Supplemental Information

Table S1: Cryo-EM Data collection, image processing and refinement for S Protein: Fab4 complex

Data Collection and Processing	**State I**	**State II**	**State III**
Magnification	54,000 kX	54,000 kX	54,000 kX
Voltage	200 kV	200 kV	200 kV
Electron exposure ($e^-/Å^2$)	50	50	50
Defocus range (μm)	-1.25 to -2.75	-1.25 to -2.75	-1.25 to -2.75
Pixel size (Å)	0.92	0.92	0.92
Symmetry Imposed	C1	C1	C1
Number of Movies	3789		
Number of Particles	1,63,492	93,959	49,160
Map Resolution (Å)	4.54	5.152	4.9
FSC threshold	0.143	0.143	0.143
Map Resolution Range	3.3-6.1	3.3-6.1	3.3-6.1
Map Sharpening B Factor ($Å^2$)	-273		
RBD Conformation	2-RBD Up	3-RBD Up	3-RBD Up

Table S2: Cryo-EM Data collection, image processing and refinement for S Protein: Fab26 Complex

Data Collection and Processing	**Single Fab Masked**	**State I**	**State II**
Magnification	54,000 kX	54,000 kX	54,000 kX
Voltage	200 kV	200 kV	200 kV
Electron exposure ($e^-/Å^2$)	45	45	45
Defocus range (μm)	-1.25 to -2.75	-1.25 to -2.75	-1.25 to -2.75
Pixel size (Å)	0.92	0.92	0.92
Symmetry Imposed	C1	C1	C1
Number of Movies	2102		
Number of Particles	1,09,596	26,719	48,584
Map Resolution (Å)	4.4	7.3	4.8
FSC threshold	0.143	0.143	0.143

Map Resolution Range	3.3-6.1	3.3-6.1	3.3-6.1
Map Sharpening B Factor ($Å^2$)	-249		
RBD Conformation	2-RBD Up	2-RBD Up	3-RBD Up

Table S3: MolProbity Score for S Protein: Fab4 Complex

	SARS-CoV-2 + Fab4		
Validation	**State I**	**State II**	**State III**
MolProbity Score	1.88	2.28	2.32
Clash Score	7.36	18.31	18.64
Rotamer Outliers (%)	0.20	0.26	0.18
Ramachandran Plot			
Outlier (%)	0.11	0.16	0.18
Allowed (%)	7.66	8.73	9.78
Favored (%)	92.23	91.11	90.04

Table S4: MolProbity Score for S Protein:Fab26 Complex

	SARS-CoV-2 + Fab26		
Validation	**Single Fab Masked**	**State I**	**State II**
MolProbity Score	1.99	3.03	2.11
Clash Score	8.77	126.30	11.39
Rotamer Outliers (%)	0.40	0.09	0.54
Ramachandran Plot			
Outlier (%)	0.14	0.10	0.07
Allowed (%)	8.98	7.30	9.43
Favored (%)	90.88	92.60	90.51

