Supporting Information

Atomistic Simulations and In Silico Mutational Profiling of Protein Stability and Binding in the SARS-CoV-2 Spike Protein Complexes with Nanobodies: Molecular Determinants of Mutational Escape Mechanisms

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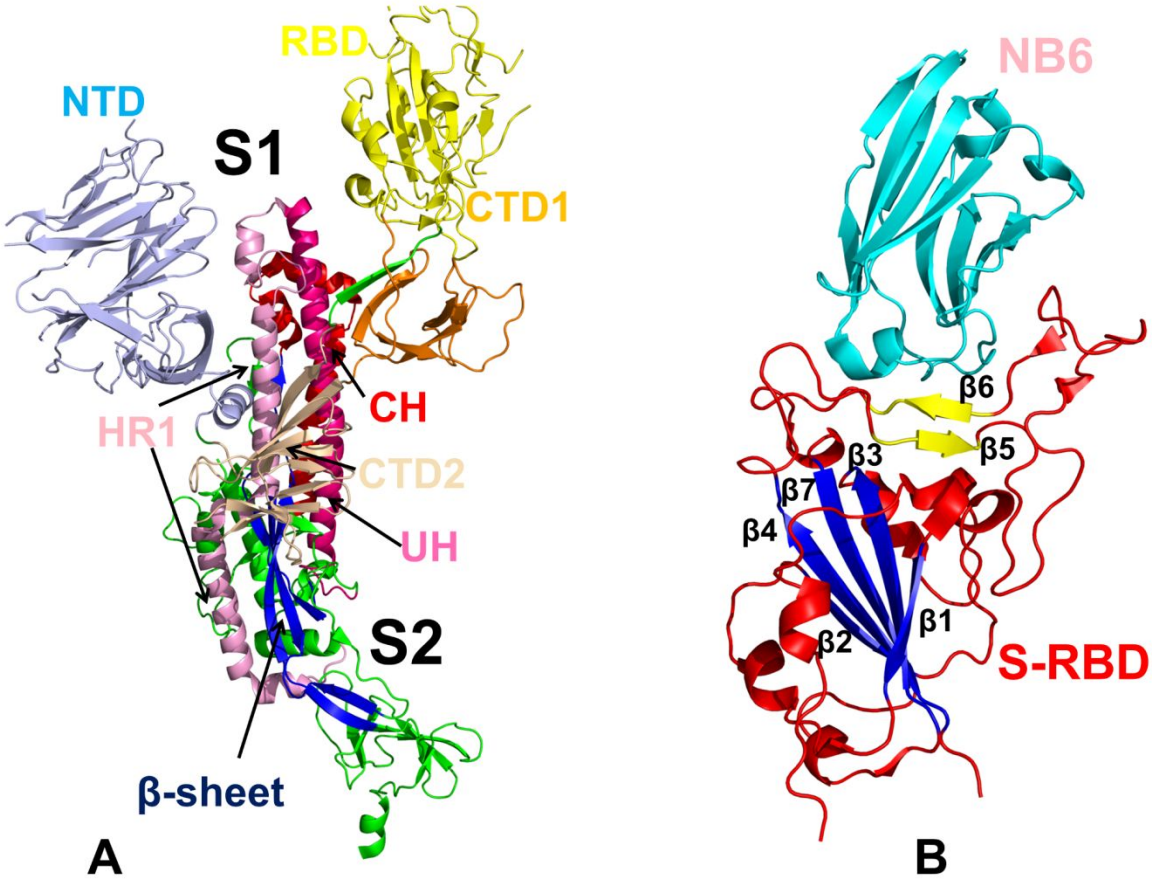

Figure S1. The domain organization for the full-length SARS-CoV-2 S protein. (A) The subunits S1 and S2 include NTD RBD, C-terminal domain 1(CTD1), C-terminal domain 2 (CTD2), fusion peptide (FP), fusion peptide proximal region (FPPR), heptad repeat 1 (HR1), central helix region (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT). The subunits S1 regions : NTD (14-306) in light blue;

RBD (331-528) in yellow; CTD1 (528-591) in orange; CTD2 (592-686) in wheat color ; upstream helix (UH) (736-781) in red; HR1 (910-985) in pink; CH (986-1035) in hot pink; core β-sheet (711-736, 1045-1076) (in blue). (B) Structural organization of the S-RBD (shown in red ribbons). The central β strands (β1 to β4 and β7) (residues 354-358, 376-380, 394-403, 431-438, 507-516) are shown in blue. β5 and β6 strands (residues 451-454 and 492-495) are shown in yellow. The bound nanobody Nb6 is shown in cyan ribbons.

