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Supplemental information

Biparatopic nanobodies targeting the receptor binding domain efficiently neutralize SARS-CoV-2

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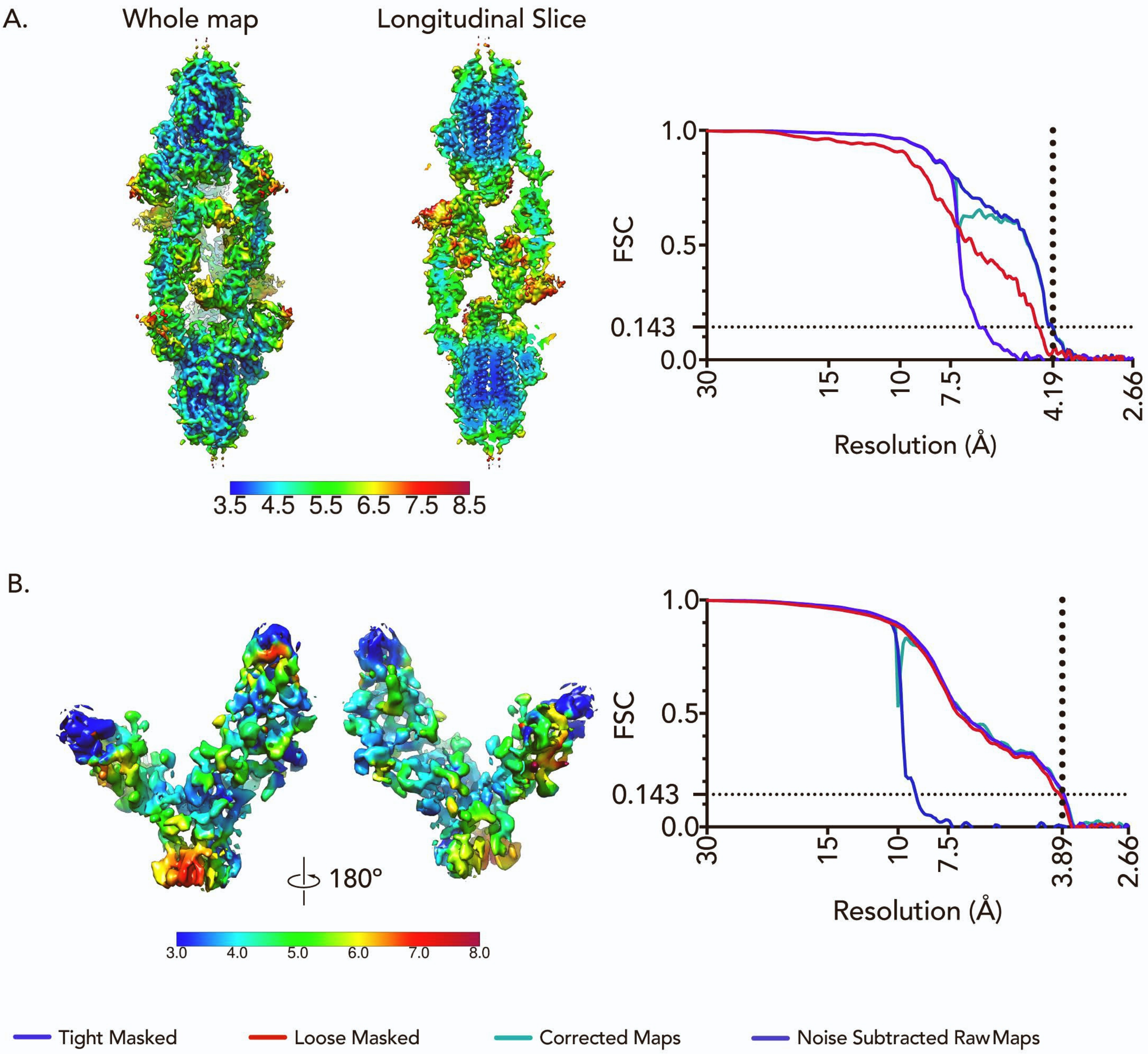

Figure S1. Cryo-EM maps coloured by local resolution of the S-BP10 complex, related to Figure 4. A. Best global refinement of the Spike-BP10 complex B. Best local refinement of a single copy of the RBD with density for two nanobodies. Highest resolution areas are coloured blue and lowest resolution areas are colured deep red. Scales and FSC curves are shown for each map. See also Figure 4.