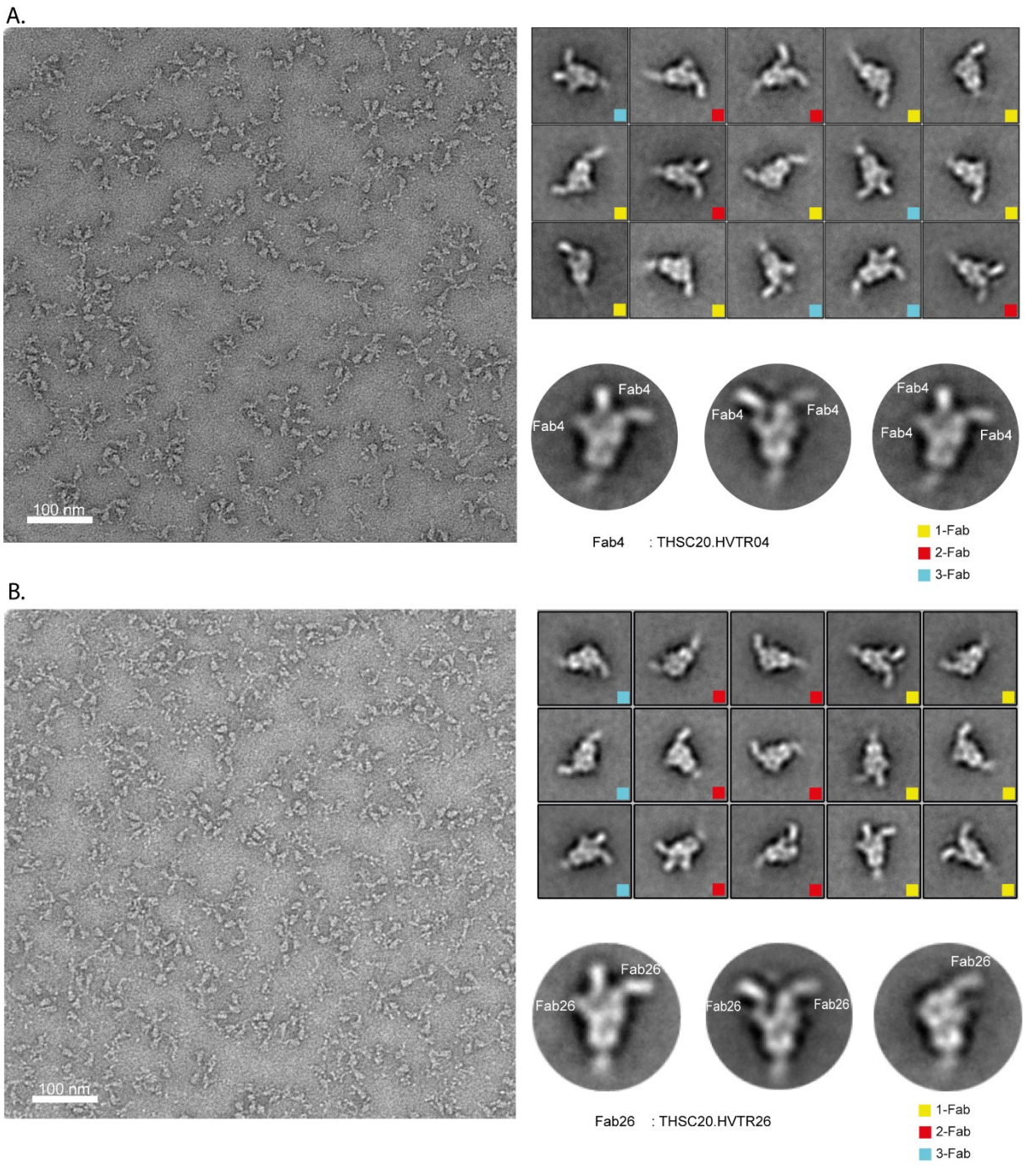

Figure S1: Negative staining micrograph and 2D class averages of S protein with A. Fab4 and B. Fab26 complexes