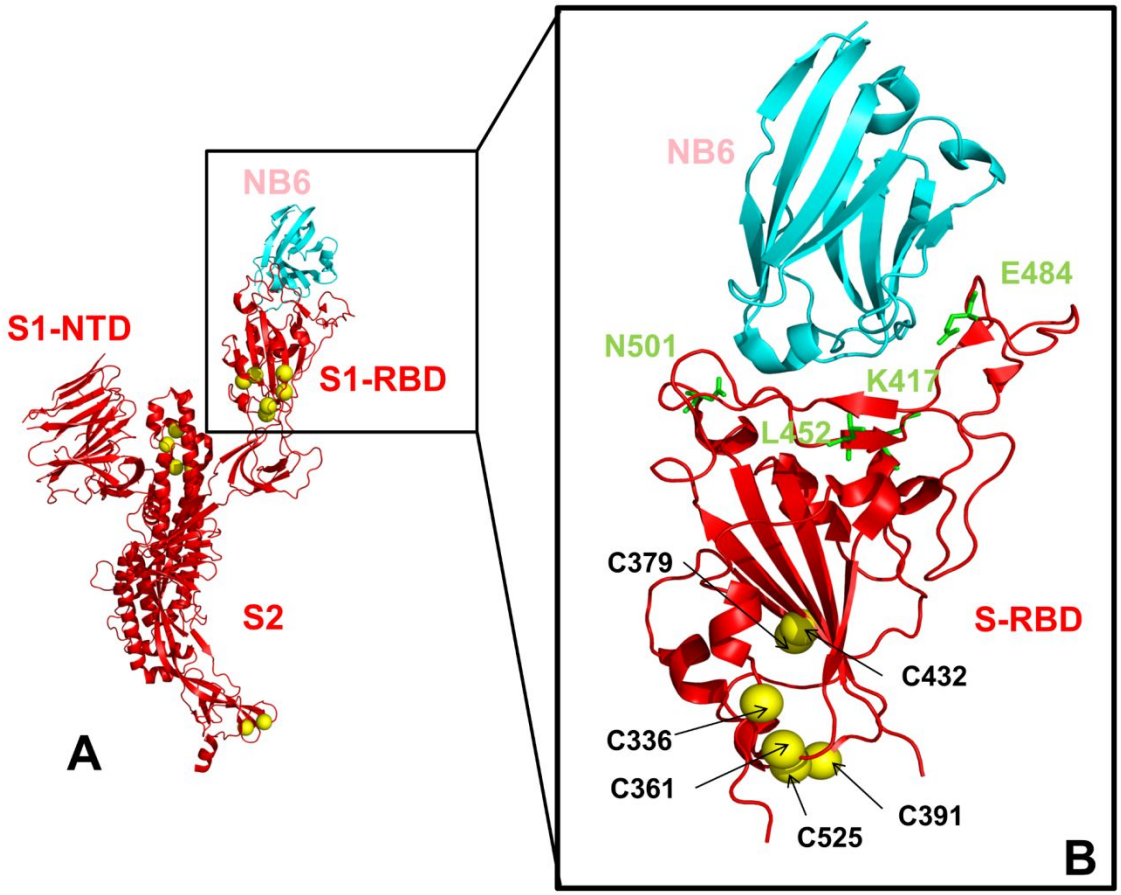

Figure S2. (A) Structural representation of the SARS-CoV-2 S protein with disulfide bonds.

The S protein monomer is shown in red ribbons and cysteine residues in S1 and S2 subdomains that form disulfide bonds are shown in yellow spheres. (B) A close-up of the S-RBD and α-helical segments (residues 367-370 and 383-388) that are tethered by C379-C432 and C391-C525 disulfide pairs. The cysteine residues forming disulfide linkages C336–C361, C379–C432 and C391–C525 in the S-RBD are shown in yellow spheres and annotated. The key RBM sites of circulating variants K417, L452, E484, and N501 are shown in green sticks.

