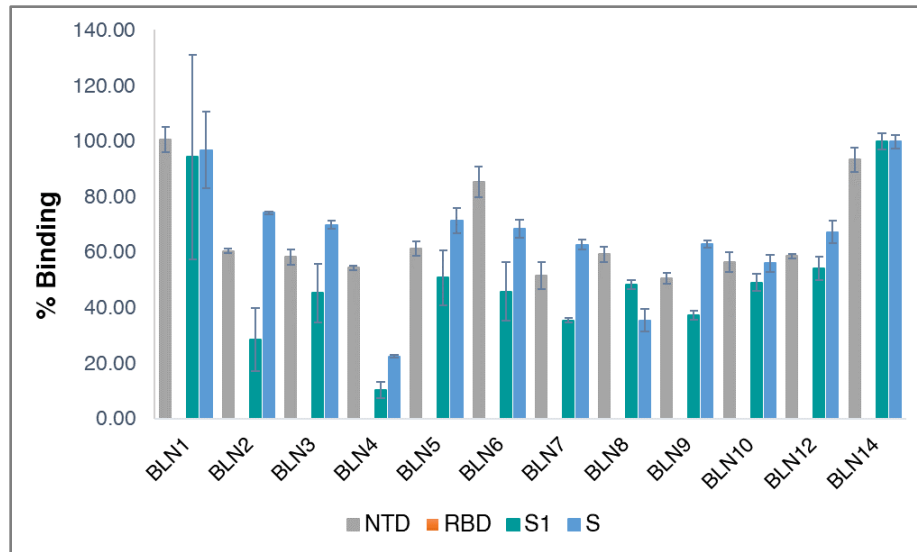


Figure S3: Normalized binding specificity of anti-NTD mAbs. Relates to Figure 1A. The specificity of each mAb was tested by ELISA using the indicated proteins as capturing antigens, at 1 $\mu\text{g/ml}$ each. The obtained O.D. values, were normalized to each antigen Bmax value. Data represent average of triplicates \pm SEM. RBD values were below detection limit.

