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## Supplemental Information

# Allosteric perspective on the mutability and druggability of the SARS-CoV-2 Spike protein

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# Allosteric perspective on the mutability and druggability of the SARS-CoV-2 Spike protein

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## Supplemental Figures

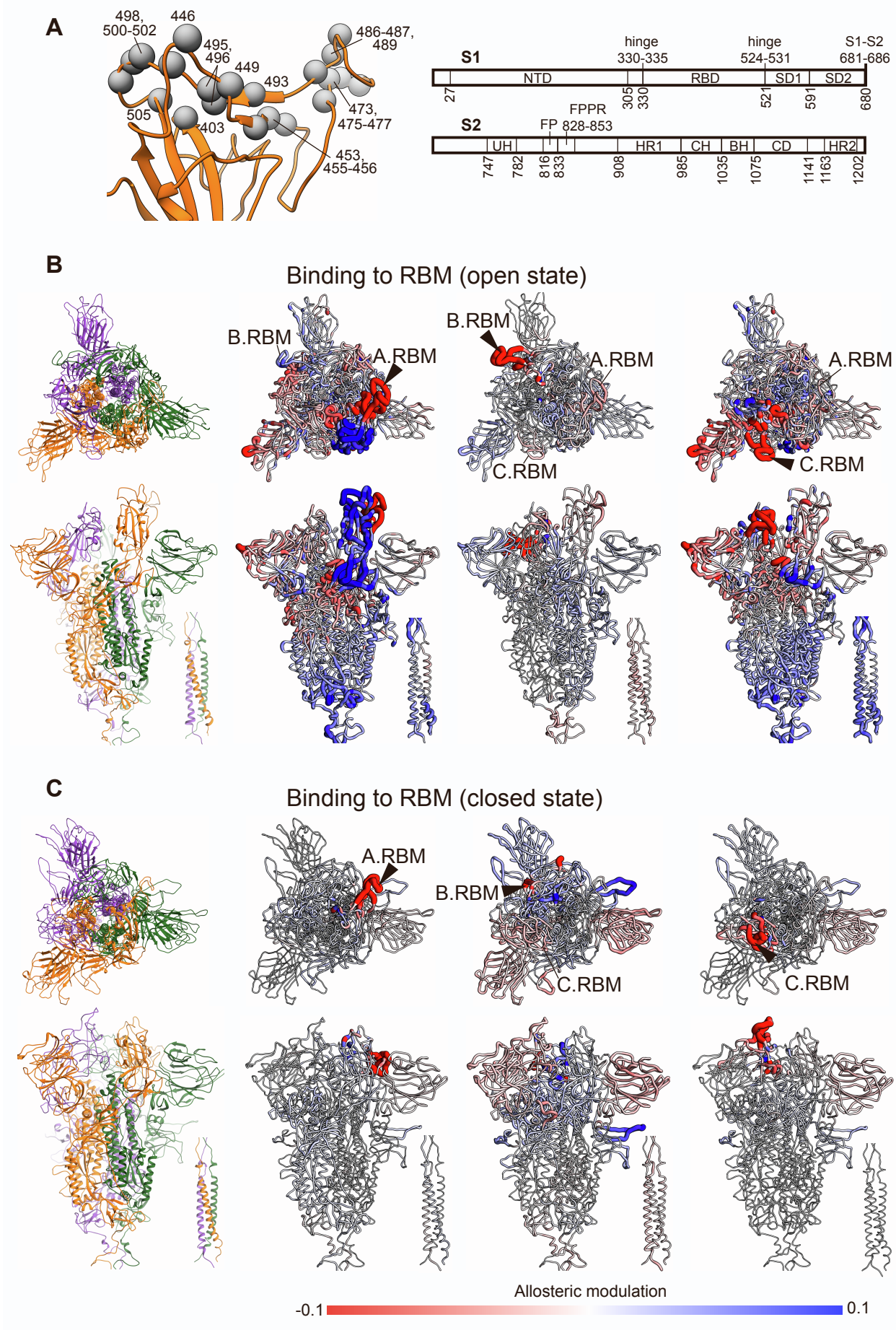
A
498,
500-502
446
495,
496
449
493
505
403
486-487,
489
473,
475-477
453,
455-456
S1
hinge
330-335
hinge
524-531
S1-S2
681-686
NTD
RBD
SD1
SD2
27
305
330
521
591
680
FPPR
S2
FP 828-853
UH
HR1
CH
BH
CD
HR2
747
782
816
833
908
985
1035
1075
1141
1163
1202
B
Binding to RBM (open state)
B.RBM
A.RBM
B.RBM
A.RBM
A.RBM
C.RBM
C
Binding to RBM (closed state)
A.RBM
B.RBM
C.RBM
C.RBM
Allosteric modulation
-0.1
0.1