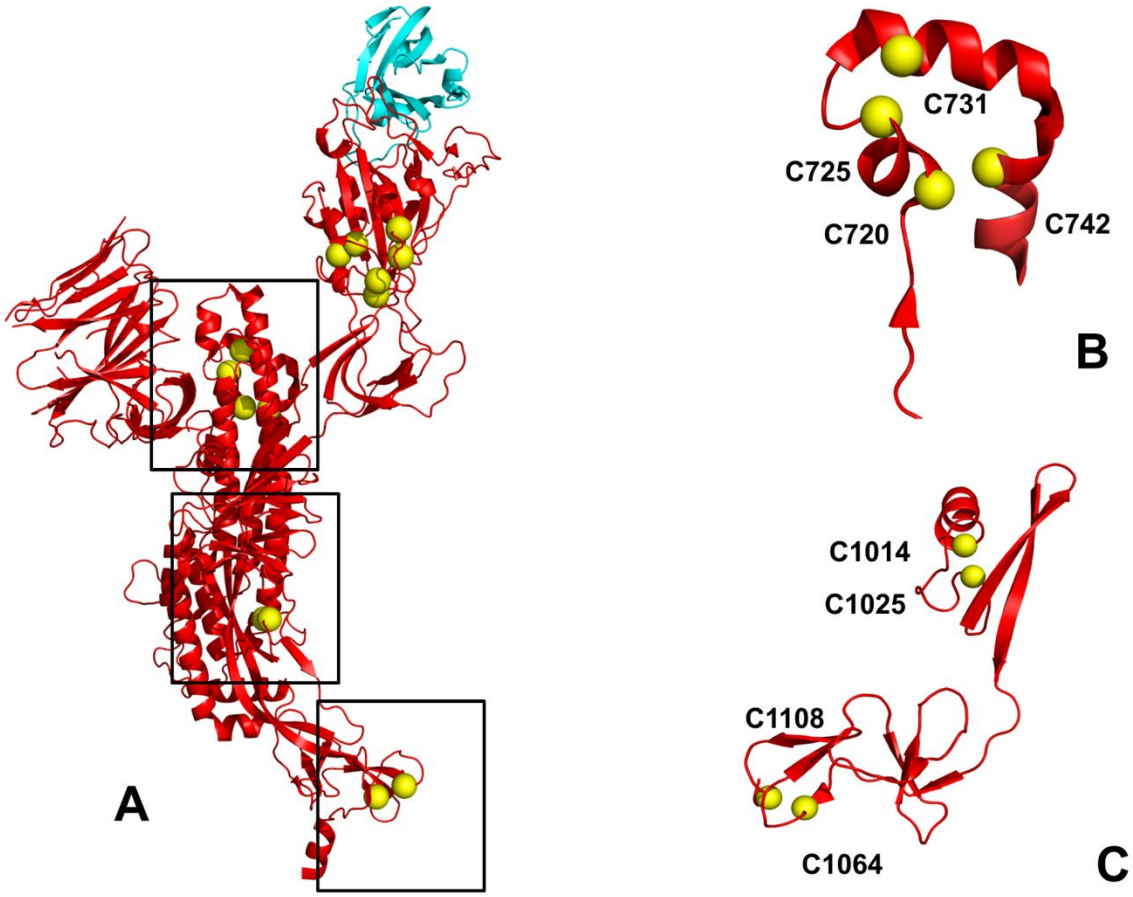

Figure S3. Structural organization of cysteine clusters in the SARS-CoV-2 spike prefusion structure. (A) The cryo-EM structure of the SARS-CoV-2 S protein in the prefusion form. The conserved cysteine clusters are shown in yellow spheres. (B) Structural arrangement of the conserved cluster in S2 subdomain formed by C720, C725, C731, and C742 in the UH region. The UH fragment is in red ribbons and cysteine sites are in yellow spheres and annotated. (C) Structural organization of the conserved cysteine cluster in the β-hairpin region of S2 subdomain

formed by C1014 and C1025, C1064 and C1108. The cysteine sites are in yellow spheres and annotated.