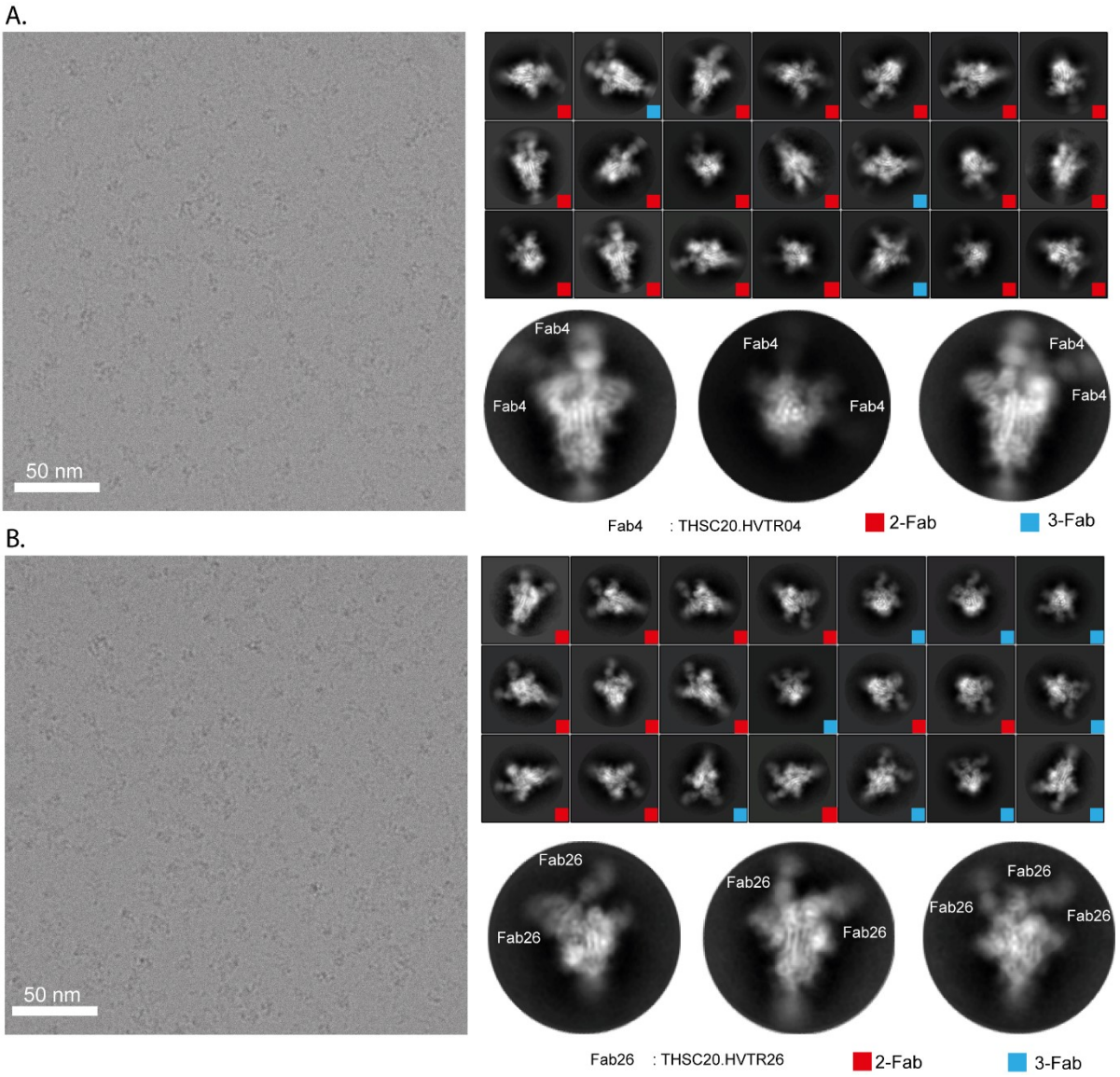

Figure S2: Cryo-electron microscopy micrograph and 2D class averages of S protein with A. Fab4 and B. Fab26 complexes

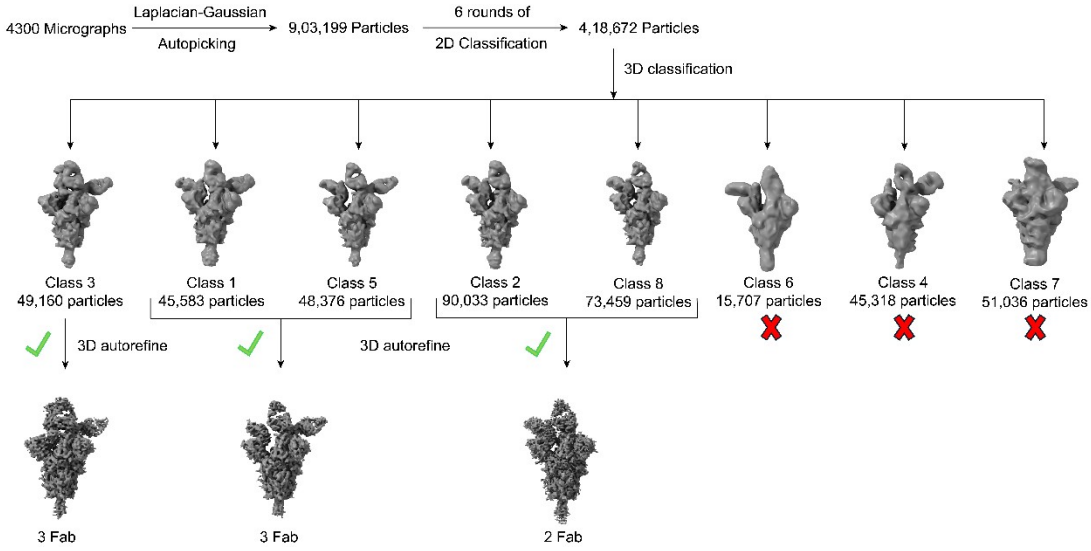

Figure S3: Workflow for the single-particle analysis (SPA) of cryo-EM for S protein: Fab4 complex: Pipeline illustrates the cryo-EM data processing, different conformations obtained and the refined structures. The green tick-marked classes were considered for refinements, whereas red cross classes were not further refined due to low-resolution.

