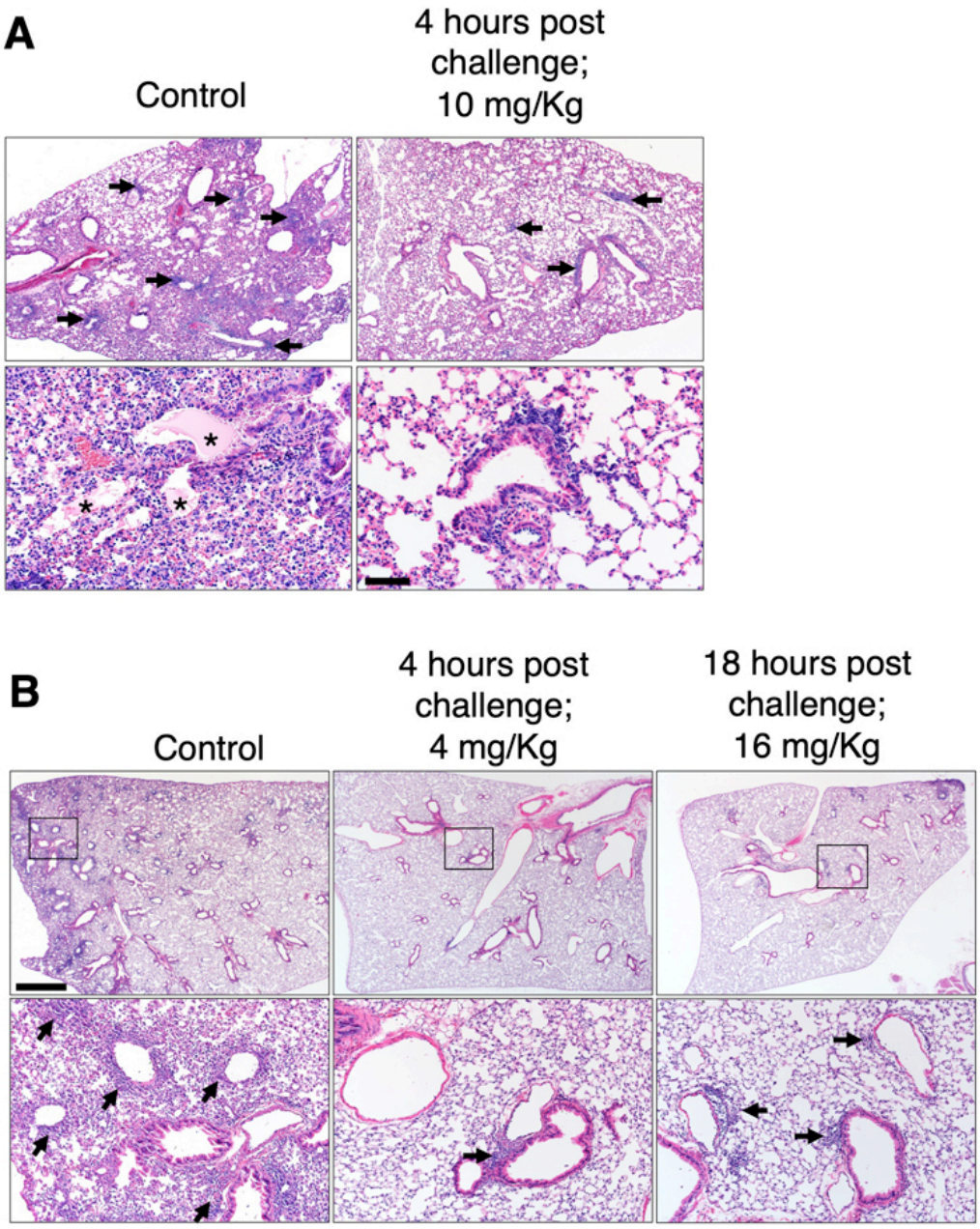

Figure S1. Pathological analysis of lung tissues in SARS-CoV-2-challenged mice. (**A**) Representative hematoxylin and eosin **(**HE) staining of lung tissues harvested on day 5 post-challenge from mice in **Figure 4A**. Inflammation (arrows, top row) and edema in airspaces (asterisks, bottom row) were much more evident in the control group (two left panels) compared to the treatment group (two right panels). (**B**) Representative HE staining of lung tissues harvested on day 5 post-challenge from mice in **Figure 4B**. The control group (two left panels) shows much more evident cellular perivascular cellular aggregates (arrows) and smaller airspaces compared to the treatment groups (two right panels).