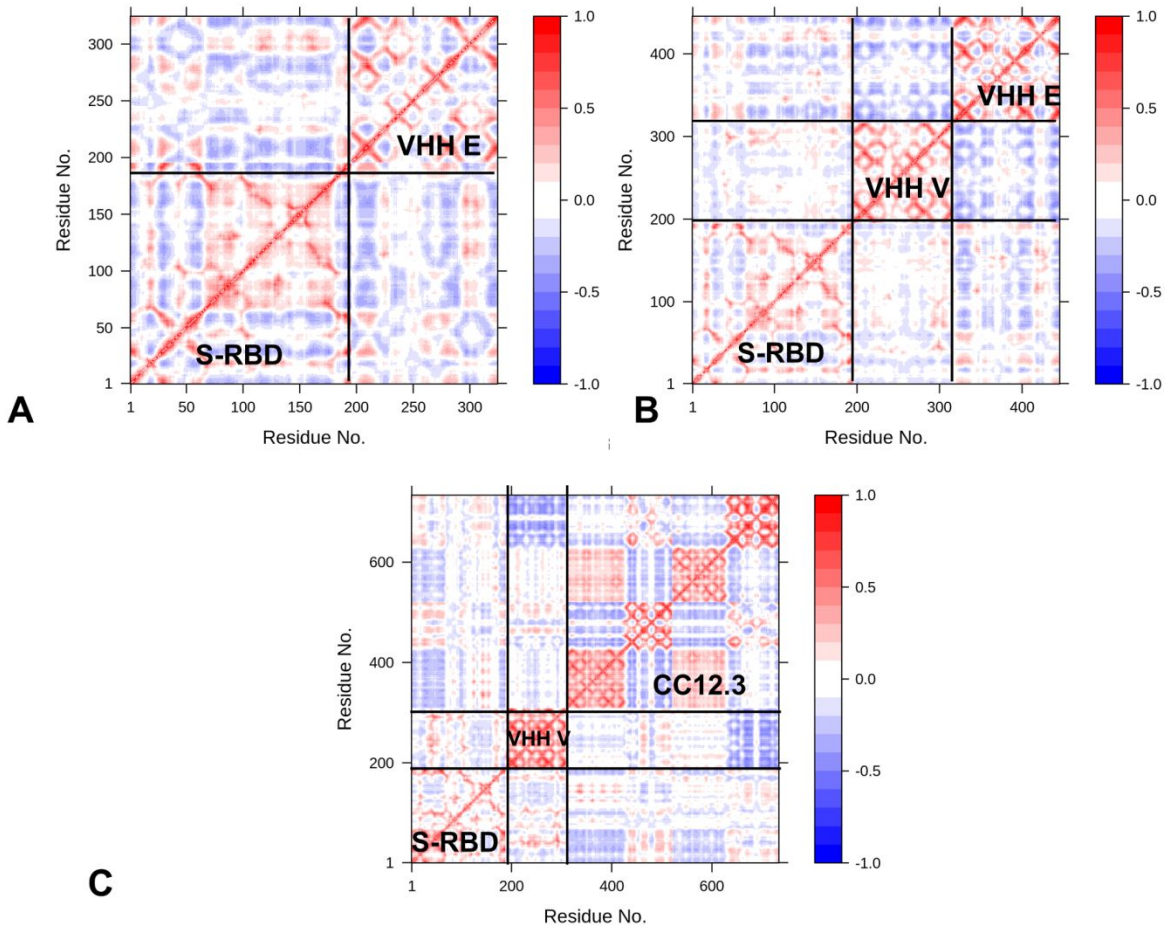

Figure S4. Conformational dynamics analysis and the covariance residue correlation matrixes for the SARS-CoV-2 S complexes with VHH E nanobody, pdb id 7lB14 (A), complex with the biparatopic nanobody VHH VE, pdb id 7B17 (B), and complex with CC12.3/VHH V combination, pdb id 7KN6 (C). The covariance matrix indicates coupling between pairs of

residues. Cross-correlations of residue-based fluctuations vary between +1 (correlated motion; fluctuation vectors in the same direction, colored in dark red) and -1 (anti-correlated motions; fluctuation vectors in the same direction, colored in dark blue). The values > 0.5 are colored in dark red and the lower bound in the color bar indicates the value of the most anti-correlated pairs.