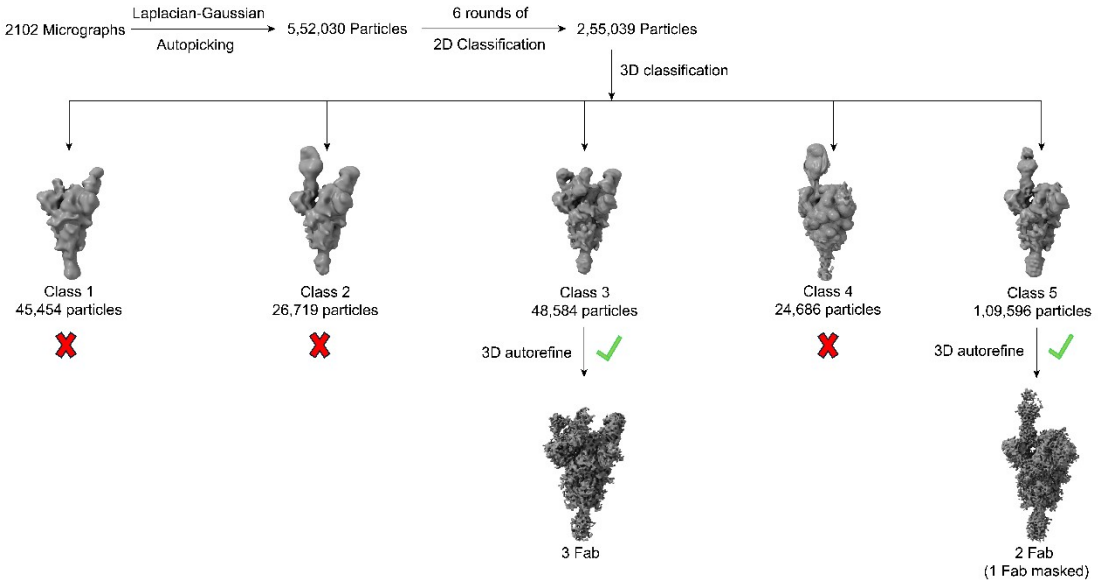

Figure S4: Workflow for the single-particle analysis (SPA) of cryo-EM for S protein: Fab26 complex: Pipeline illustrates the cryo-EM data processing, different conformations obtained and the refined structures. The green tick marked classes were considered for refinements, whereas red cross marked were not further refined due to low-resolution.

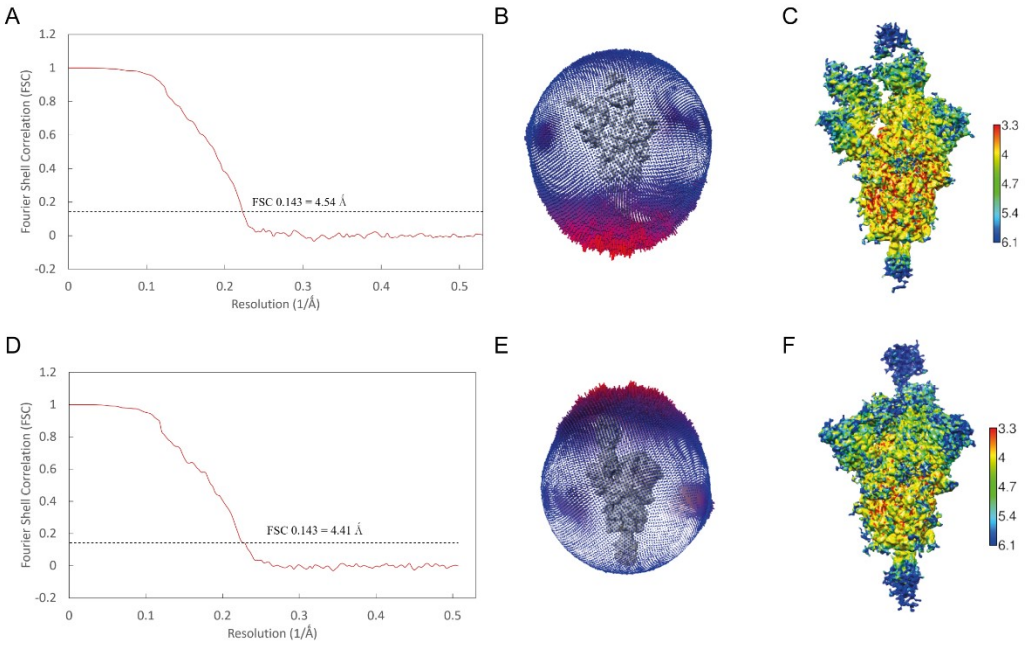

Figure S5: A, D) Gold standard Fourier shell correlation (FSC) curve calculated from the independent half maps results in 4.54 and 4.4 Å global resolution EM density map for the S protein complex with Fab4 and Fab26 respectively. B, E) Angular distribution plot for the final refined EM maps of S protein: Fab4/Fab26 complexes respectively without imposing symmetry (C1). C, F) Local resolution calculated for the high-resolution EM maps of Spike with Fab4 and Fab26 complexes respectively (C1 symmetry), coloured according to resolution calculated using ResMap.