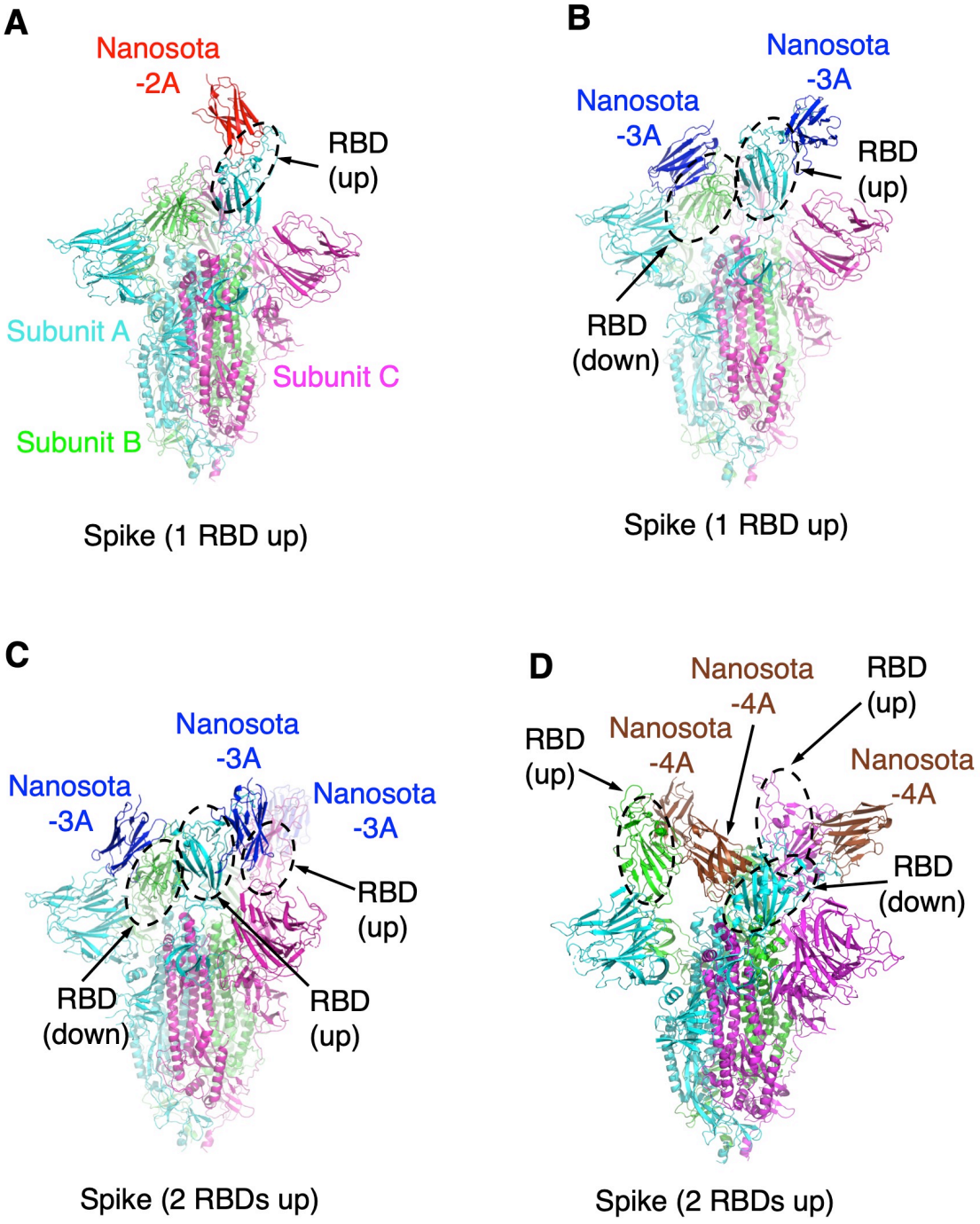

Figure S2. Structures of prototypic SARS-CoV-2 spike complexed with either Nanosota-2A, -3A or -4A. (**A**) SARS-CoV-2 spike (with one RBD up) complexed with Nanosota-2A. (**B**) SARS-CoV-2 spike (with one RBD up) complexed with two Nanosota-3A molecules. (**C**) SARS-CoV-2 spike (with two RBDs up) complexed with three Nanosota-3A molecules. (**D**) SARS-CoV-2 spike (with two RBDs up) complexed with three Nanosota-4A molecules. The spike trimer is colored by different subunits: subunit A in cyan, subunit B in green and subunit C in magenta.

