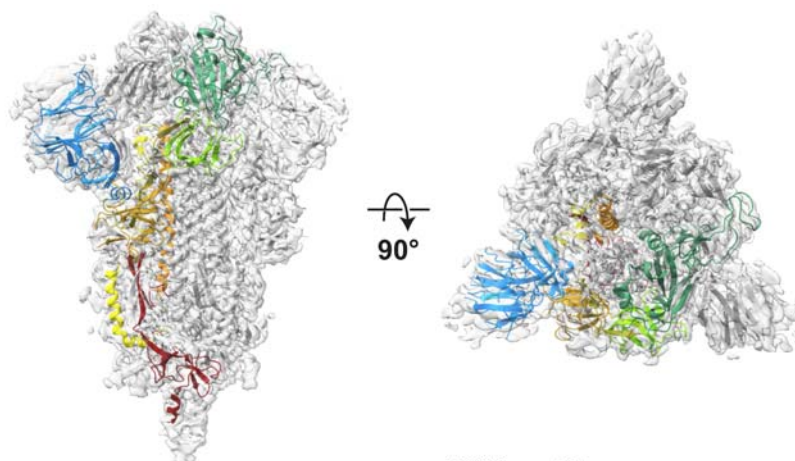
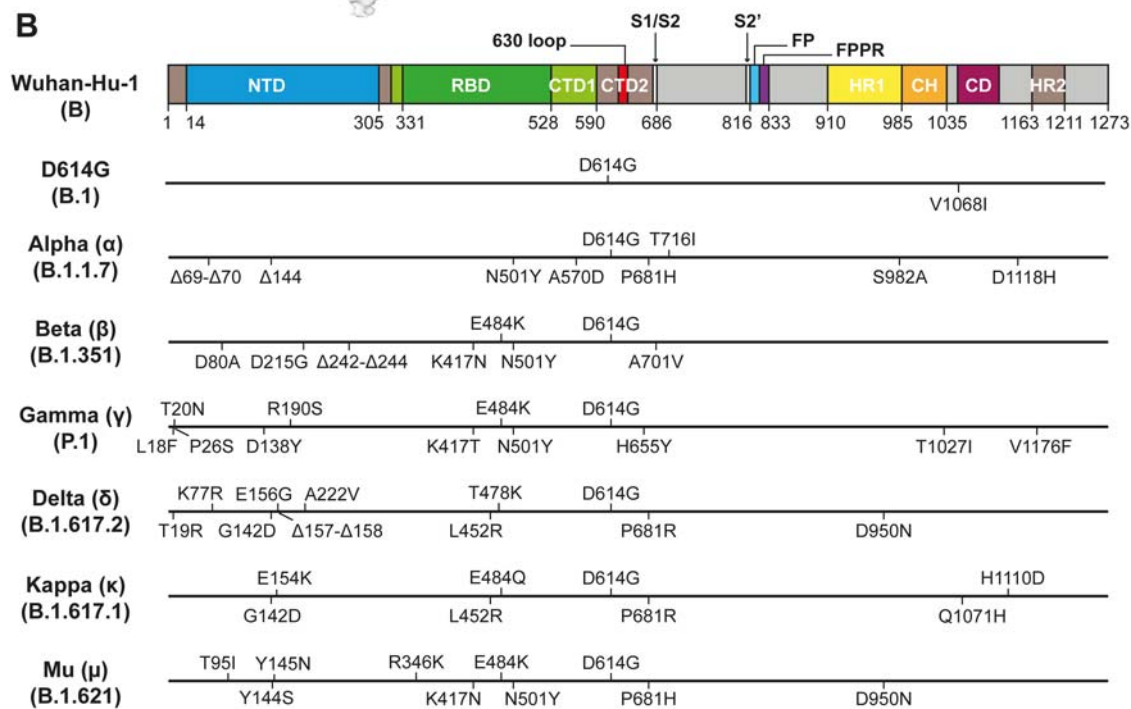
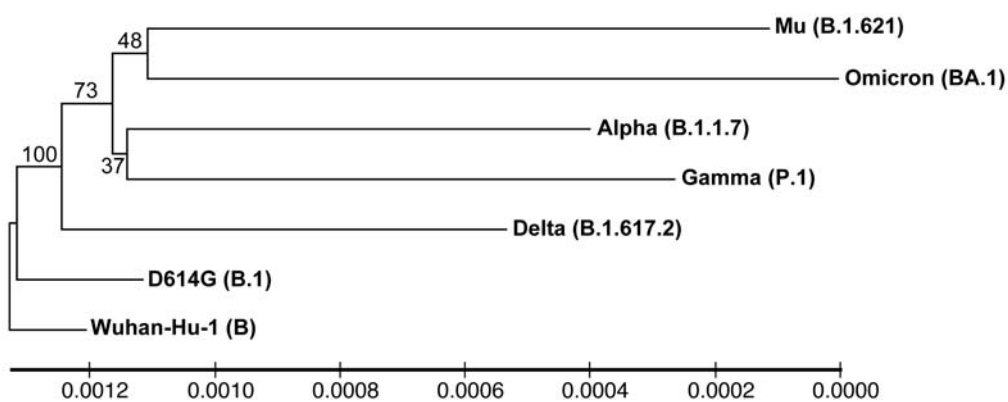