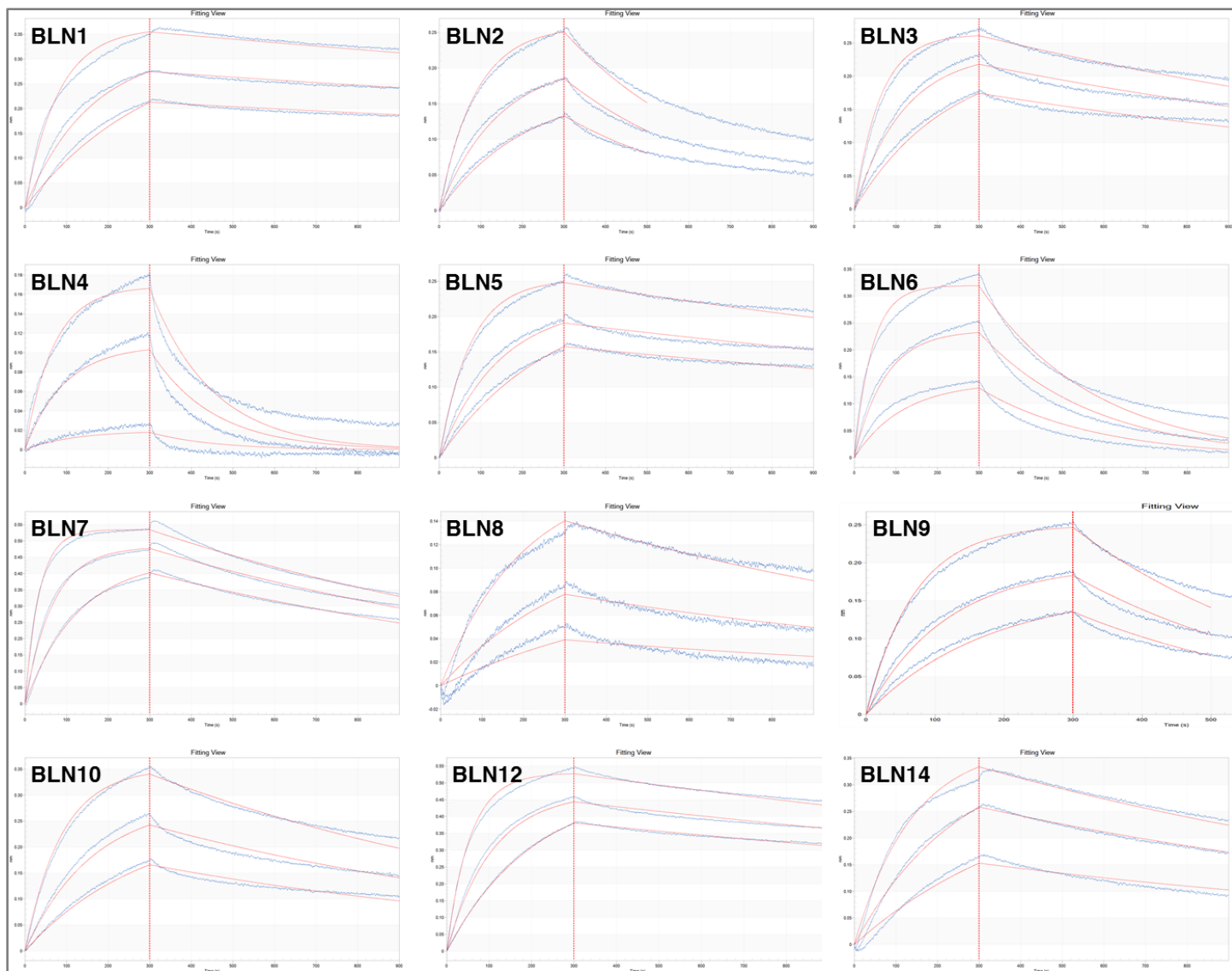


Figure S4. NTD-specific mAbs affinity determination by BLI. Relates to Table 2.

Biolayer interferometry analysis used for the determination of the 12 selected anti-NTD mAbs. Streptavidin-coated biosensors were loaded with each biotinylated mAb and reacted for 300 s with the indicated concentrations of the recombinant NTD (association phase) and then transferred to buffer-containing wells for another 600 s (dissociation phase). Sensograms (after subtraction of parallel measurements from unloaded biosensors) were fitted with 1:1 binding model (red curves) using the Octet data analysis software 8.1.