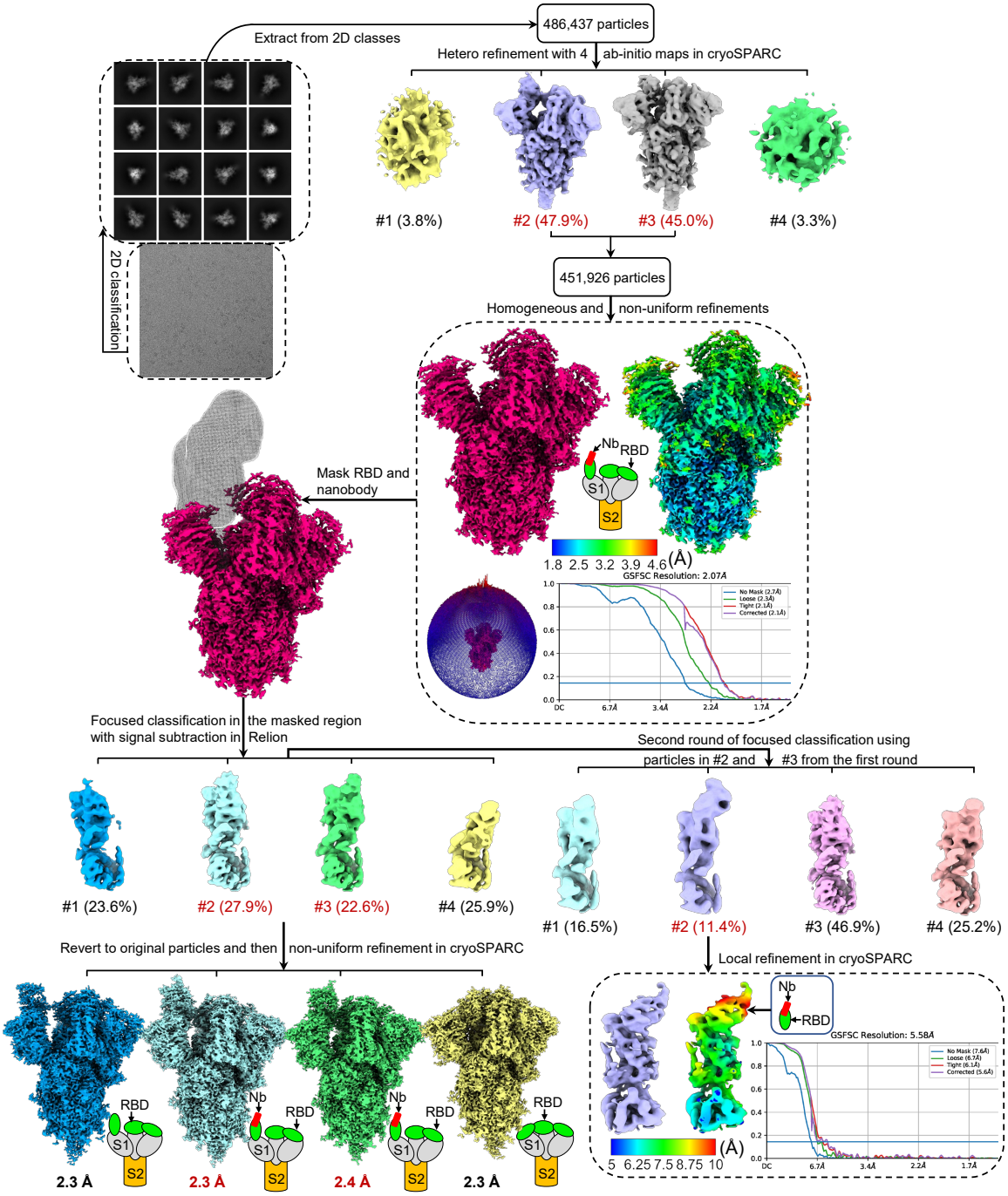

Figure S3. Flow chart of cryo-EM image processing and 3D reconstruction for prototypic SARS-CoV-2 spike/Nanosota-2A complex. Representative raw cryo-EM image and 2D classes are presented. 3D refinements using all the particles from good 3D classes generated a 2.1 Å map. Two rounds of masked 3D classification and further local refinement visualized the density for the bound nanobody. Angular distribution plot, the final maps, half-map FSC curves and accompanying local resolution illustrations are enclosed in the dashed black boxes.