Expanded View Figures

Figure EV1. SARS-CoV-2 S mutations and the phylogenetic tree.

(A) S structure side and top views. One of the three chains is color-coded according to the color scheme in (B) to illustrate the positions of the structural features that are discussed in the manuscript. (B) Mutations in S for the variants investigated in this study. Variants are indicated using both WHO labels (Greek letters) and PANGO lineage nomenclatures. Δ indicates amino acid deletions. Secondary structures are color-coded in the index strain (Wuhan-Hu-1) and the residue numbers are marked in the panel. NTD, N-terminal domain; RBD, receptor binding domain; CTD, C-terminal domain; 630 loop, a loop which contains residues 617-644; S1/S2, furin cleavage site; S2', S2' cleavage site; FP, fusion peptide; FPPR, furin peptide proximal region; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2. (C) Neighbor-joining phylogenetic tree of SARS-CoV-2 variants investigated in this study, together with the Omicron BA.1 variant. Scale bar refers to a phylogenetic distance of nucleotide substitutions per site.

A**B****C**

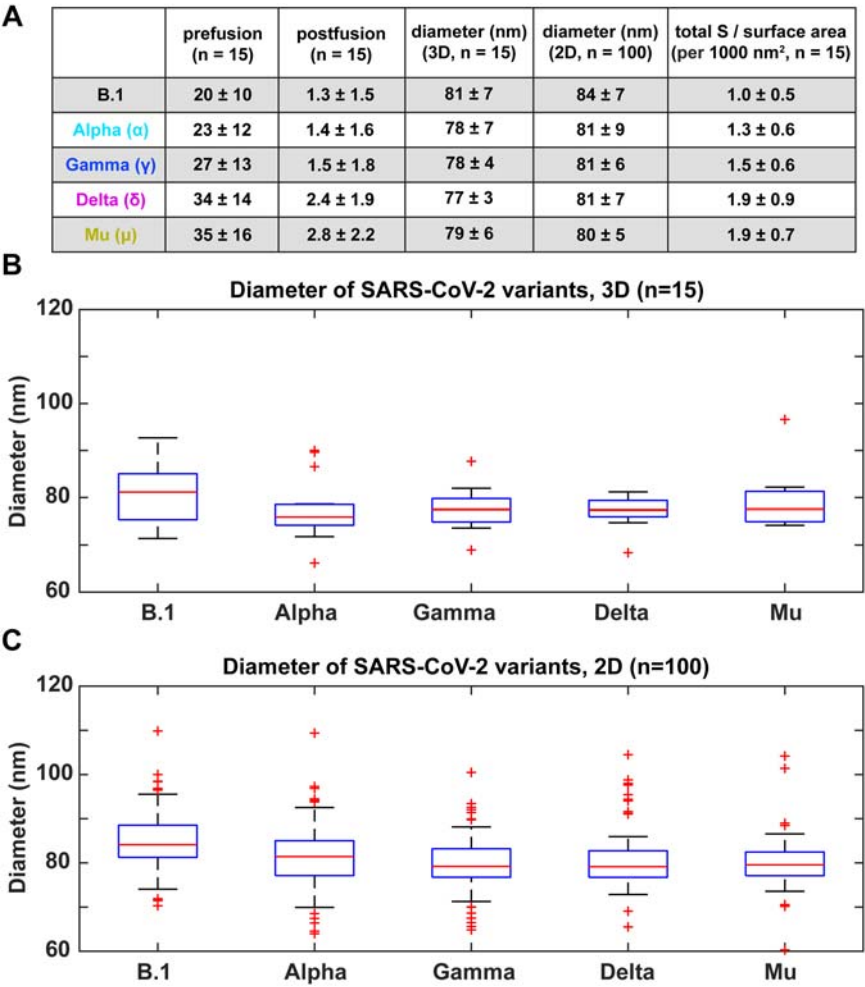


Figure EV2. Virion diameter quantification.

(A) The table summarizes the mean numbers of prefusion S, postfusion S, virion diameter (in 3D and 2D measurements), and number of S trimers per unit surface area (per 1000 nm²). Values are presented as mean ± SD; The number of virions used for quantification is in the column header. (B, C) Virion diameter measurements from 3D tomographic reconstructions (B) and 2D projections (C). The n indicates number of virions used for quantification. The box plot represents mean ± SD. A comparative statistical analysis between strains was not performed, because we cannot take possible variation between virus preparations into account. On each box, the central mark (red line) indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers. Outliers are plotted individually using the '+' marker symbol.