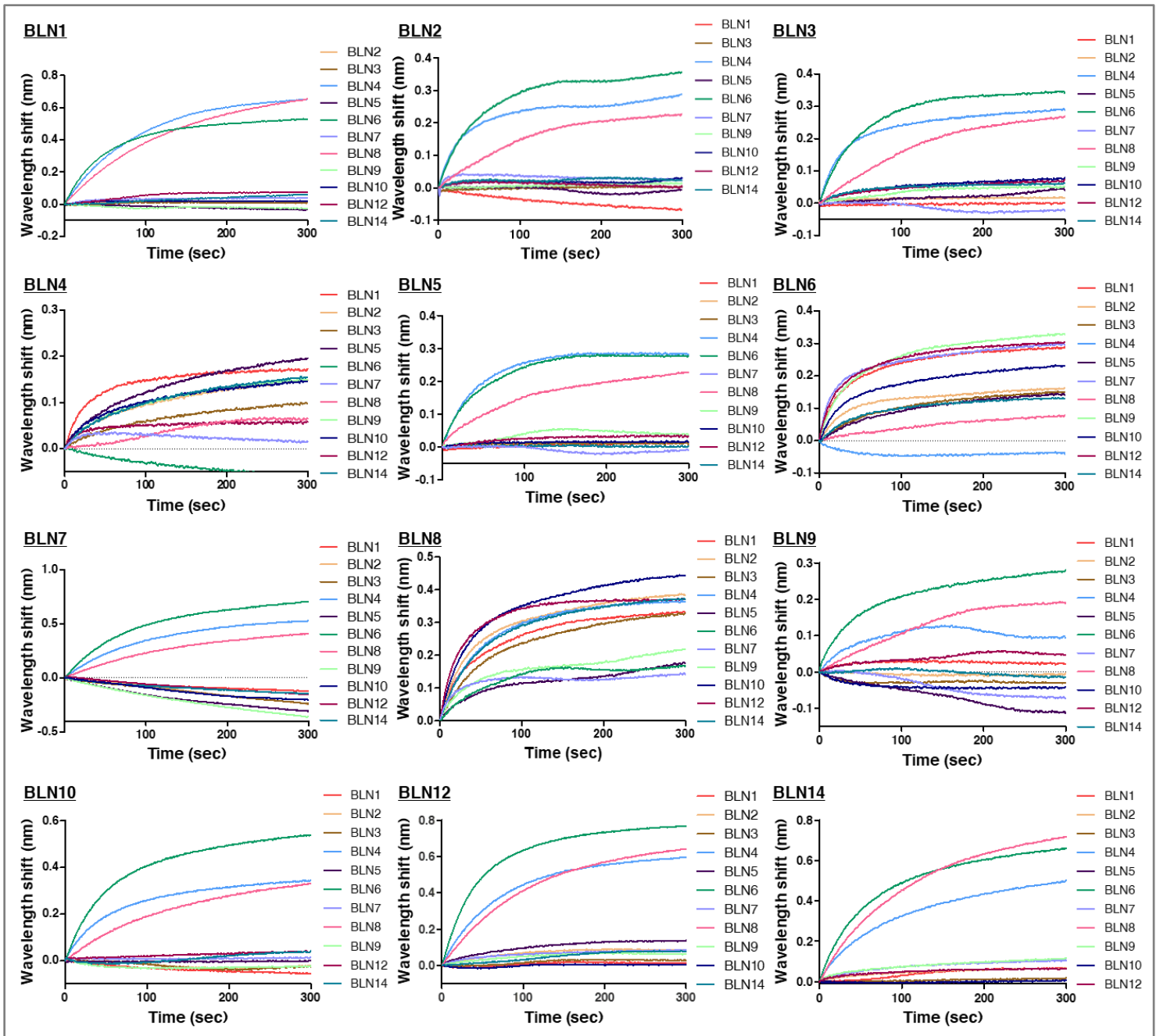


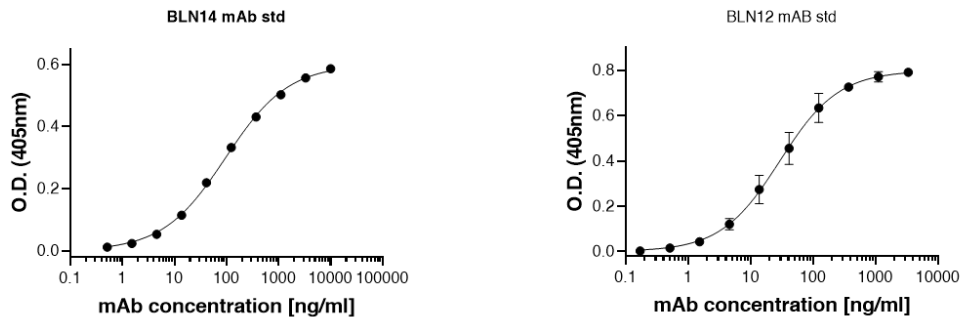
Figure S5. Epitope binning of the selected anti-NTD mAbs, by Biolayer interferometry (BLI). Relates to Figure 2. Each purified antibody (indicated on the left upper side of each sensogram) was biotinylated, immobilized on a streptavidin biosensor and saturated with S1. The complex (mAb-S1; Time 0) was then incubated for 300 s with each one of the other antibodies (colored differently according to the mAb, as indicated within the panels). Competition of each mAb with itself, served as a negative control, subtracted from the data before generation of the depicted sensograms.

A

Blanked Data [OD 405]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.003	0	0	0.003	0.004	0.002	0.004	0.004	0.003	0.001	-0.003	-0.004
B	0.001	0.005	0.004	0.008	0.005	0.003	0	0	0.005	0.003	-0.003	-0.004
C	0.004	0.007	0.012	0.02	0.011	0.004	0.006	0.005	0.011	0.008	0.007	0.001
D	0.004	0.017	0.016	0.022	0.015	0.016	0.014	0.017	0.015	0.014	0.011	0.003
E	0.01	0.018	0.016	0.016	0.019	0.021	0.015	0.014	0.01	0.01	0.009	0.014
F	0.59	0.55	0.51	0.44	0.34	0.23	0.13	0.07	0.03	0.02	0.01	0.003
G	0.59	0.56	0.50	0.44	0.33	0.21	0.11	0.05	0.02	0.01	0.01	0.001
H	0.58	0.55	0.49	0.42	0.33	0.22	0.11	0.04	0.02	0.01	0.00	-0.004
												BLN14 treated mice sera
Plate description												
	1	2	3	4	5	6	7	8	9	10	11	12
A	M1 1:10	M1 1:20	M1 1:40	M1 1:80	M6 1:10	M6 1:20	M6 1:40	M6 1:80	M11 1:10	M11 1:20	M11 1:40	M11 1:80
B	M2 1:10	M2 1:20	M2 1:40	M2 1:80	M7 1:10	M7 1:20	M7 1:40	M7 1:80	M12 1:10	M12 1:20	M12 1:40	M12 1:80
C	M3 1:10	M3 1:20	M3 1:40	M3 1:80	M8 1:10	M8 1:20	M8 1:40	M8 1:80	M13 1:10	M13 1:20	M13 1:40	M13 1:80
D	M4 1:10	M4 1:20	M4 1:40	M4 1:80	M9 1:10	M9 1:20	M9 1:40	M9 1:80	M14 1:10	M14 1:20	M14 1:40	M14 1:80
E	M5 1:10	M5 1:20	M5 1:40	M5 1:80	M10 1:10	M10 1:20	M10 1:40	M10 1:80	M15 1:10	M15 1:20	M15 1:40	M15 1:80
F	10,000.00	3,333.33	1,111.11	370.37	123.46	41.15	13.72	4.57	1.52	0.51	0.17	Blk
G	10,000.00	3,333.33	1,111.11	370.37	123.46	41.15	13.72	4.57	1.52	0.51	0.17	Blk
H	10,000.00	3,333.33	1,111.11	370.37	123.46	41.15	13.72	4.57	1.52	0.51	0.17	Blk
												BLN14 Std 10,000-0.17 ng/ml
M1-M10: 0.1 mg BLN14 treated mice												
M11-M15 0.01 mg BLN14 treated mice												