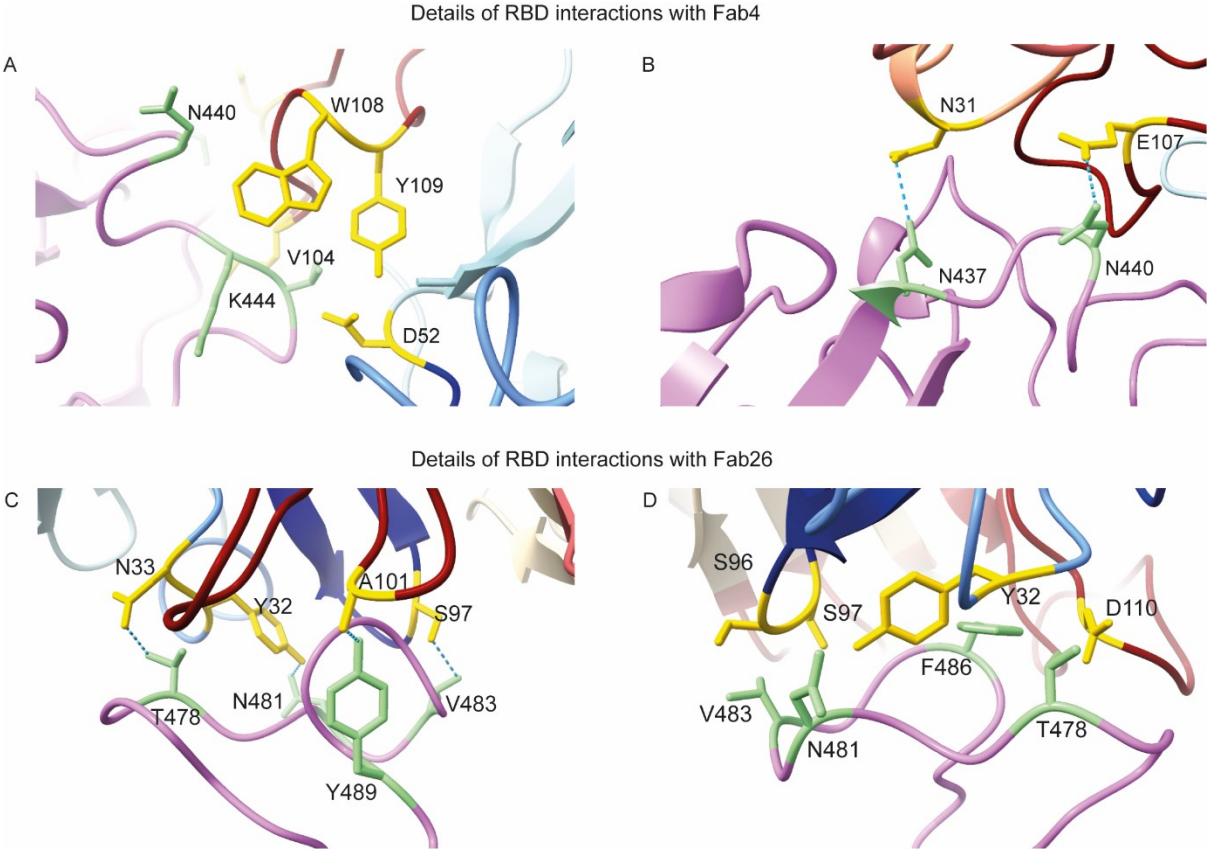

Figure S6: Interacting residues between Fabs and S trimer: **A**. Interaction by hydrogen bonding between Fab4 and RBD and the residues involved in the RBD region such as N437, and N440 are interacting with N31, and E107 respectively. **B.** Interacting partners of Fab4 and RBD involved in non-hydrogen bonding i.e Van-Der-Waals distance. **C.** For Fab26 complex, hydrogen bonding residues involved are T478, N481, Y489, and V483 which are interacting with N33, Y32, A101, and S97 respectively. Interacting residues of Fabs and RBD are colored in gold and pale green respectively. **D.** shows various interacting partners of Fab26 and RBD in the Van-Der-Waals distance.