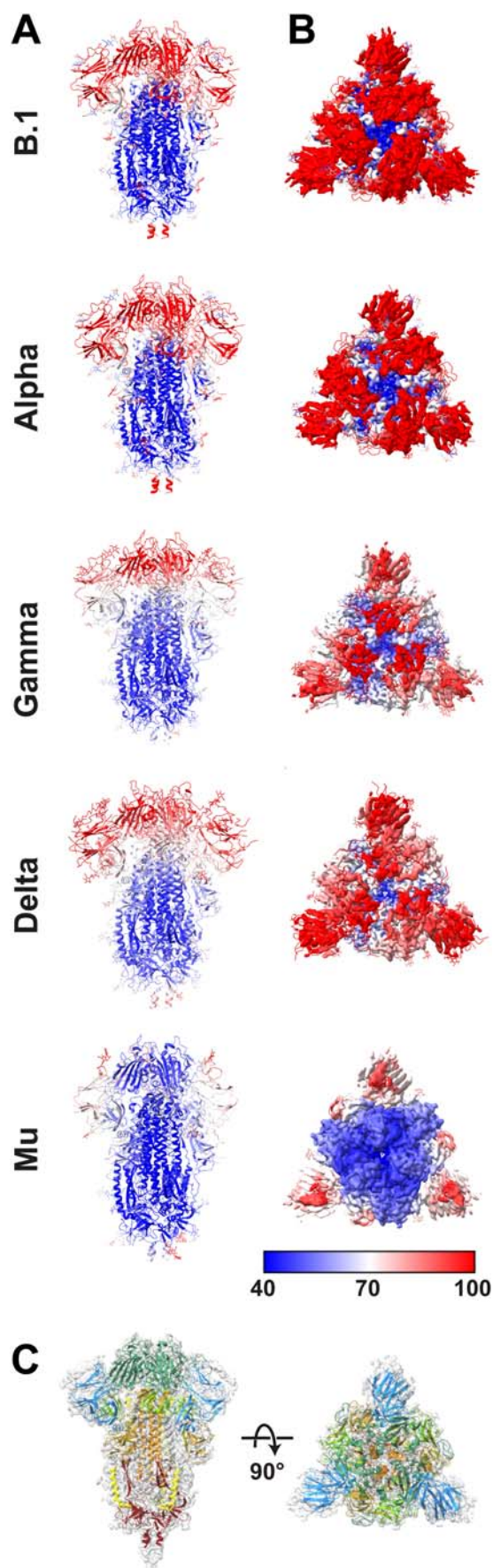


 Figure EV3. B-factor analysis of S structures.

(A, B) The modeled structures are color-coded according to the residue specific B-factors from 40 Å² (blue) to 100 Å² (red). S is shown from the side in cartoon (left column, A) and as a top view with the EM map surface colored by b-factor (right column, B). The lower the B-factor, the more rigid the protein is. In general, the S2 region is mostly rigid across all the variants, indicated by the low B-factor (blue), while the NTD and RBD are more flexible, indicated by the high B-factor (red). Note, that the Mu variant has a relatively rigid RBD while its NTD remains flexible. (C) The secondary structure of S is color-coded according to Fig. EV1.