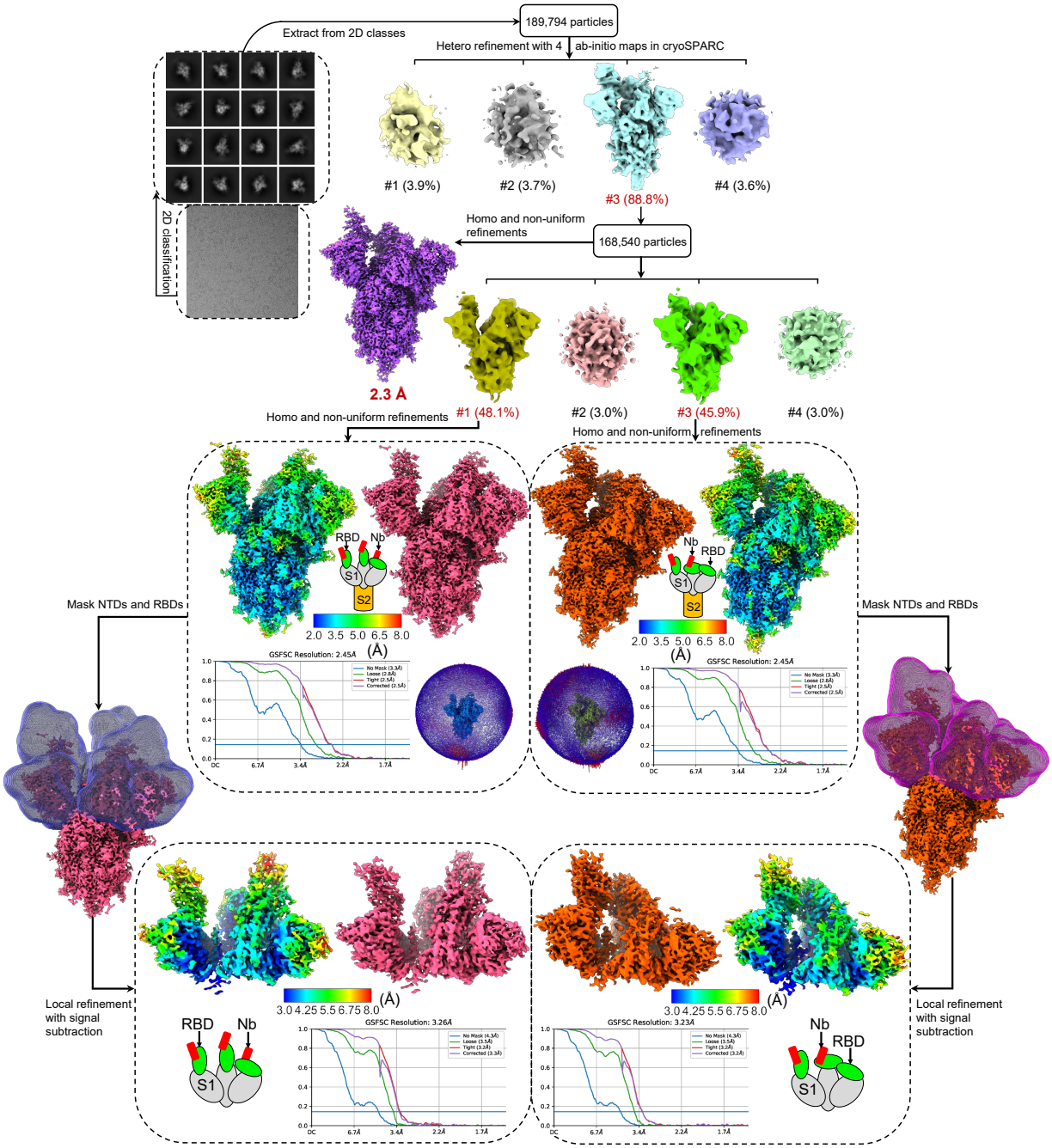

Figure S4. Flow chart of cryo-EM image processing and 3D reconstruction for prototypic SARS-CoV-2 spike/Nanosota-3A complex. Representative raw cryo-EM image and 2D classes are presented. 3D refinements using the particles from individual good 3D classes generated 2.5 Å maps. Further local refinements improved the density for the bound nanobodies. The final maps, half-map FSC curves, angular distribution plot and accompanying local resolution illustrations are enclosed in the dashed black boxes.

