Supplementary Materials for

Short-term instantaneous prophylaxis and efficient treatment against SARS-CoV-2 in hACE2 mice conferred by an intranasal nanobody (Nb22)

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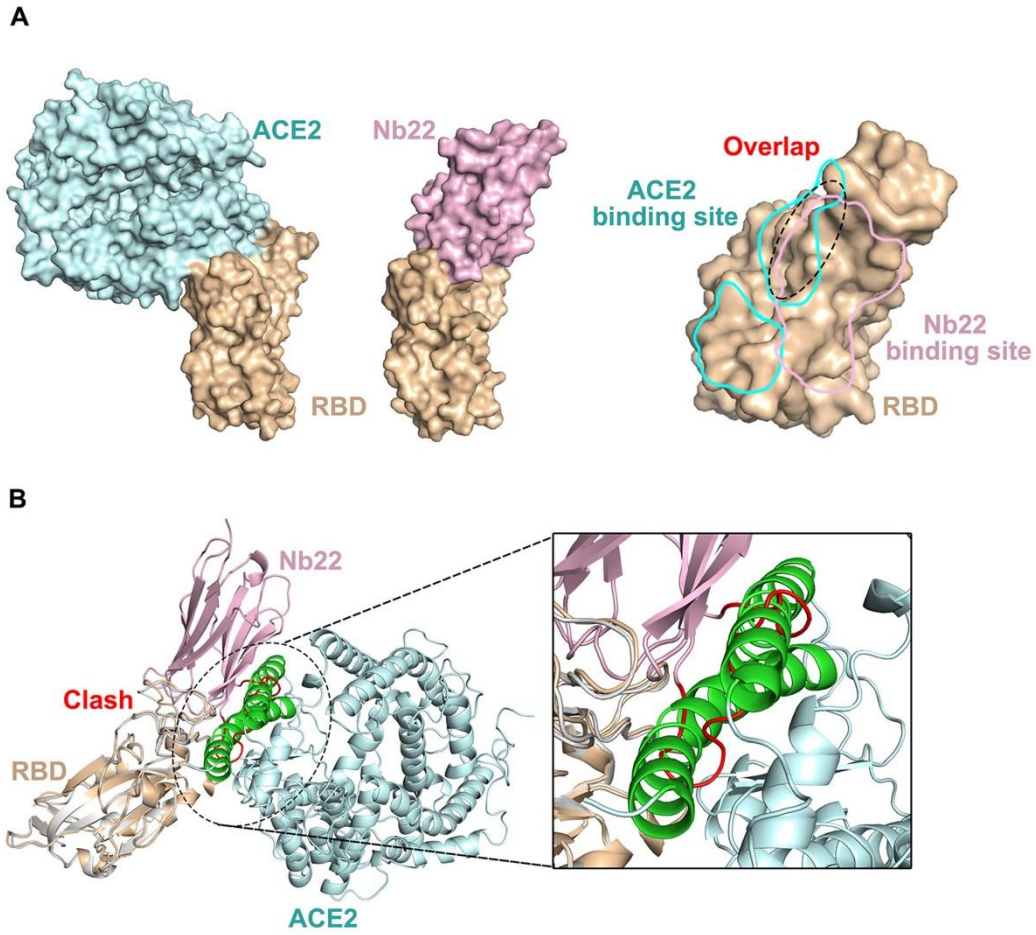
Supplementary Figure 1. Nb22 blocks the binding of hACE2 to WH01 RBD. (**A**) Overlap of Nb22 and hACE2 binding sites on WH01 RBD. hACE2 binding site on WH01 RBD is shown in cyan line. Nb22 binding site is shown in pink line. The overlap region is represented by ellipses with dashed lines. (**B**) The loop (V102-Y117) of Nb22 is clashed with the two helixes on N-terminal of hACE2. The loop is colored in red and helixes are colored in green.