B

Blanked Data [OD 405]												
	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.004	-0.001	0.009	0.016	0.021	0.02	0.019	0.017	0.01			-0.003
B	0.005	0.012	0.021	0.013	0.017	0.013	0.009	0.006	0.005			0.007
C	0.005	0.018	0.02	0.015	0.015	0.011	0.004	0.009	0.01			0.01
D	0.012	0.014	0.021	0.021	0.018	0.01	0.013	0.016	0.02			0.013
E	0.004	0.014	0.019	0.02	0.015	0.014	0.016	0.017	0.014			0.01
F	0.008	0.012	0.013	0.013	0.011	0.007	0.003	0.002	0.003			0.006
G	0.83	0.78	0.76	0.72	0.59	0.41	0.23	0.10	0.04	0.01	0.00	0.001
H	0.82	0.80	0.79	0.73	0.68	0.51	0.32	0.14	0.05	0.02	0.00	-0.001
												BLN12 Std 10,000-0.17 ng/ml
Plate description												
	1	2	3	4	5	6	7	8	9	10	11	12
A	M1 1:10	M2 1:10	M3 1:10	M4 1:10	M5 1:10	M6 1:10	M7 1:10	M8 1:10	M9 1:10		NC 1:10	
B	M1 1:20	M2 1:20	M3 1:20	M4 1:20	M5 1:20	M6 1:20	M7 1:20	M8 1:20	M9 1:20		NC 1:20	
C	M1 1:40	M2 1:40	M3 1:40	M4 1:40	M5 1:40	M6 1:40	M7 1:40	M8 1:40	M9 1:40		NC 1:40	
D	M1 1:80	M2 1:80	M3 1:80	M4 1:80	M5 1:80	M6 1:80	M7 1:80	M8 1:80	M9 1:80		NC 1:80	
E	M1 1:160	M2 1:160	M3 1:160	M4 1:160	M5 1:160	M6 1:160	M7 1:160	M8 1:160	M9 1:160		NC 1:160	
F	M1 1:320	M2 1:320	M3 1:320	M4 1:320	M5 1:320	M6 1:320	M7 1:320	M8 1:320	M9 1:320		NC 1:320	
G	10,000.00	3,333.33	1,111.11	370.37	123.46	41.15	13.72	4.57	1.52	0.51	0.17	Blk
H	10,000.00	3,333.33	1,111.11	370.37	123.46	41.15	13.72	4.57	1.52	0.51	0.17	Blk
												BLN12 Std 10,000-0.17 ng/ml
M1-M4: 0.1 mg BLN12 treated mice												
M5-M9: 0.01 mg BLN12 treated mice												

C**Figure S7: Quantification of human antibodies in mice sera, at 21 dpi. Relates to Figure 4.**

Mice were treated with BLN12 and BLN14 mAbs at 2 dpi and their residual amount in the serum was evaluated at 21 dpi, by ELISA, using anti-human specific secondary antibody. **A-B.** No residual human antibodies could be detected in the mice serum, tested at the indicated dilutions. **C.** A standard curve, was constructed for each mAb, in the range of 10,000-0.17 ng/ml, showing a detection limit of 1.5 ng/ml and 4.6 ng/ml for BLN12 and BLN14, respectively. These concentrations are also below the limit of detection of the neutralization assay used to evaluate these sera samples.