**Figure S1. Definition of the receptor-binding motif (RBM) and the allosteric response upon binding to the RBM.** Related to Figure 1. (A) Left, residues forming the receptor-binding motif (RBM) are shown as spheres on A.RBD of the open spike. The residues are defined based on a $C_\alpha$-$C_\alpha$ distance cutoff at 5 Å from any residue of the ACE2 receptor, using a structure (PDB: 6M17) of the RBD-ACE2 complex. Right, the residues forming each studied spike region. (B, C) Allosteric modulation of the spike in the open and closed states upon simulated binding to a RBM (marked by a triangle).

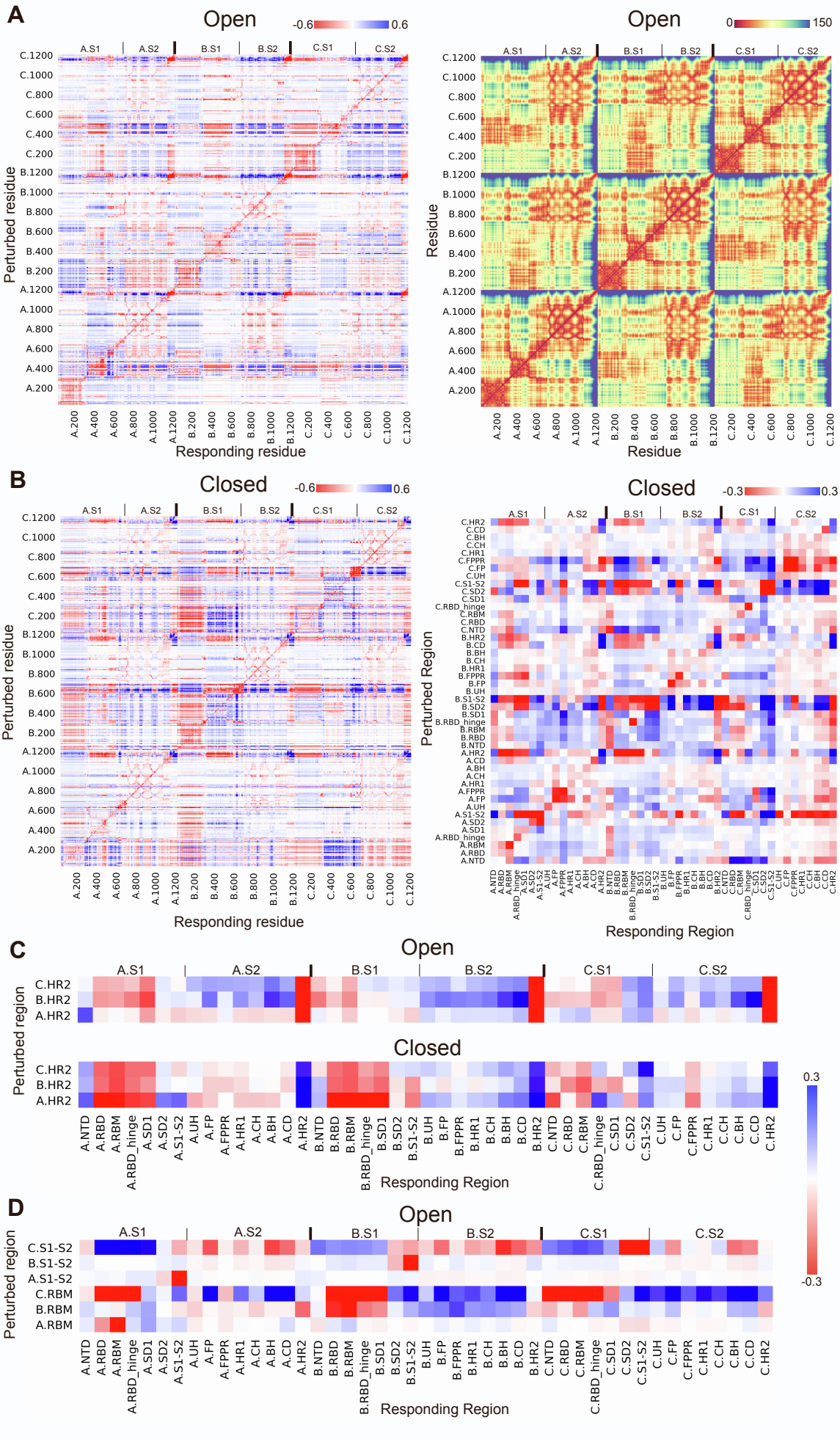
A
Open
-0.6
0.6
Perturbed residue
Responding residue
Open
0
150
Residue
B
Closed
-0.6
0.6
Perturbed residue
Responding residue
Closed
-0.3
0.3
Perturbed Region
Responding Region
C
Open
Perturbed region
Closed
Responding Region
0.3
-0.3
D
Open
Perturbed region
Responding Region