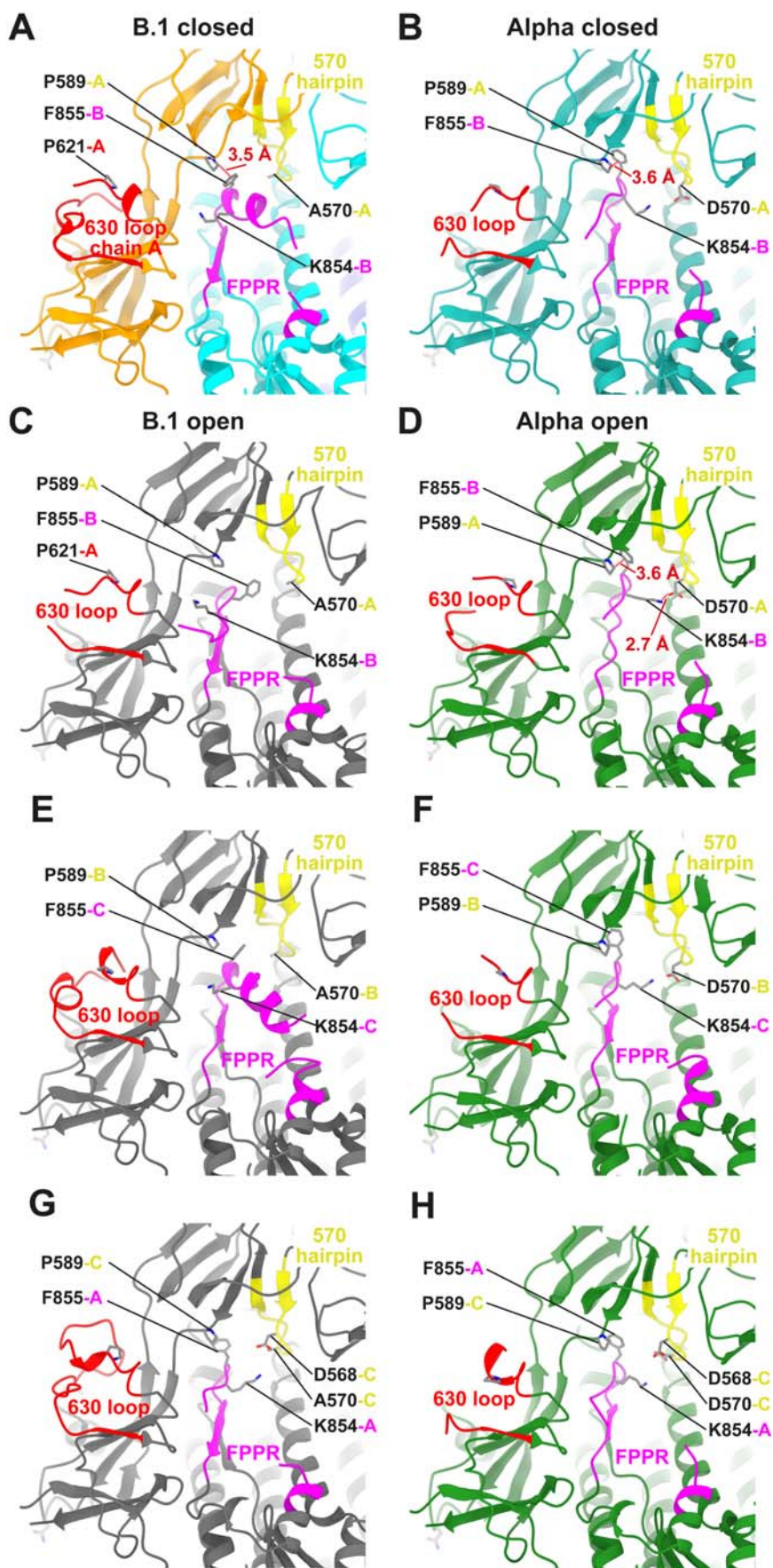


Figure EV4. Structural changes modulated by A570D illustrated for all chains.

The three individual chains (ABC) of the 4 structures (B.1 and Alpha, closed and open states) are illustrated here. Color schemes are the same as in Fig. 5. The positions of the three chains are illustrated in (A): chain A is in cyan, chain B is in orange, chain C is in blue. (A–D) Panels duplicated from Fig. 5 for comparison. (E–H) Contacts made by the other chains for B.1 and Alpha open states. The FPPR and interacting residues from each of the three chains from the B.1-open structure are illustrated in (C, E, G); the three chains from the Alpha open structure are illustrated in (D, F, H).