Supplementary Figure 2. Spational distribution of Nb22 labeled with dye YF®750 SE. Mice were dissected and detected by NightOwl LB 983 after 200 μg Nb22-YF®750 SE infusion into mice as indicated in figure 5C. The fluorescence intensity was measured at 2 hours (0d-Nb22), 7d (7d-Nb22), 14d (14d-Nb22), 21d（21d-Nb22） and 28d（8d-Nb22） after infusion of Nb22 via i.n., respectively. Blank, blank mice without infusion of any antibody, was taken as blank control. The fluorescence intensity of various organs including trachea (Tr), lung (Lu), heart (H) , stomach(St), intestine (In), liver(Li), spleen (Sp), kidney (Ki), bladder (B), were analyzed.

Supplementary Figure 3. Body weight of mice. Body weight of mice in figure 6 was recorded at the indicated time point.

Supplementary Table 1. Data collection and refinement statistics

Supplementary Table 2. Residues contributed to interaction between Nb22 and RBD were identified by PISA at the European Bioinformatics Institute.



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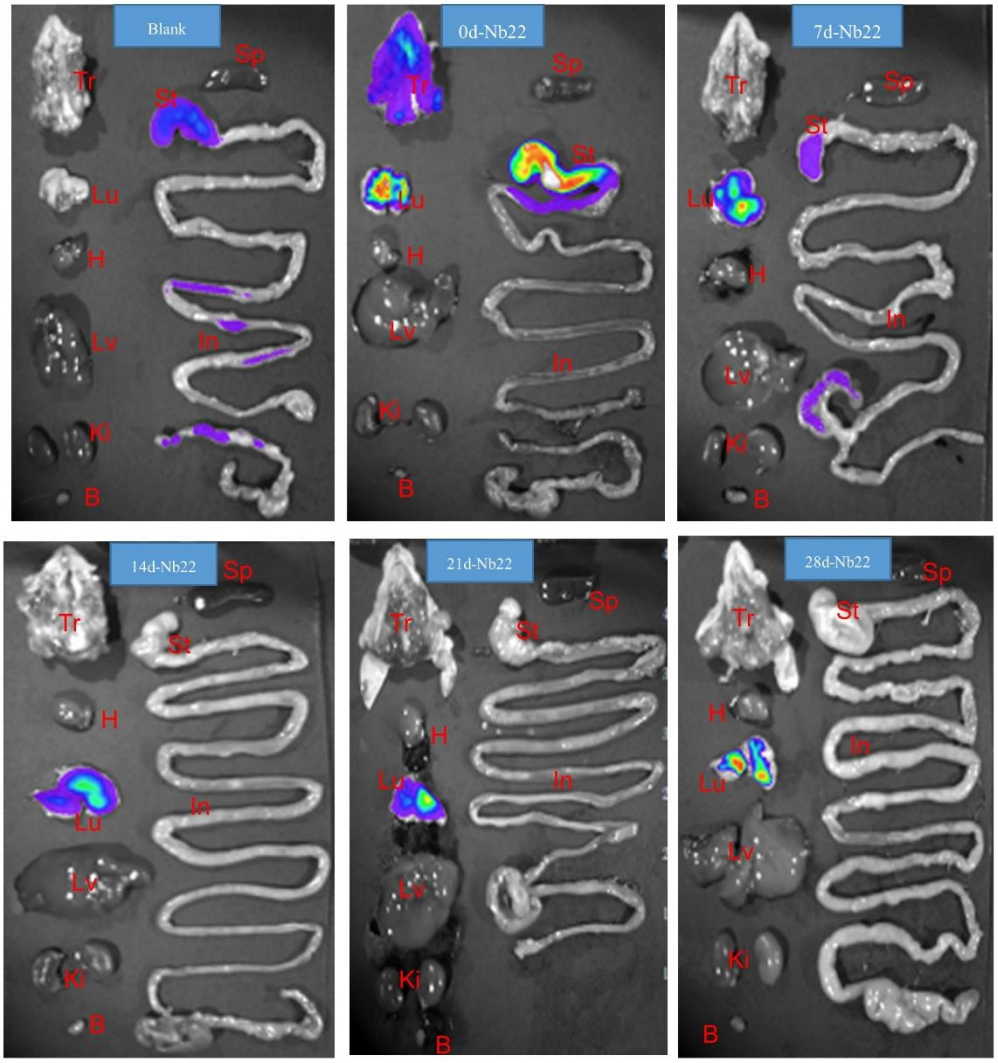