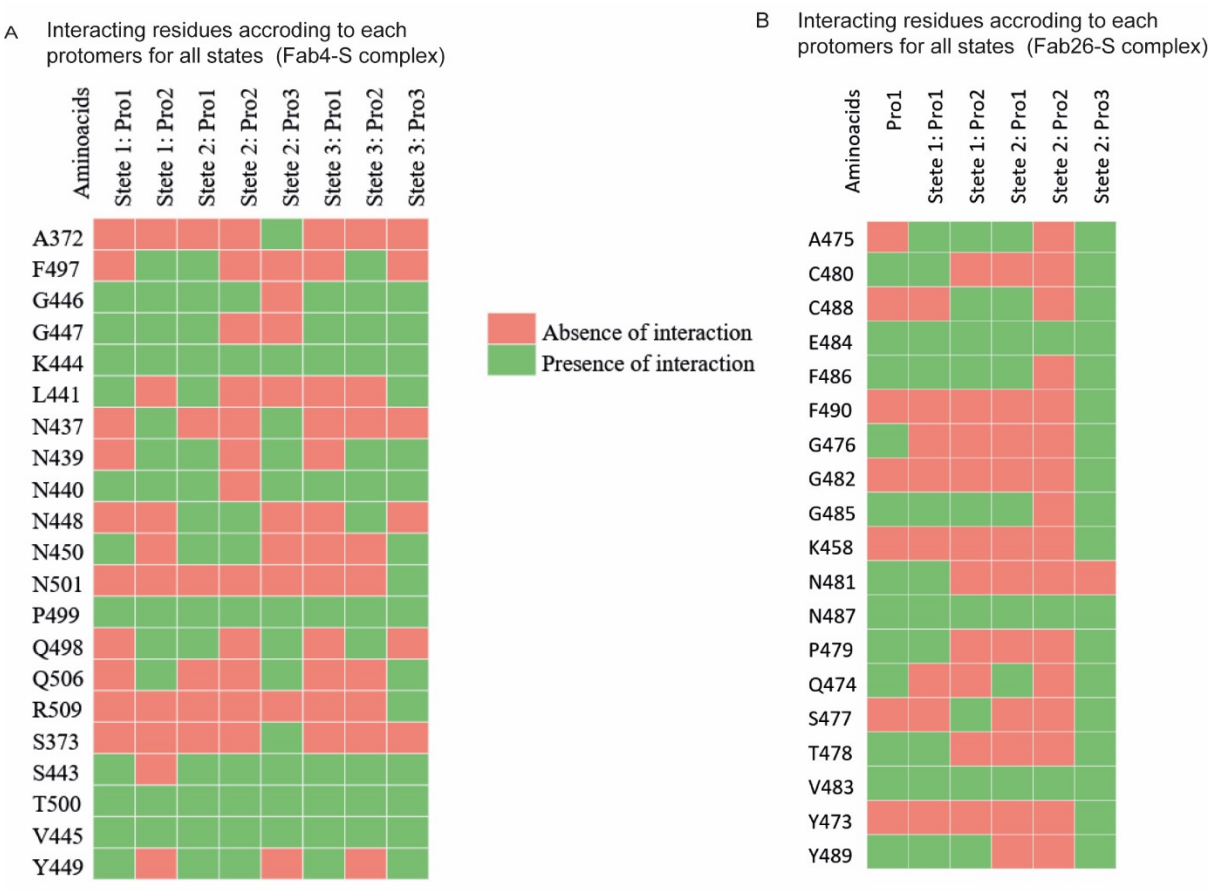

Figure S7: Heat map represents the interacting residues obtained from each protomer of various states captured for Fab4 and Fab26 complexes, **A, B** respectively.