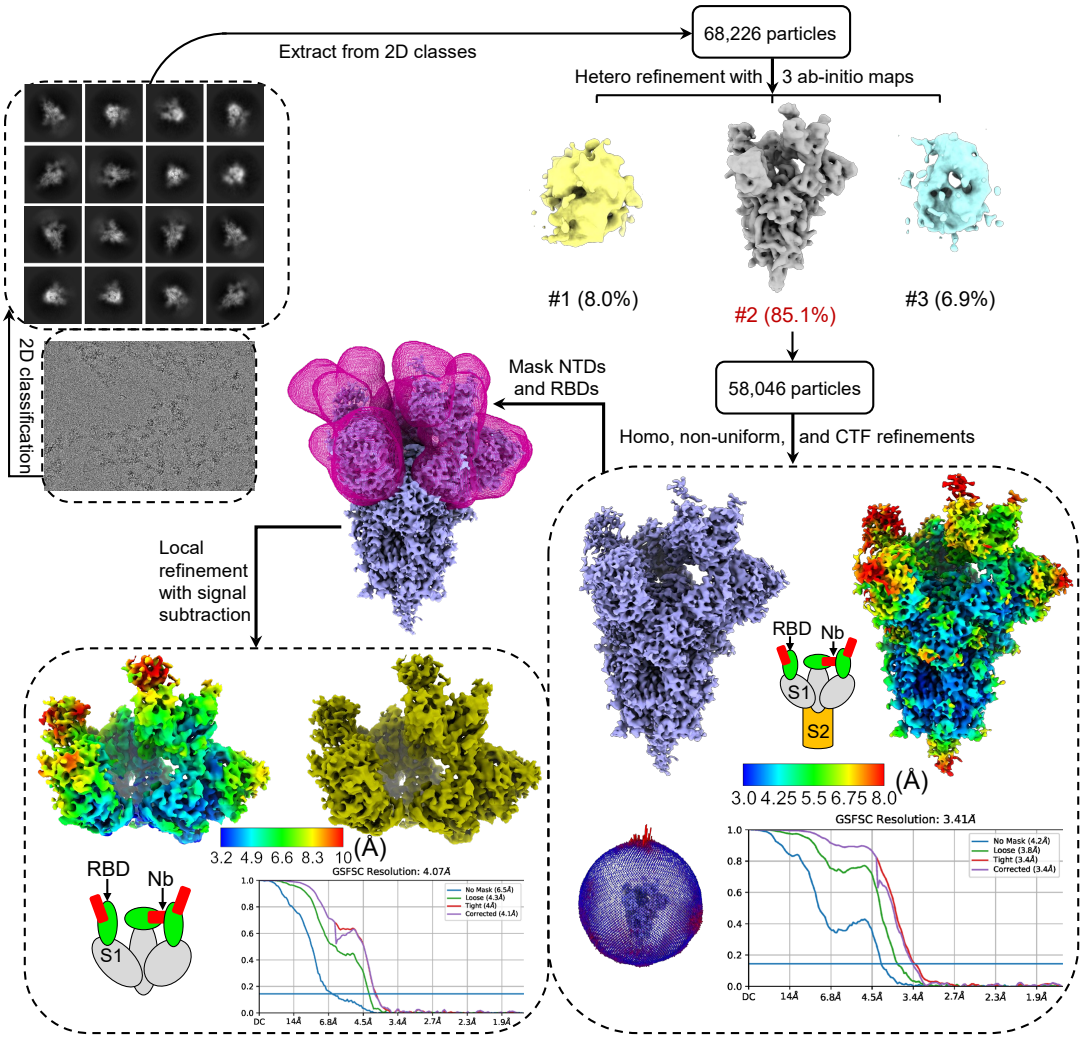

Figure S5. Flow chart of cryo-EM image processing and 3D reconstruction for prototypic SARS-CoV-2 spike/Nanosota-4A complex. Representative raw cryo-EM image and 2D classes are presented. 3D refinements using the particles in the good 3D class generated a 3.4 Å map. Further local refinements improved the density for the bound nanobodies. The final maps, half-map FSC curves, angular distribution plot and accompanying local resolution illustrations are enclosed in the dashed black boxes.

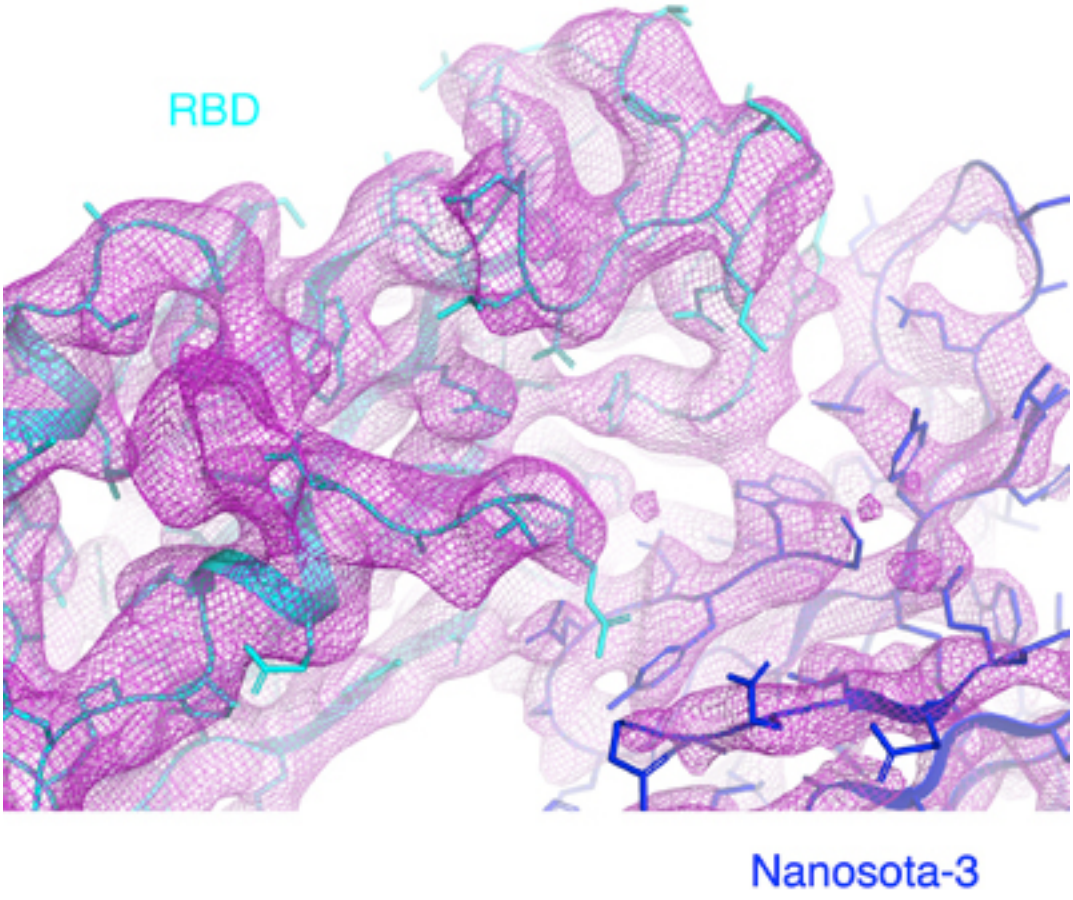

Figure S6. Cryo-EM densities (after local refinement) of the interface between the lying-down RBD and Nanosota-3A. RBD is colored in cyan, Nanosota-3A is colored in blue, and cryo-EM densities are colored in magenta.