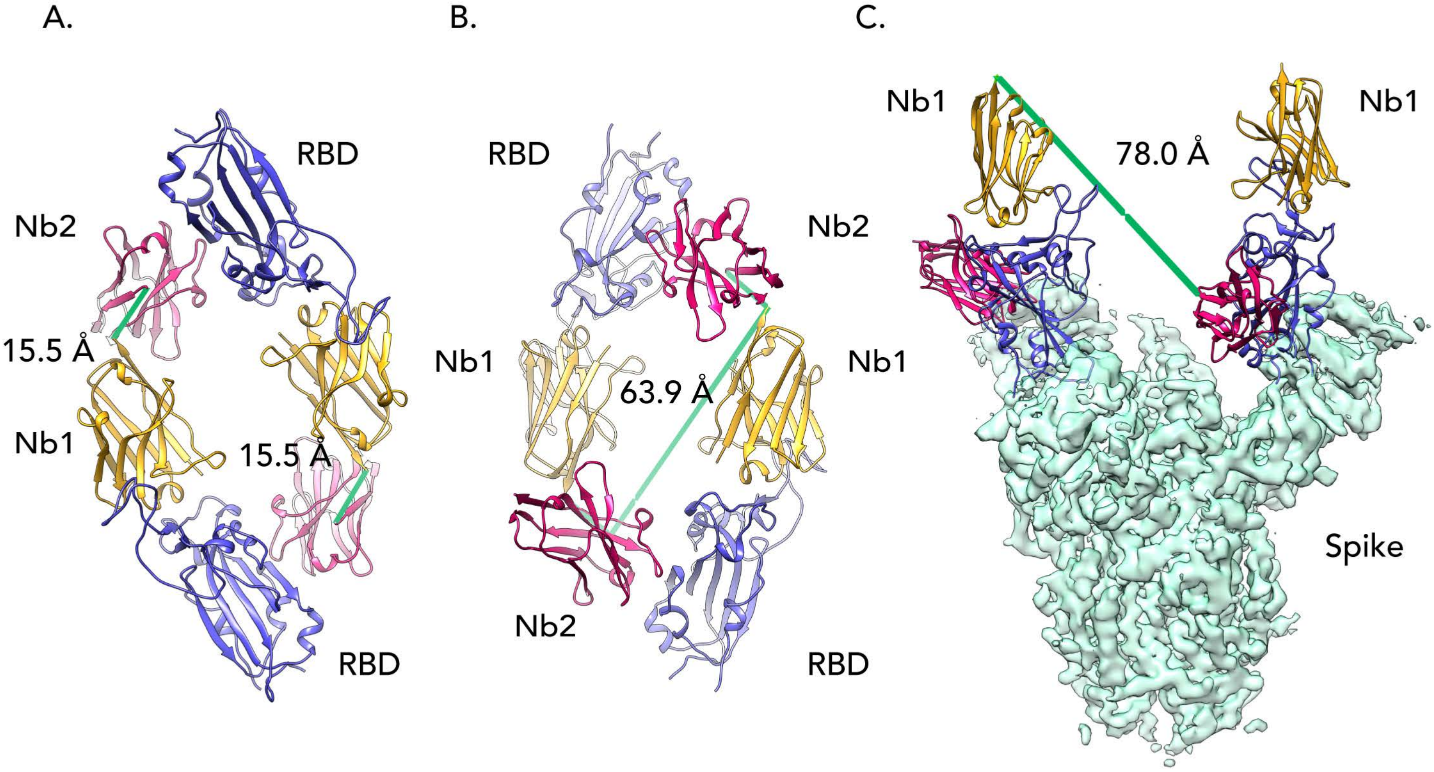

Figure S2. Linker length requirements for connecting nanobodies in the S-BP10 complex, related to figure 4. Cryo-EM maps of the BP10-spike complex showing spike (green), RBDs (purple-blue, PDB ID: 7LX5) and Nanobodies 1 (gold, PDB ID: 7KKK) and 2 (pink, PDB ID: 5IMK) distance measurements are coloured bright green. A. Distance between Nanobody 1 C-terminus and nanobody 2 N-terminus linking RBD's from different cpoies of Spike. B. Distance between Nanobody 1 C-terminus and nanobody 2 N-terminus on the same RBD. C. Distance between Nanobody 1 C-terminus and nanobody 2 N-terminus on adjacent RBD's on the same copy of Spike. See also Figure 4.