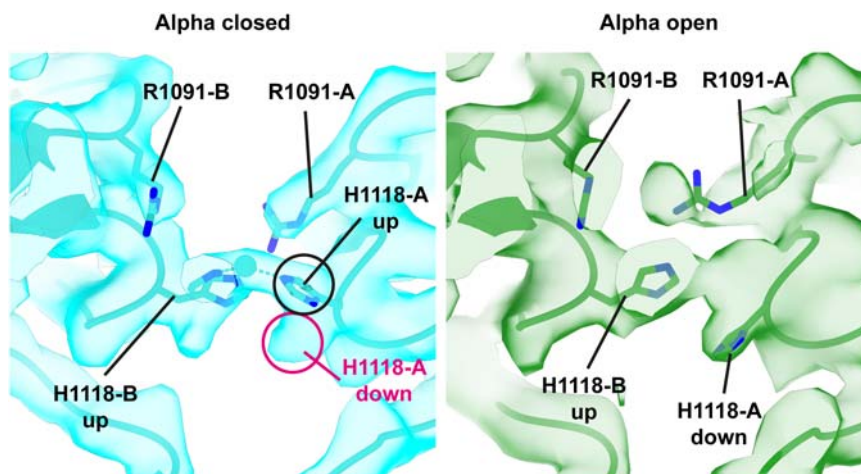


Figure EV5. Structural comparison of S structures in the closed and open conformation at mutation D118H from Alpha variant.

Left: S structure from Alpha closed state illustrates that H1118 has two conformers, one points upwards (black circle) away from the membrane, and an alternate conformation points downwards (unoccupied magenta circle) towards the membrane. Right: In the open conformation H1118 in chain A (open chain) points primarily downwards and chains B and C points upwards, while R1091 (chain A) has rotated.