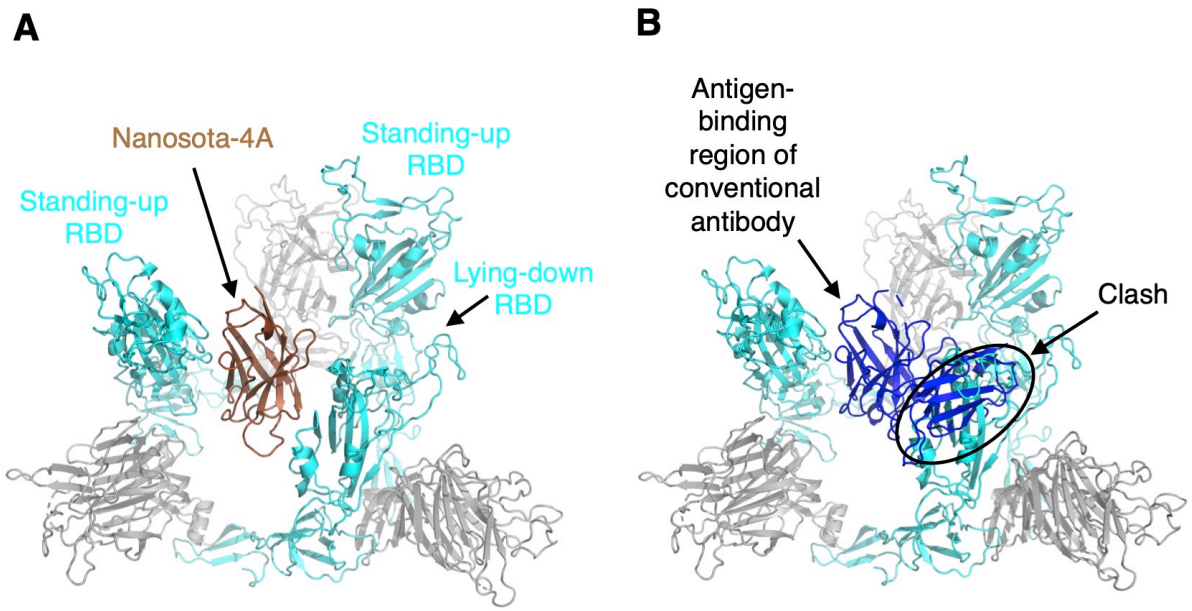

Figure S7. Nanosota-4A fits into a cavity in the trimeric spike, which would not accommodate conventional antibodies. (A) Nanosota-4A (consisting of only the heavy chain) binds to the lying-down RBD by fitting into a cavity in the trimeric spike. Only the three receptor-binding subunits are shown. RBD is colored in cyan. Nanosota-4A is colored in brown. **(B)** The antigen-binding region (consisting of both heavy and light chains) of a conventional antibody (PDB 7DEO) would not fit into the cavity and hence would not bind to the lying-down RBD in the same way as Nanosota-4A does. The clash of the antibody and the spike protein is labeled.

Table S1. *In vitro* anti-SARS-CoV-2 potency of antibodies/nanobodies from the literature

Reference	Nanobody	Conventional antibody	Pseudovirus	Live virus	Animal testing?
Current study	Nanosota-2A-Fc		0.50 ng/ml = 6.2 pM (WT) 0.56 ng/ml = 6.9 pM (Alpha) 1.1 ng/ml = 14 pM (Delta)	**0.16 ng/ml = 2 pM (WT)**	Yes
Current study	Nanosota-3A-Fc		5.7 ng/ml = 74 pM (WT) 1.2 ng/ml = 16 pM (Alpha) 3.6 ng/ml = 47 pM (Omicron)	2.3 ng/ml = 30 pM (Omicron)	Yes
(1)		A23-58.1, B1.182.1	0.3-11 ng/ml (23 variants; no Omicron)	2.1-4.8 ng/ml (WT)	No
(2)		35B5		1.6 ng/ml (WT)	Yes
		32C7		8.6 ng/ml (WT)	
(3)		J08, I14		1-10 ng/ml (D614G, Alpha)	Yes
(4)		JMB2002	150 ng/ml (omicron)		No
(5)		35B5	2.4 ng/ml (Delta) 6.9 ng/ml (Delta) 15 ng/ml (Omicron)	50 ng/ml (Omicron)	No
(6)		S2E12	1.4 ng/ml (WT) 38 ng/ml (Omicron)		No
		LY-CoV1404	3.0 ng/ml (WT) 5.1 ng/ml (Omicron)		
(7)		P17	165 pM (WT)	195 pM (WT)	Yes
(8)		Clone2	0.54 ng/ml (WT);37.8 ng/ml (Beta)	7.70 ng/ml (WT); 31.51 ng/ml (Delta)	Yes
		Clone6	4.2 ng/ml (WT);435.8 ng/ml (Beta)	6.14 ng/ml (WT); 111.34 ng/ml (Delta)	
		Clone13A	6.2 ng/ml (WT)	59.28 ng/ml (WT); 120.10 ng/ml (Delta)	Yes
(9)		STE90-C11		0.56 nM (WT)	Yes
(10)		WRAIR NTD mAbs	6-27 ng/ml (WT)	32-215 ng/ml (WT)	Yes
		WRAIR RBD mAbs	4-17 ng/ml (WT)	33-172 ng/ml (WT)	
(11)		31 RBD mAbs	2-800 ng/ml (WT, Alpha, Beta, Gamma, Delta, Lambda); < 20 ng/ml (Omicron)		Yes
(12)	Nanosota-1C-Fc		0.27 ug/ml (WT)	0.16 ug/ml (WT)	Yes
(13)	ANTE-CoV2-Nab21T$_{GS}$		1.32 pM (WT)	6.04 pM (SARS-CoV-2 Munich strain)	No
(14)	mNb6-tri		5.0 ng/ml = 120 pM (WT)	2.3 ng/ml = 54 pM (WT)	No
(15)	C5-Trimer			20 pM (WT) 25 pM (Alpha)	Yes