**Figure S2. The complete allosteric signalling maps and some relevant parts.**

Related to Figure 2. (A) ASM (left) and the pairwise $C_\alpha$-$C_\alpha$ distance map (right, Å) of the open spike. (B) The ASM for the closed spike at the per-residue and per-site levels. Additionally to those described in main text, examples of signalling between different parts of the S protein: positive modulation by the cluster of A.FP, A.FPPR, A.HR1 and C.SD1 regions in the open state, while only A.FP positively modulates A.RBM in the closed state; the A.RBM is more strongly modulated by HR2 in the S2 stalk in the closed state compared to the open state. Trends in signalling to A.RBM from the same sites of chains B and C are, in general, similar, but become weaker upon movement from B to C (Figures 2A and S2). (C, D) The parts of the ASMs (Figures 2A and S2B, right) corresponding to the results described in Figure 2D and 2E.

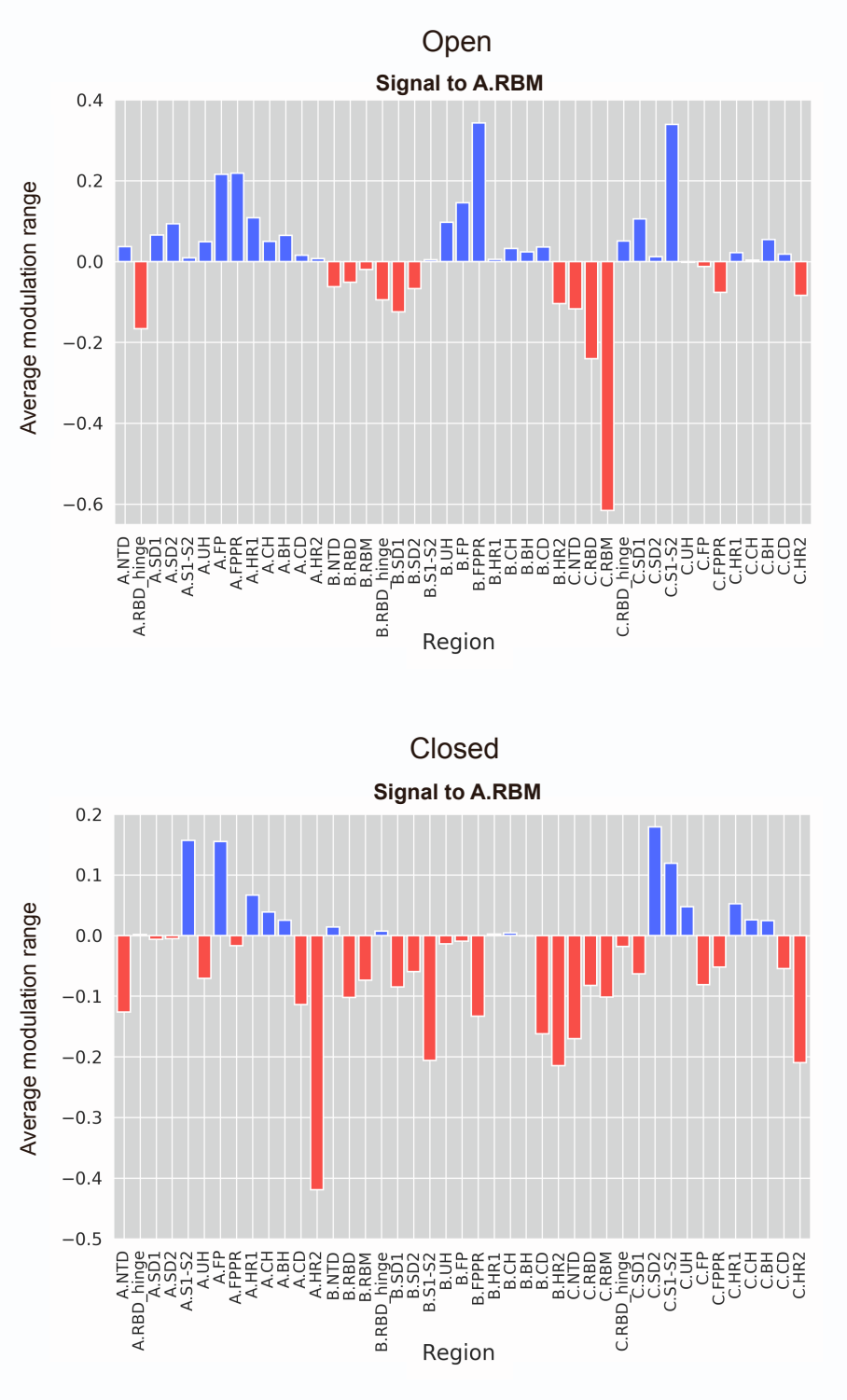


**Figure S3. The average modulation range in A.RBM caused by single mutations in every region (except A.RBM and A.RBD).** Related to Figure 3. The FPPR loop adopts different conformations — packed below SD1 in the open structure (Figure 3B, top panel, center), but sandwiched between the loops connecting SD1 to SD2 and the helices forming the S2 core in the closed state (Figure 3D, bottom panel, right).