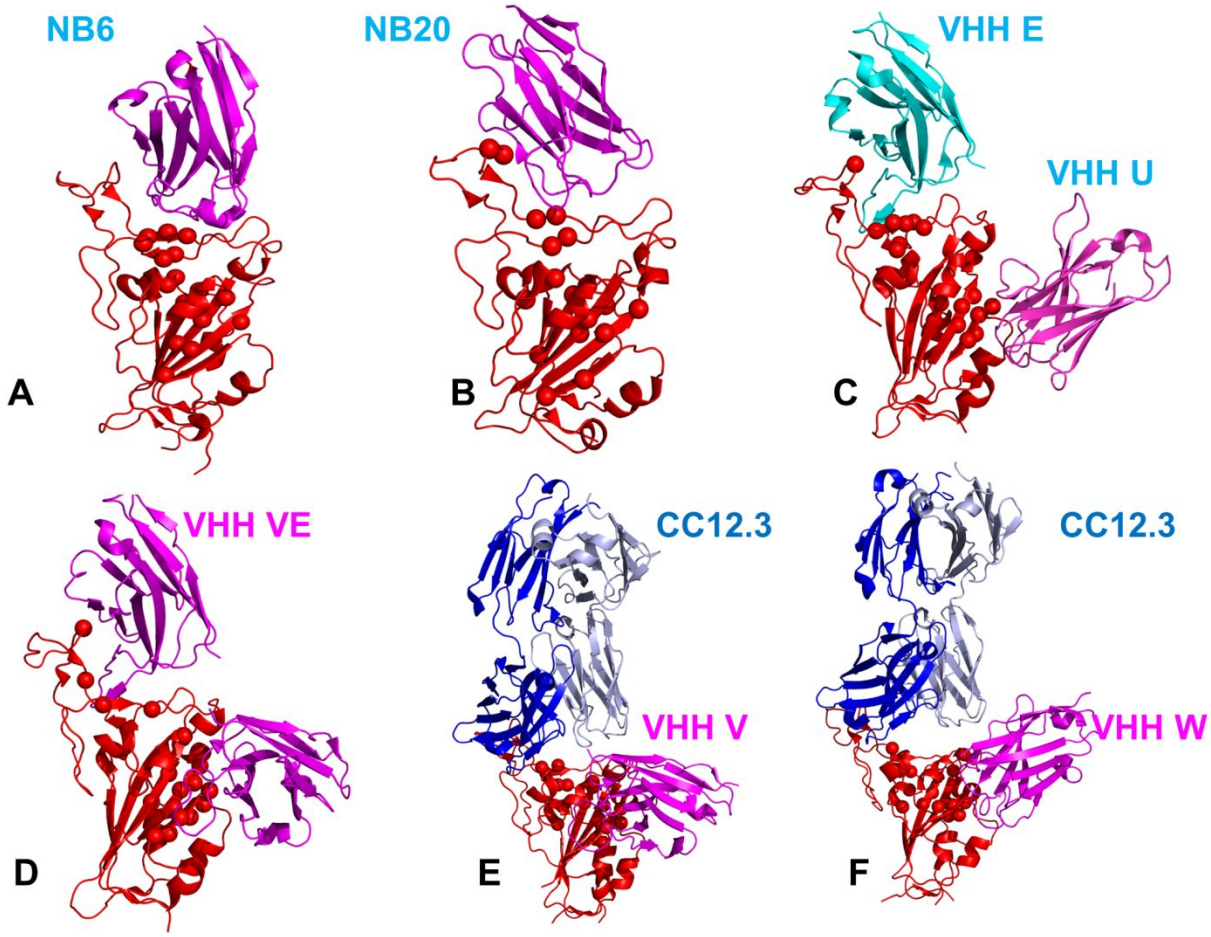

Figure S5. Structural mapping of protein stability centers for the SARS-CoV-2 S complexes with Nb6 (A), Nb20(B), VHH E/VHH U pair (C), biparatopic nanobody VHH VE (D), CC12.3/VHH V pair (E), and CC12.3/VHH pair (F). The S-RBD is shown in red ribbons. The bound nanobodies are shown in magenta-colored ribbons. The heavy chain of CC12.3 antibody is in blue ribbons and the light chain is in cyan-colored ribbons.