(16)	Nb 19 trimer		0.7 ng/ml = 9 pM (WT)		No
	Nb 56 trimer		3.9 ng/ml = 55 pM (WT)	0.2 ng/ml = 3 pM (WT) 0.6 ng/ml = 8 pM (Alpha) 1.3 ng/ml = 18 pM (Beta) 0.2 ng/ml = 3 pM (Gamma)	
(17)	WNb-Fc 36			100 pM (WT) 110 pM (N501Y; D614G)	Yes
(18)	aRBD-2-5-Fc		20 pM (Omicron)	83 pM (WT) 44 pM (Beta) 27 pM (Delta) 29 pM (Omicron)	Yes
(19)	8A2+7A3		1 nM (WT) 400 pM (Alpha) 300 pM (Beta) 200 pM (Gamma)	1 nM (WT) 6 nM (614G) 2 nM (Alpha) 870 pM (Beta) 140 pM (Gamma) 19 nM (Delta)	Yes
(20)	2-67(A)		80 pM (WT) 80 pM (Alpha) 180 pM (Delta) 110 pM (Omicron)	310 pM (Delta)	No
(21)	V_H-Fc ab8		30 ng/ml (WT)	40 ng/ml (WT)	Yes
(22)	humVHH_S56A/LALAPG-Fc/Gen2			110 ng/ml (Beta)	Yes
	(humVHH_S56A)2/LALAPG-Fc/Gen2			20 ng/ml (Beta)	
(23)	BP10-Fc			90 ± 20 nM (WT)	Yes
	BP19-Fc			112 ± 18 nM (WT)	
	BP39-Fc			34 ± 2 nM (WT)	
(24)	BiShAb0201		24 ng/ml (WT) 26 ng/ml (Beta) 5 ng/ml (Delta) 27 ng/ml (BA.1) 35 ng/ml (BA.5)		Yes
	ShAb01H02K		< 23 ng/ml (WT) 21 ng/ml (Beta) 2 ng/ml (Delta) 391 ng/ml (BA.1) 524 ng/ml (BA.5)		

(25)	MR14		5.3 ± 0.45 ng/ml (WT) 5.0 ± 1.7 ng/ml (Delta) 0.16 ± 0.044 ng/ml (BA.1) 0.17 ± 0.082 ng/ml (BA.2) 0.69 ± 0.63 ng/ml (BA.3) 18.7 ± 9.7 ng/ml (BA.4/5)	91 ng/ml (WT) 13 ng/ml (Delta) 6.6 ng/ml (BA.1) 3.4 ng/ml (BA.2)	Yes
	MS43		179 ± 7.4 ng/ml (WT) 30 ± 17 ng/ml (Delta) 1.5 ± 0.037 ng/ml (BA.1) 52520 ±43208 ng/ml (BA.2) 50 ± 25 ng/ml (BA.3) 4405 ± 2672 ng/ml (BA.4/5)	1117 ng/ml (WT) 20 ng/ml (Delta) 30 ng/ml (BA.1) 2937 ng/ml (BA.2)	
(26)	W25-Fc			9.01 nM (WT) 0.38 nM (Alpha) 1.31 (Beta) 0.29 (Gamma) 1.45 nM (BA.1) 2.07 (BA.2)	Yes

WT: the original SARS-CoV-2 Wuhan strain isolated in early 2020
D614G: the original SARS-CoV-2 Wuhan strain with the added D614G mutation

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Table S2. *In vivo* anti-SARS-CoV-2 potency of discovered antibodies in the literature

Reference	Nanobody	Conventional Antibody	Animal	Route	Treatment time (hpi)	Dosage (mg/kg)	Virus
Current study	Nanosota-2A-Fc		Mouse	IP	4	4 10	WT
					18	16	
Current study	Nanosota-3A-Fc		Mouse	IP	4	10	Omicron
(1)		35B5 32C7	Mouse	IP	6	20	WT
					4	30	614G, Beta, Delta
(2)		J08, I14	Hamster	IP	24	4	WT
(3)		P17	Mouse	IP	-12 (pre-)	20	WT
					4		
(4)		Clone2	Mouse	IP	-24 (pre-) 18	20	WT
		Clone6					
		Clone13A			-24 (pre-)		Delta
(5)		STE90-C11	Mouse	IV	1	6 30 60 120	WT
			Hamster	IP	2	3.7 37	
(6)		WRAIR NTD mAbs	Mouse	IV	-24 (pre-)	0.25	WT
		WRAIR RBD mAbs				1	
		Cocktail			24	0.625 (Partial protective)	
						2.5	
(7)		31 RBD mAbs	Mouse	IP	1	5	Beta
					2	30	Omicron
(8)	Nanosota-1C-Fc		Mouse	IP	-24 (pre-)	20	WT
					4	10 20	
(9)	C5-Trimer		Hamster	IP	24	4	WT
(10)	WNb-Fc 36		Mouse	IP	-24 (pre-)	0.2	WT (N501Y D614G)
(11)	aRBD-2-5-Fc		Mouse	IP	-24 (pre-)	10	WT
			Hamster		-24 (pre-) 3		Omicron
(12)	8A2+7A3		Mouse	IP	-2 (pre-)	5	Beta