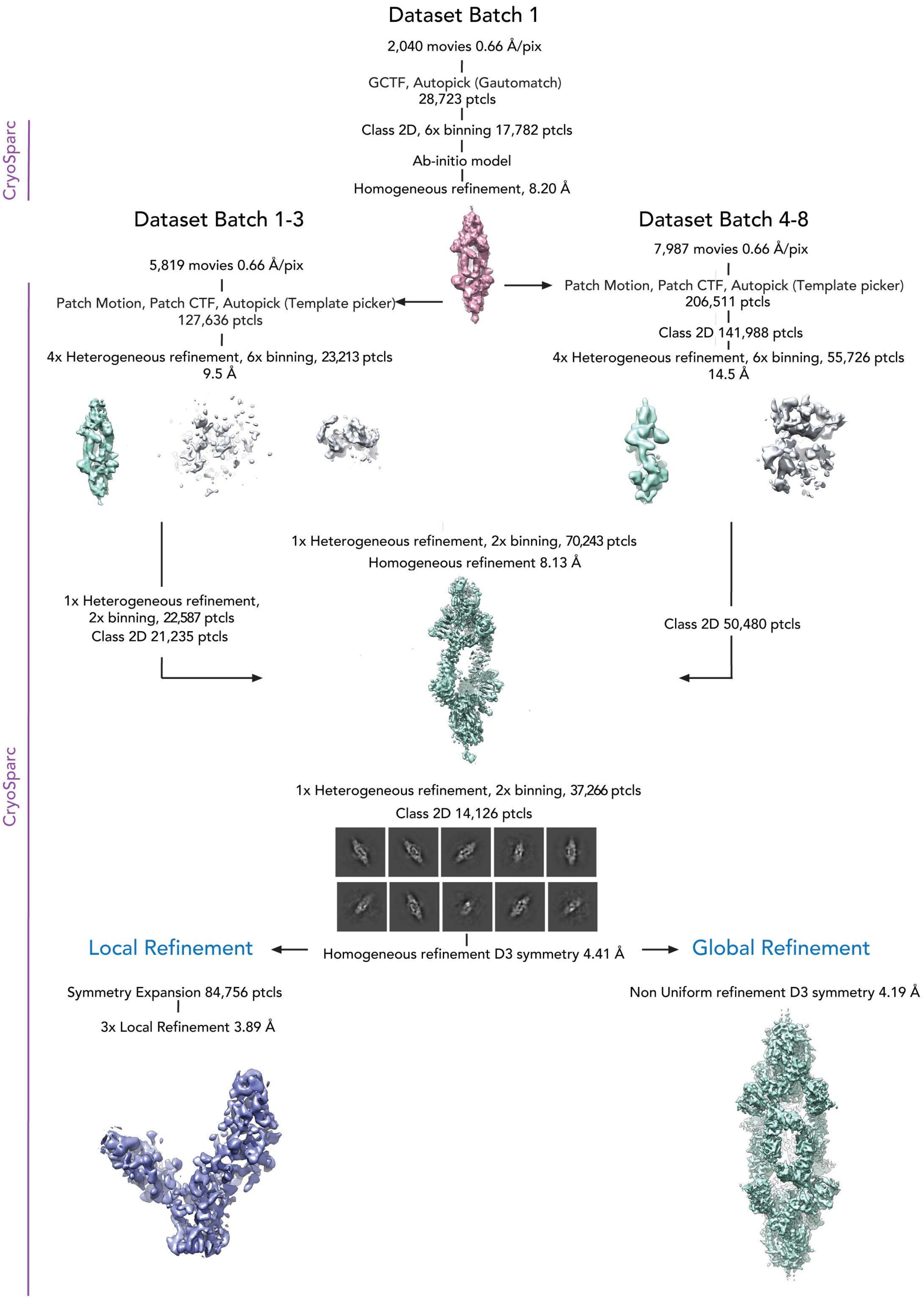

Figure S3. Cryo-EM data processing workflow for the S-BP10 complex, related to Figure 4. Details of workflow are fully described in materials and methods. See also Figure 4.

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SARS-CoV-2              RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYAD  80
SARS-CoV-2 Alpha        ................................................................................  80
SARS-CoV-2 Delta        ................................................................................  80
SARS-CoV-2 Lambda       ................................................................................  80
SARS-CoV-2 Beta         ................................................................................  80
SARS-CoV-2 gamma        ................................................................................  80
SARS-CoV-2 Delta plus   ................................................................................  80
SARS-CoV-2 Mu           ...........................K....................................................  80
Bat CoV BANAL-52        ...........................T.........................T..........................  80
Pangolin CoV GD-1       ...........................T.........................T..........................  80
Bat CoV BANAL-236       .....D.....................T.........................T..........................  80
SARS-CoV-2 Omicron BA.1 ....................D...............................L.P.F.......................  80
SARS-CoV-2 Omicron BA.2 ....................D...............................F.P.FA......................  80
Bat CoV RaTG13          .....D.....................T.........................T..........................  80
Pangolin CoV GX-P5L     .....I....................SK.........................T..........................  80
Bat CoV WIV-1           ..A.SKEV...................T.P.....E.................T...........A........S.....  80
Bat CoV RsSHC014        ..A.SKEV...................T.P.....E............I....T...........A........S.....  80
Bat CoV Rs2018B         ..A.SKEV...................T.P.....E.................T...........A........S.....  80
Bat CoV LYRa11          ..S.SKEV...................T.P.....E.................T...........AI.......S.....  80