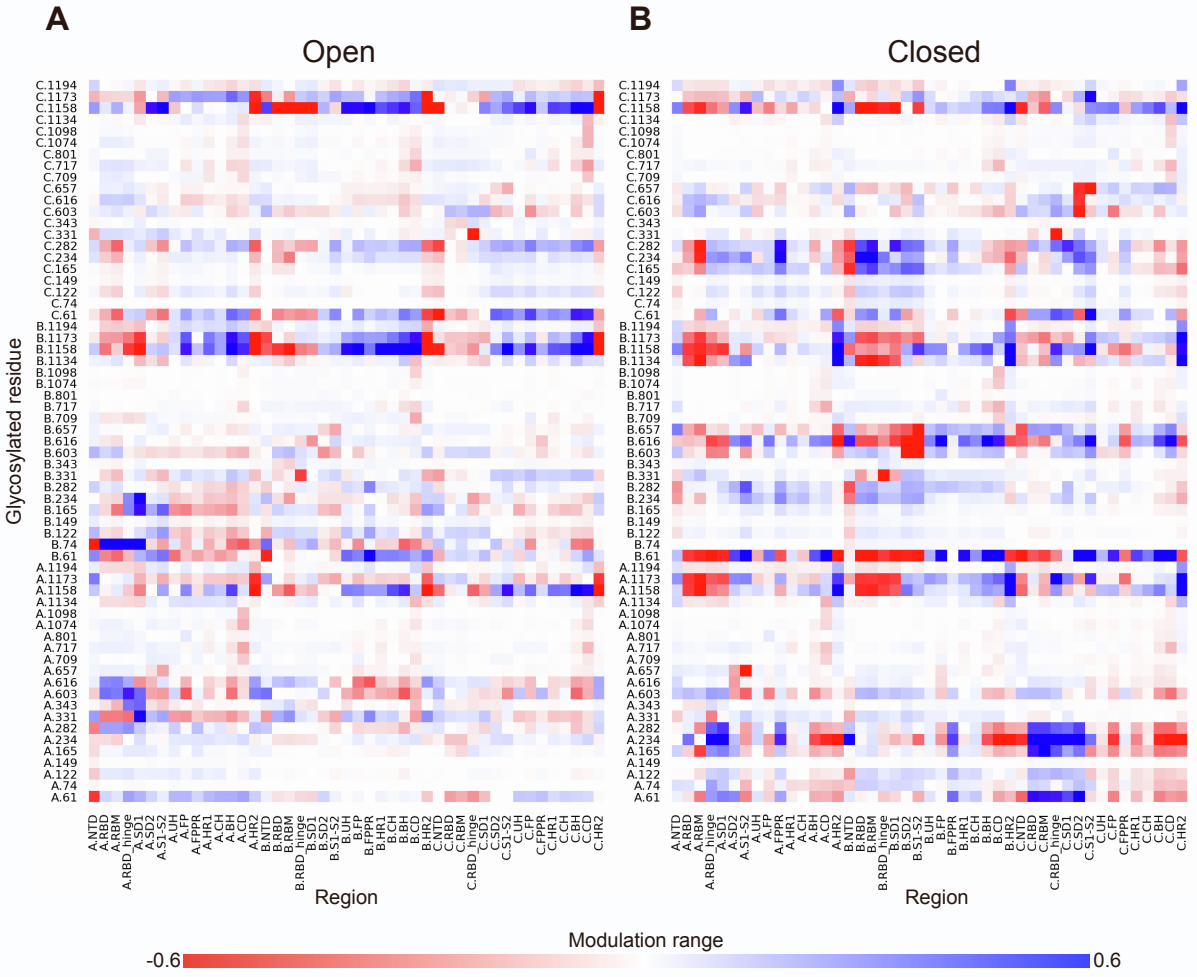


**Figure S4. The average modulation range resulting in every responding site/region due to signalling from each glycosylated position in the open/closed S homotrimer.** Related to Figure 4.