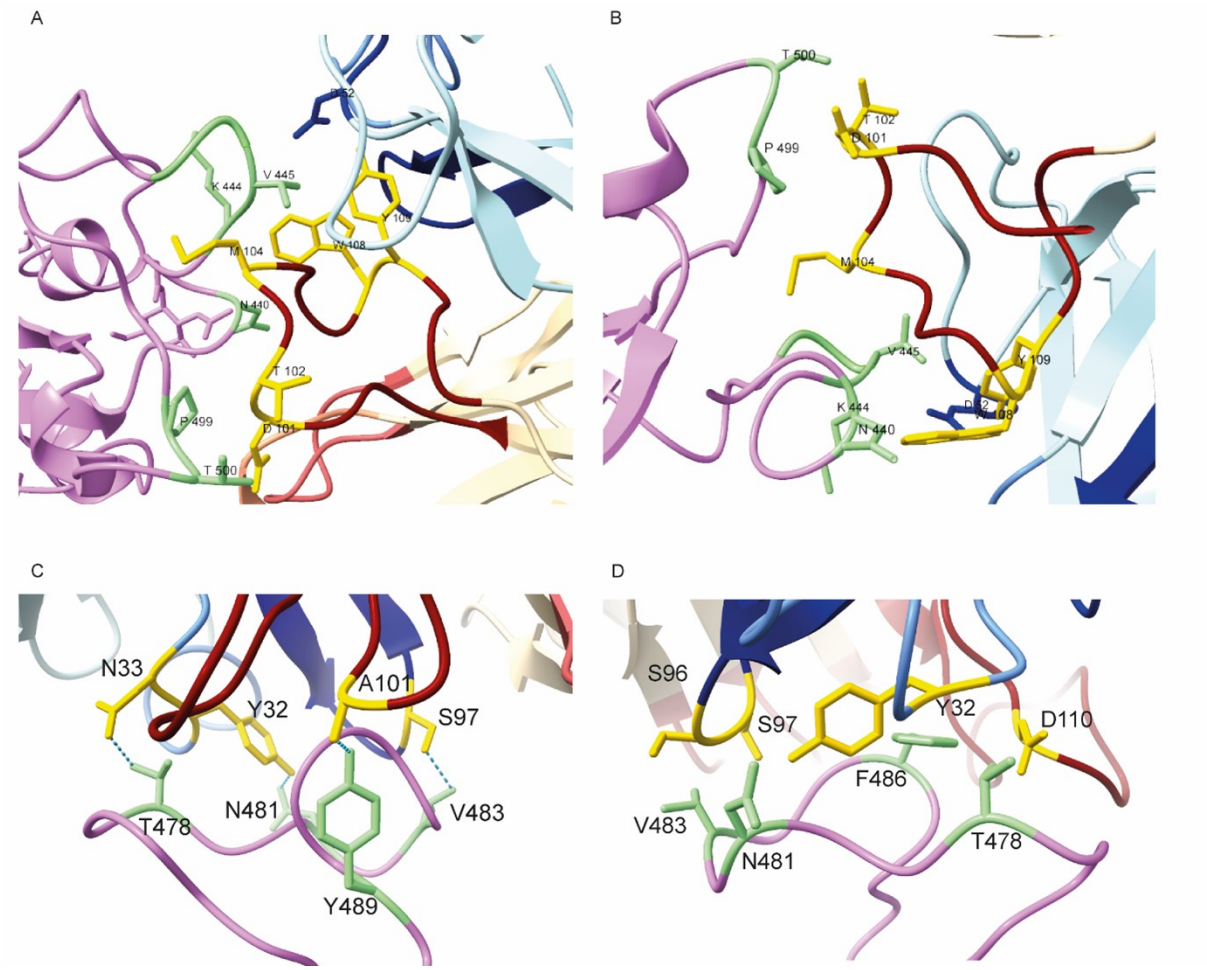

Figure S8: Potential key epitope residues interacting with Fab4 and 26 from the various states captured in cryo-EM analysis.