SARS-CoV-1              ..V.SGDV...................K.P.....E..K..............TF..........A........S.....  80

SARS-CoV-2              SFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGST 160
SARS-CoV-2 Alpha        ................................................................................ 160
SARS-CoV-2 Delta        .....................................................R.........................K 160
SARS-CoV-2 Lambda       .....................................................R.......................... 160
SARS-CoV-2 Beta         ..................N............................................................. 160
SARS-CoV-2 gamma        ..................T............................................................. 160
SARS-CoV-2 Delta plus   ..................N..................................R.........................K 160
SARS-CoV-2 Mu           ................................................................................ 160
Bat CoV BANAL-52        ...V............................................................................ 160
Pangolin CoV GD-1       ...V..............R............................................................. 160
Bat CoV BANAL-236       ...V............................................................................ 160
SARS-CoV-2 Omicron BA.1 ..................N......................K.....S..............................NK 160
SARS-CoV-2 Omicron BA.2 ......N..S........N......................K....................................NK 160
Bat CoV RaTG13          ....T...................................KHI.A.E...F.........A..................K 160
Pangolin CoV GX-P5L     ...VK.............V.....................VKQ.ALT....G.........K.................. 160
Bat CoV WIV-1           ...VK..D..........V................L...TR.I.ATQT.....K..SL.HGK.R.......NVPFSPDGK 160
Bat CoV RsSHC014        ...VK..D..........V............L...L...T.SK..STS........WV.R.K.N.Y...L.ND..SP.GQ 160
Bat CoV Rs2018B         ...VK..D..........V............M...L...TR.I.ATST.....K..SL.HGK.R.......NVPFSPDGK 160
Bat CoV LYRa11          ...VK..D..........V............M...L...TR.I.ATSS..F..K..SL.HGK.R.......NVPFSPDGK 160
SARS-CoV-1              ...VK..D..........V............M...L...TR.I.ATST.....K..YL.HGK.R.......NVPFSPDGK 160