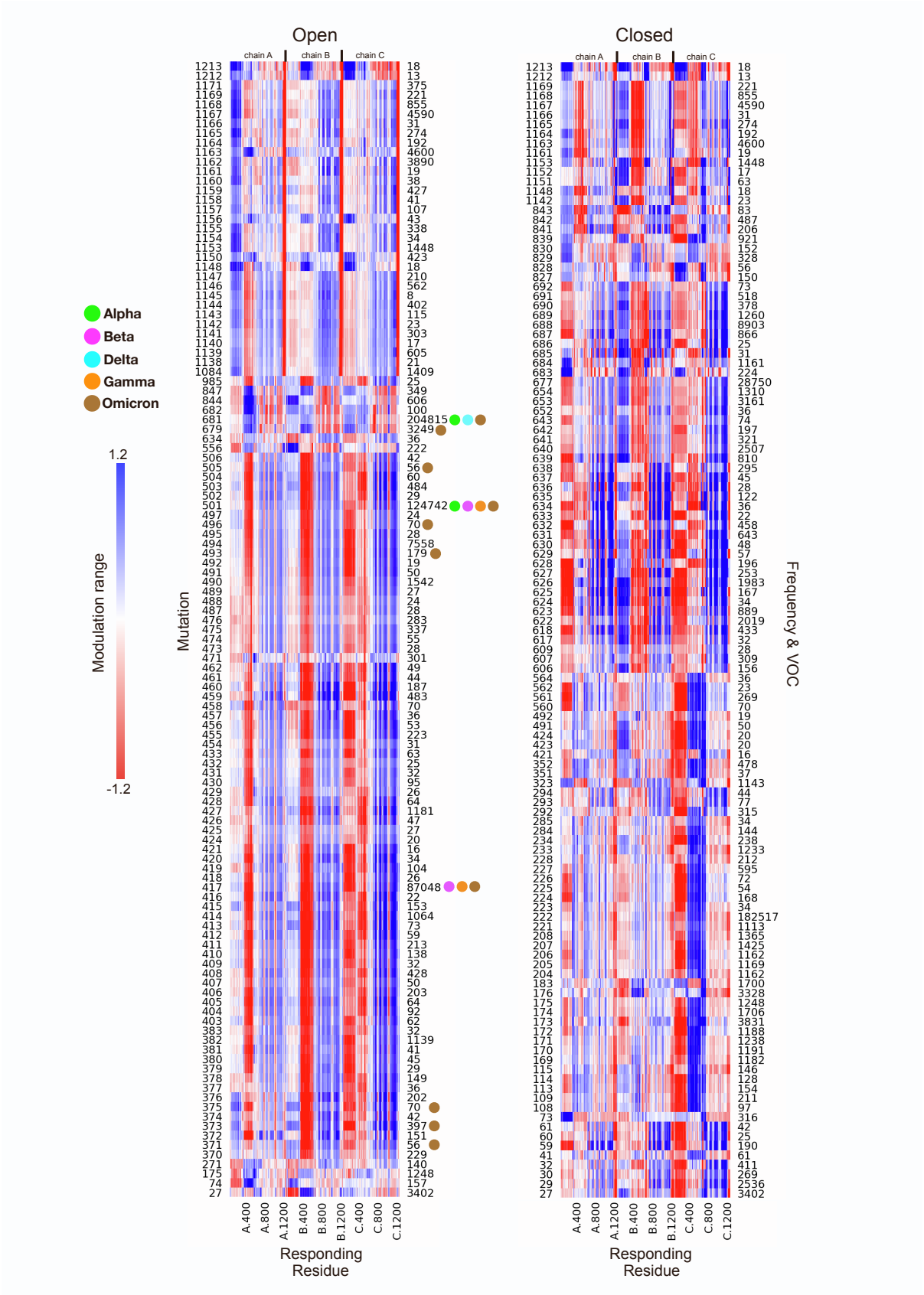


**Figure S5. Agnostic analysis of the ASMs.** Related to Figure 5.

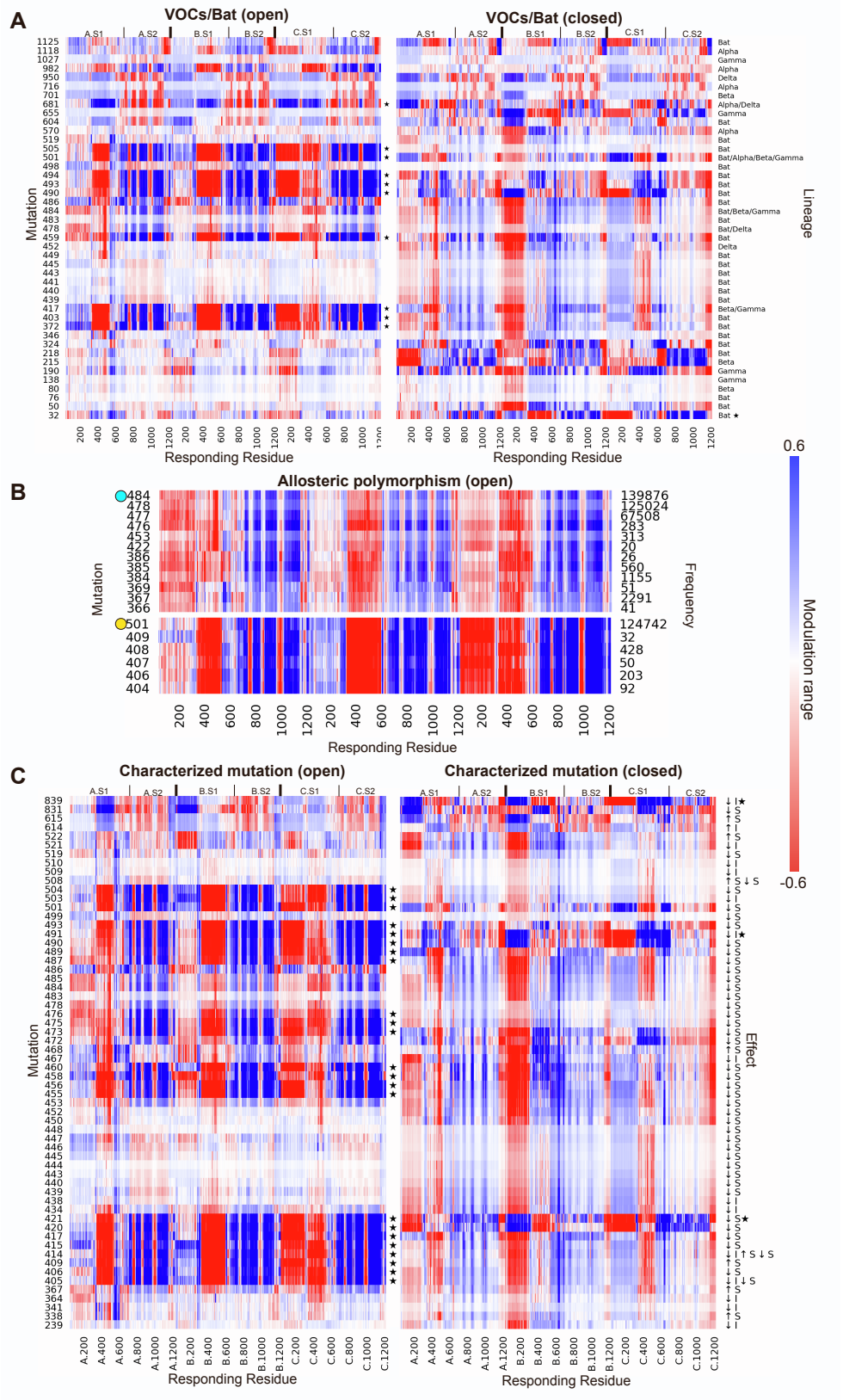


**Figure S6. Modulation ranges caused by mutations in open/closed spike.** Related to Figure 6. Modulation ranges originated from (A) mutated positions in VOCs/Bat, (B) positions 484/501 as well as those causing a similar response, and (C) experimentally characterized mutations. In (C), mutations of residues in the β4-loop-β5 segment and the β7

strand (residues 434-453 and 508-519) of the RBD core, for instance residues 438 and 510, typically result in very weak modulation (Figures 6A, S6C). This suggests an orthosteric mode of action in the case of the S438F and V510L mutations, which lead to decreased infectivity (↓I in Figure 6A, (Li et al., 2020a)).