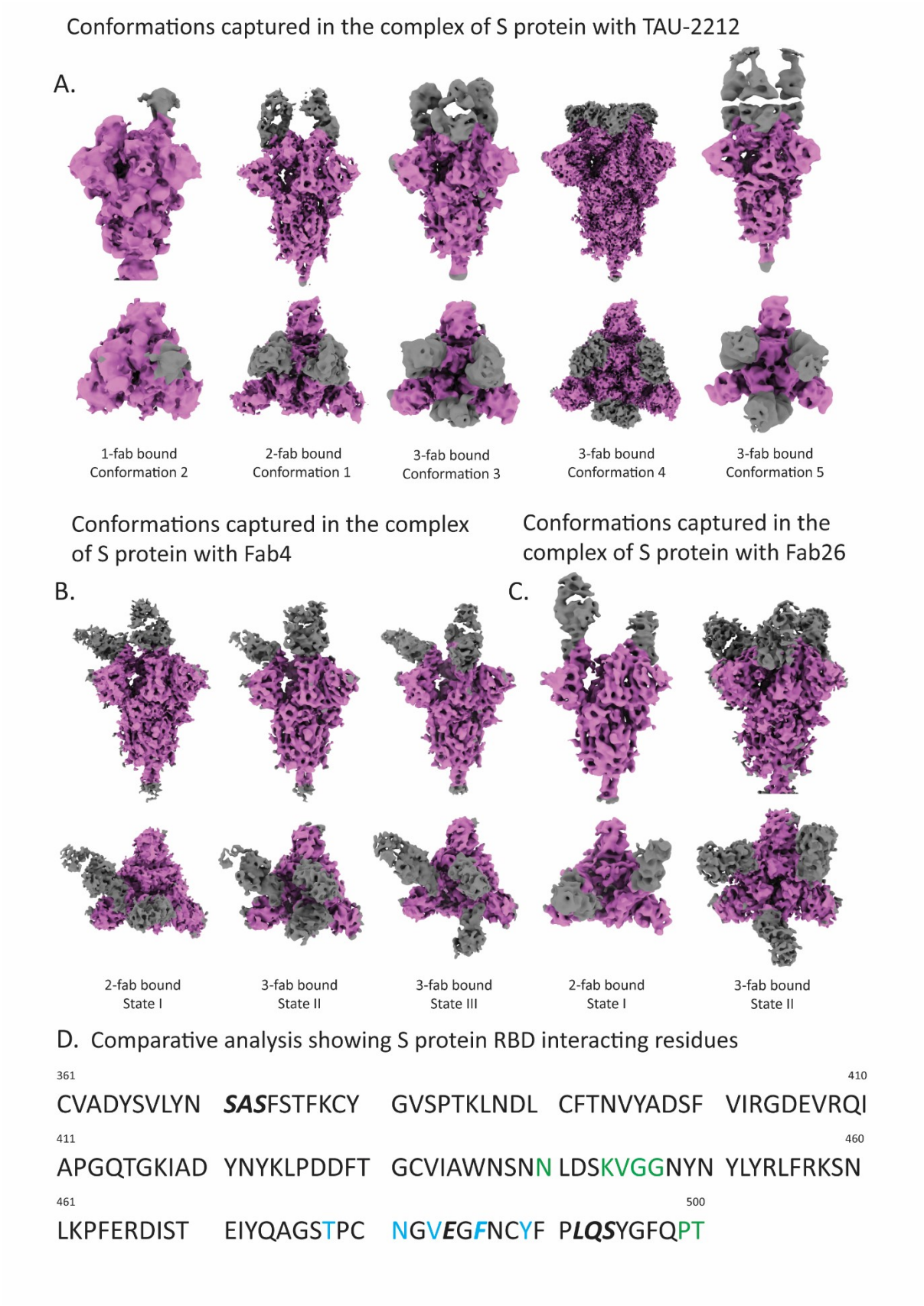

Figure S9: Comparative analysis showing conformational flexibilities S protein with reported Fab (TAU-2212) (Li, Ruofan, et al., *Commun Biol*, 2022) and our antibodies (Fab4 and Fab26): **A.** Five different conformations observed in neutralization of TAU-2212. Conformations 2 and 1 show single-

and dual-fab bound forms respectively, while the others have three-fab bound. **B.** Three different conformations captured in complex with Fab4: state I have a dual-fab binder, and the rest are three-fab bound. **C.** Likewise, for the Fab26 complex, two states were seen showing dual and three-binders. S proteins are colored in purple, and the grey region of the map shows the Fab. Top and bottom views are shown. **D.** Sequence analysis of S protein RBD region (361-500) shows the interacting residues involved in Fab binding (Fab4, Fab26 and TAU-2212). Interacting residues of Fab4 and Fab26 are highlighted in green and cyan, respectively, while TAU-2212 are in bold italics.

Cryo-EM density maps of the Wuhan SARS-CoV-2 trimeric spike protein complexed with monoclonal antibodies (THSC20.HVTR04 & THSC20.HVTR26) are deposited in the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB). The various conformational states captured for each antibody and its identifiers at EMDB and PDB are as follows:

	EMDB accession code(s)	PDB ID(s)
Spike- THSC20.HVTR04(Fab4) complexes		
State - I: Spike 2-up RBD with THSC20.HVTR04 (Fab4)	EMD-34546	8YBS
State - II: Spike 3-up RBD with THSC20.HVTR04 (Fab4)	EMD-34547	NA
State - III: Spike 3-up RBD with THSC20.HVTR04 (Fab4)	EMD-34548	NA
Spike- THSC20.HVTR26(Fab26) complexes		
State - I: Spike 2-up RBD with THSC20.HVTR26 (Fab26)	EMD-34563	8YBY
State - I: Spike 2-up RBD with THSC20.HVTR26 (Fab26)	EMD-34602	NA
State - II: Spike 3-up RBD with THSC20.HVTR26 (Fab26)	EMD-34603	8YBZ