(13)	V_H-Fc ab8		Mouse	IP	-12 (pre-)	8 36	WT (Q498T/P499Y)
			Hamster		-24 (pre-) 6	10	WT
(14)	VHH72-Fc		Mouse	IP	-7 (pre-)	5	Muc-IMB-1/2020
				IN		1	
	HumVHH_S56A/LALAPG-Fc/Gen2		Hamster	IP	-24 (pre-)	20	Beta
					4	2 7 20	
	(humVHH_S56A)$_2$/LALAPG-Fc/Gen2			IP	4	2 7 20	
(15)	ShAb01		Mouse	IP	-24 (pre-)	10	WT
	ShAb02						
(16)	MR14		Mouse	IP IN	-6 (pre-)	5	BA.2
				IN (3 dose)	6, 30, 54		
				IP	6		
(17)	W25-Fc		Mouse	IP	-4 (pre-) 24	5	Beta
				IP	24	5	BA.1
				IN		1	

Pre-: time refers to hours pre-challenge
IP: intraperitoneally
IN: intranasally
IV: intravenously

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Table S3: Cryo-EM data collection, refinement and validation statistics.

	Spike/2A complex with 2 RBDs up	Local refinement of Spike/2A complex with 2 RBDs up	Spike/3A complex with 2 RBDs up and 3 Nbs bound	Local refinement of Spike/3A complex with 2 RBDs up and 3 Nbs bound	Spike/3A complex with 1 RBD up and 2 Nbs bound	Local refinement of Spike/3A complex with 1 RBD up and 2 Nbs bound	Spike/4A complex with 2 RBDs up and 3 Nbs bound	Local refinement of spike/4A complex with 2 RBDs up and 3 Nbs bound
Data collection and processing								
Magnification	165,000		165,000		165,000		75,300	
Voltage (kV)	300		300		300		300	
Electron exposure (e–/Å^2)	40.00		40.00		40.00		50.00	
Defocus range (μm)	0.8–2.4		0.8–2.4		0.8–2.4		0.75–2.5	
Pixel size (Å)	0.73		0.73		0.73		0.664	
Symmetry imposed	C1		C1		C1		C1	
Initial particle images (no.)	486,437	486,437	189,794	189,794	189,794	189,794	68,226	68,226
Final particle images (no.)	451,926	25124	81,068	81,068	77,360	77,360	58,046	58,046
Map resolution (Å)	2.1	5.6	2.5	3.3	2.5	3.2	3.4	4.1
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	1.8–4.6	5-10	2.0–8.0	3.0-8.0	2.0–8.0	3.0-8.0	3.0-8.0	3.2-10
Refinement								
Initial model used (PDB code)	7TGX	7TGX	7TGX	7TGX	7TGX	7TGX	7TGX	7TGX
Model resolution (Å)	2.7	9.4	2.9	4.0	2.9	3.6	3.8	7.4
FSC threshold	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Model resolution range (Å)	44.3-2.1	23.4-5.0	51.4-2.4	43.3-3.2	52.0-2.4	40.0-3.2	63.0-3.4	54.4-4.0
Map sharpening *B* factor (Å^2)	-44.9	-348.4	-43.2	-66.0	-42.7	-70.3	-81.2	-97.3
Model composition								
Non-hydrogen atoms	25761	3037	28420	16237	27543	15406	28711	17466
Protein residues	3217	338	3562	2046	3447	1928	3598	2193
Ligands	45		47	2	47	7	47	12
B factors (Å^2)								
Protein	106.09	228.15	128.96	173.60	129.02	126.74	159.30	144.99
Nucleotide								
Ligand	115.62		118.50	172.91	129.21	150.68	144.99	126.54
R.m.s. deviations								
Bond lengths (Å)	0.006	0.004	0.005	0.006	0.005	0.004	0.016	0.005
Bond angles (°)	0.949	0.852	0.843	0.831	0.839	0.724	1.028	0.767
Validation								
MolProbity score	2.33	1.78	2.17	2.18	2.09	1.96	1.81	2.04
Clashscore	32.58	15.75	21.22	24.55	20.71	14.00	14.17	17.42
Poor rotamers (%)	1.24	0.00	1.26	0.61	1.16	0.29	0.54	0.05
Ramachandran plot								
Favored (%)	96.20	97.64	95.97	95.65	96.48	95.59	97.19	95.68
Allowed (%)	3.45	2.36	3.77	4.10	3.37	4.25	2.70	4.09
Disallowed (%)	0.35	0.00	0.26	0.25	0.15	0.16	0.11	0.23

2A, 3A and 4A represent Nanosota-2A, -3A and -4A, respectively.