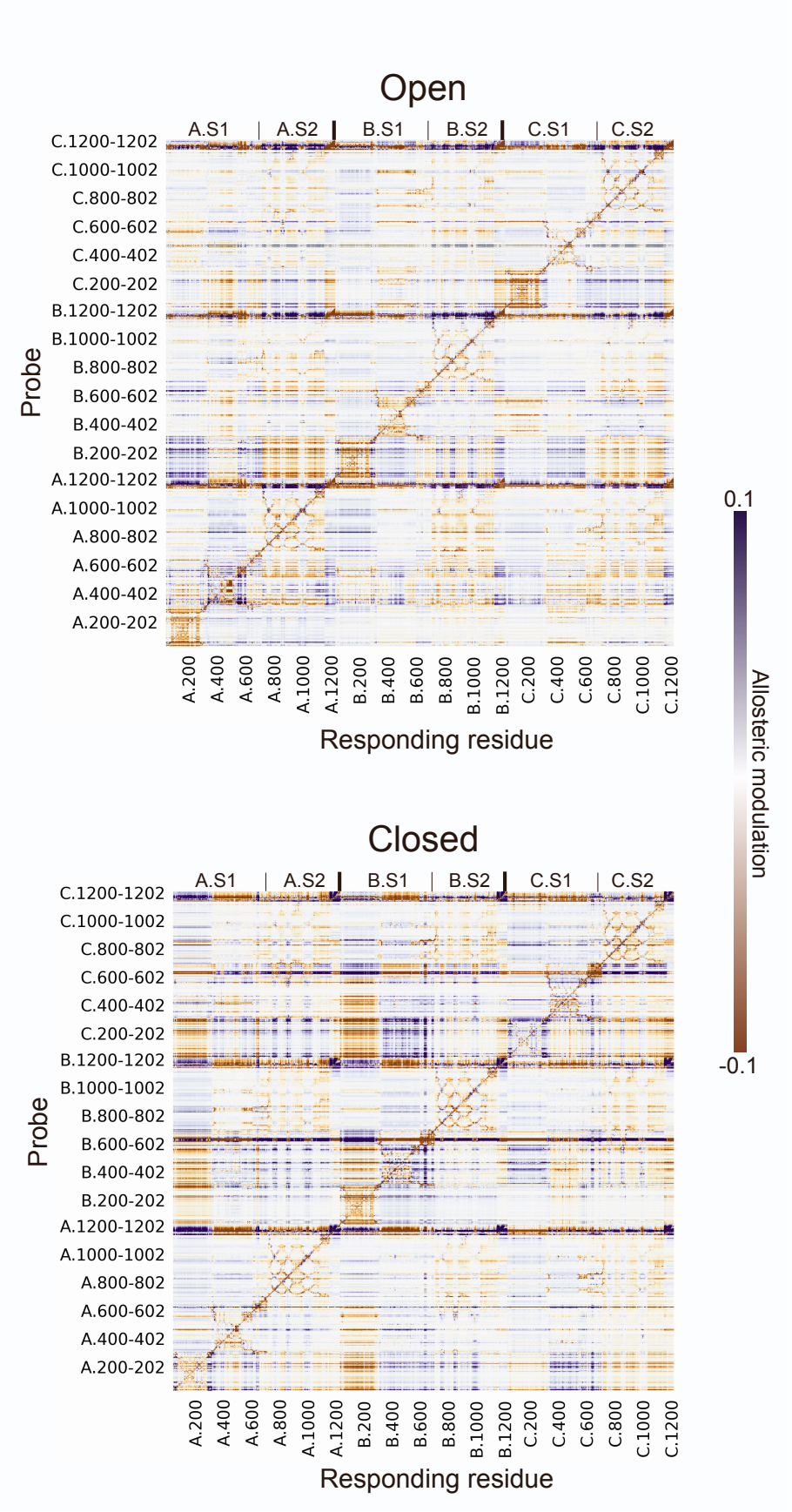


**Figure S7. Allosteric probing maps.** Related to Figure 7.