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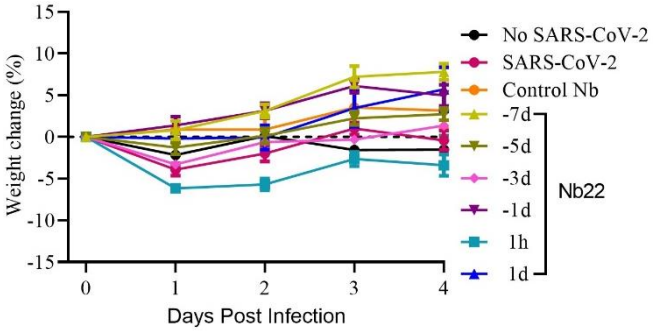

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Supplementary Table 1. Data collection and refinement statistics

Parameters	WH01 RBD-Nb22	Delta RBD-Nb22
X-ray Source	BL18U1	BL02U1
Wavelength (Å)	0.97915	0.97918
Space group	$P2_12_12_1$	$C2$
Unit cell parameters (Å)	a=73.8, b=88.7, c=172.5	a=151.8, b=108.5, c=115.3 α=90.0, β=126.2, γ=90.0
Resolution range (Å)	50.0-2.7 (2.75-2.7)*	93.0-2.9 (3.0-2.9)*
Unique reflections	32,672 (1,579)	30,823 (3,106)
Completeness (%)	99.8 (99.2)	93.8 (95.7)
Redundancy	7.2 (4.8)	3.2 (3.1)
I/σ(I)	12.3 (1.7)	6.9 (2.5)
R_{merge} (%)	13.7 (64.4)	11.0 (41.6)
R_{meas} (%)	14.8 (72.2)	13.1 (50.0)
R_{pim} (%)	5.5 (32.1)	7.1 (27.4)
$CC_{1/2}$	0.999 (0.763)	0.985 (0.836)
Refinement statistics		
Resolution range (Å)	37.23- 2.7 (2.796-2.7)	41.2-2.9 (3.0-2.9)
Reflections used in refinement	31,683 (3,093)	30,797 (3,104)
Reflections used for R-free	1,663 (162)	1,495 (140)
R_{work} (%)	19.6 (27.3)	20.5 (27.2)
R_{free} (%)	24.4 (35.4)	25.5 (32.1)
Number of non-hydrogen atoms	7,710	7,539
Protein	972	972
Solvent	155	/
Ligand	13	/
Average B-factors	59.1	85.7
Protein	59.4	85.7
Solvent	40.7	/
Ligand	52.0	/
r.m.s. deviations		
Bond lengths (Å)	0.003	0.009
Bond angles (°)	0.54	1.11
Ramachandran		
Favored (%)	94.9	94.3
Allowed (%)	5.1	5.7
Outliers (%)	0.0	0.0

*Numbers in the brackets are for the highest resolution shell.

Supplementary Table 2. Residues contributed to interaction between Nb22 and RBD were identified by PISA at the European Bioinformatics Institute.

Nb22	Distance (Å)	WH01 RBD	Distance (Å)	Delta RBD
GLY 1[O]	2.9	ASN 450[ND2]	2.8	ASN 450[ND2]
THR 30[N]	2.9	SER 494[OG]	3.0	SER 494[OG]
THR 30[OG1]	/	ARG 452[NE]	2.9	ARG 452[NE]
SER 33[OG]	/	GLN 493[OE1]	2.8	GLN 493[OE1]
SER 33[OG]	2.6	SER 494[N]	3.0	SER 494[N]
ASN 57[ND2]	2.9	GLY 485[O]	2.9	GLY 485[O]
SER 75[N]	2.4	GLU 484[OE2]	2.6	GLU 484[OE2]
SER 75[OG]	2.8	GLU 484[OE1]	2.8	GLU 484[OE1]
TYR 119[OH]	2.8	GLY 446[O]	2.8	GLY 446[O]