SARS-CoV-2              PCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNF                  223
SARS-CoV-2 Alpha        ......................Y........................................                  223
SARS-CoV-2 Delta        ...............................................................                  223
SARS-CoV-2 Lambda       ...........S...................................................                  223
SARS-CoV-2 Beta         .....K................Y........................................                  223
SARS-CoV-2 gamma        .....K................Y........................................                  223
SARS-CoV-2 Delta plus   ...............................................................                  223
SARS-CoV-2 Mu           .....K................Y........................................                  223
Bat CoV BANAL-52        ...................H....................N..............I.......                  223
Pangolin CoV GD-1       ...................H....................N.........Q............                  223
Bat CoV BANAL-236       ..............K....H....................N..............I.......                  223
SARS-CoV-2 Omicron BA.1 .....A........R..S.R..Y...H....................................                  223
SARS-CoV-2 Omicron BA.2 .....A........R....R..Y...H....................................                  223
Bat CoV RaTG13          ....QT.L...Y..YR...Y..D...H.............N......................                  223
Pangolin CoV GX-P5L     ....QV.L...Y..ER...H..T..N...F..........NG........L..T...D.....                  223
Bat CoV WIV-1           ..TP-PA....W..ND...YI...I...............N.........L..D.I..Q....                  222
Bat CoV RsSHC014        S.SA-V.P...N..RP...FT.A...H.............N.........L..D.I..Q....                  222
Bat CoV Rs2018B         ..TP-PA....W..ND...FT...I...............N.........L..D.I..Q....                  222
Bat CoV LYRa11          ..TP-PA....W..ND...YT...I...............N.........L..D.IT.Q....                  222
SARS-CoV-1              ..TP-PAL...W..ND...YT.T.I...............N.........L..D.I..Q....                  222
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Figure S4. Amino acid sequence alignment of RBD variants used in sVNT assay, related to Figure 5.

Table S1. List of RBD mutants used in the alanine-scan assay, related to Figure 5.

	Mutation
1	L335A
2	G339A
3	N354A
4	E324A
5	S359A
6	S366A
7	V367A
8	Y369A
9	A372S
10	S375A
11	S383A
12	P384A
13	T385A
14	K386A
15	N388A
16	D389A
17	L390A
18	F392A
19	T393A
20	N394A
21	D405A
22	R408A
23	Q414A
24	D427A
25	I434A
26	A435S
27	N439A
28	N440A
29	L441A
30	S433A
31	K444A
32	V445A
33	G446A
34	T449A
35	N450A
36	L452A
37	L455A
38	F456A
39	K458A
40	S459A
41	N460A
42	K462A
43	P463A
44	S477A
45	T478A
46	E484A
47	F486A
48	F490A
49	Q493A
50	S494A
51	G496A
52	Q498A
53	T500A
54	N501A
55	G502A
56	V503A
57	G504A
58	Y505A
59	S514A
60	L517A
61	L518A
62	H519A
63	A520S
64	P521A
65	A522A
66	P527A

Table S2. Cryo-EM data collection, refinement and validation statistics, related to Figure 4.

	Best Entire Spike-BP10 Complex (EMDB-28189)	RBD+BP10-Nb1+Nb2 (localized reconstruction) (EMDB-28190)
Data collection and processing		
Magnification	155K	155K
Voltage (kV)	300 keV	300 keV
Electron exposure (e–/Å^2)	1.25	1.25
Defocus range (μm)		
Pixel size (Å)	0.66	0.66
Symmetry imposed	D3	C1
Initial particle images (no.)	334,147	334,147
Final particle images (no.)	14,126	84,756 (symmetry expanded)
Map resolution (Å) FSC threshold	4.19 0.143	3.89 0.143
Map resolution range (Å)	2.9-12.8	2.9-10.6