Supplementary Data

Differential Interactions Between Human ACE2 and Spike RBD of SARS-CoV-2 Variants of Concern

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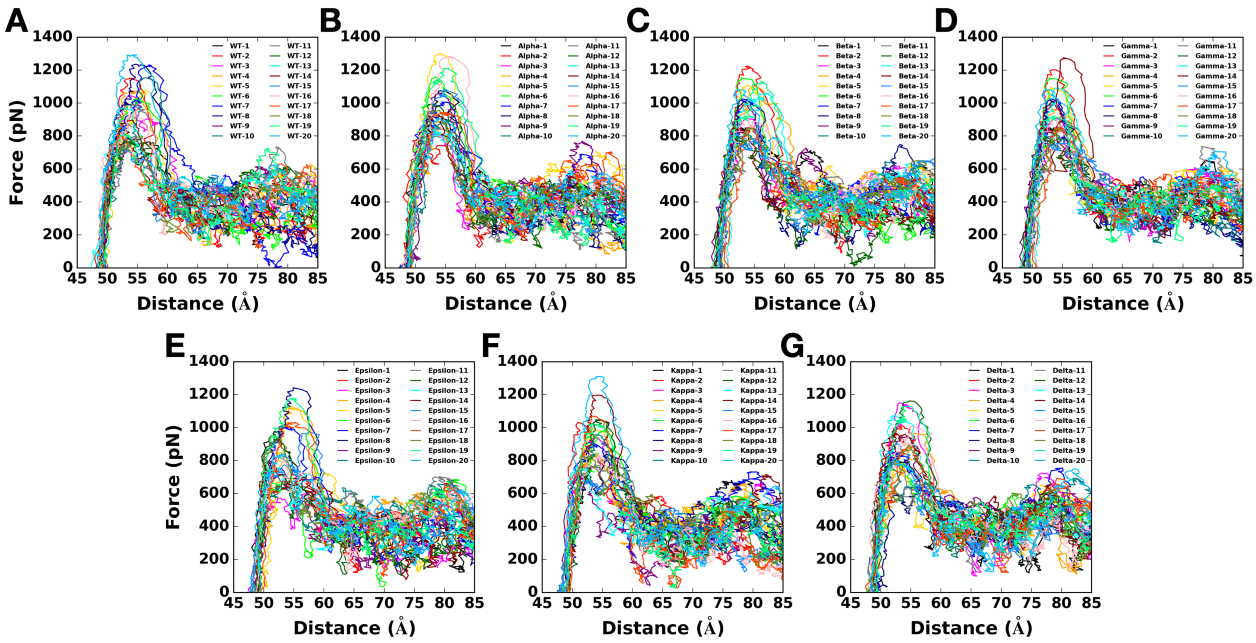


Figure S1. Force profiles of 20 replicas of (A) WT, (B) Alpha, (C) Beta, (D) Gamma, (E) Epsilon, (F) Kappa, and (G) Delta as a function of the distance between the COMs of RBD and ACE2.

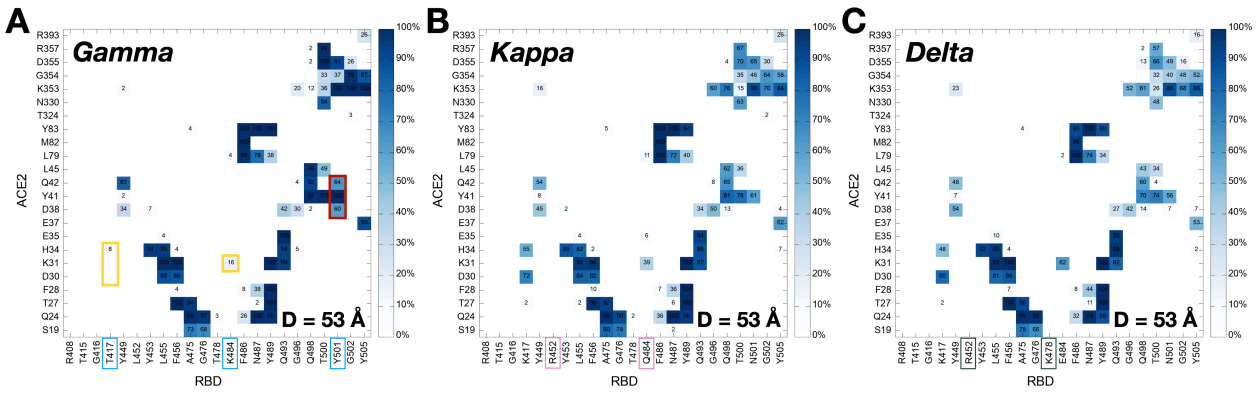


Figure S2. Two-dimensional contact maps at D = 53 Å. Mutated RBD residues are labeled as colored boxes: sky blue for Gamma, pink for Kappa, and gray for Delta. The contact frequency is numbered with colors from light blue to dark blue. Dark red and yellow colors on the map respectively represent increased and decreased interactions between RBD and ACE2 upon mutations.

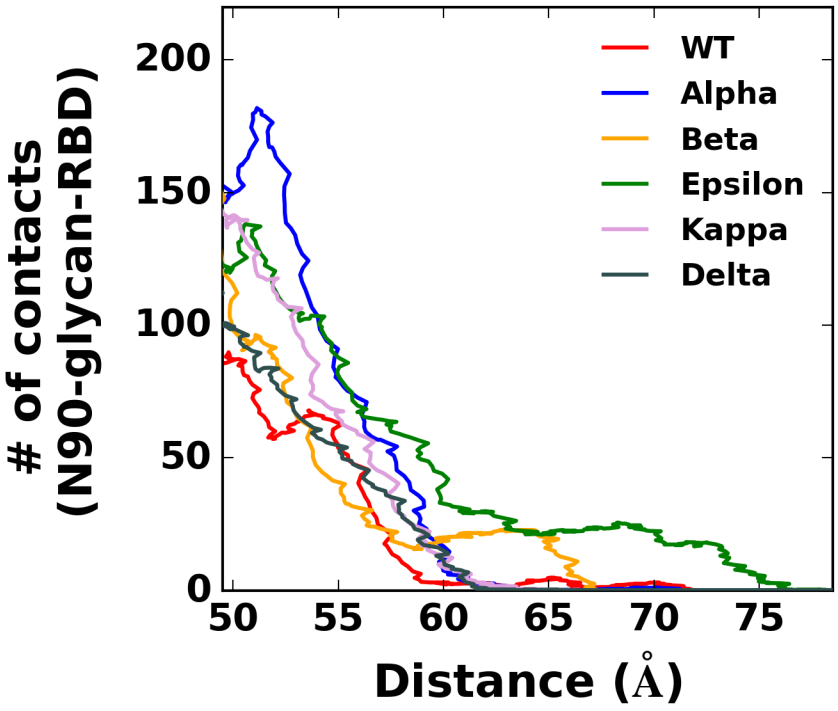


Figure S3. The number of heavy atom contacts between RBD and ACE2 N90-glycan. The numbers are counted if any heavy atom of N90-glycan is within 4.5 Å of any residue in RBD. The color scheme is the same as in **Figure 1A**.

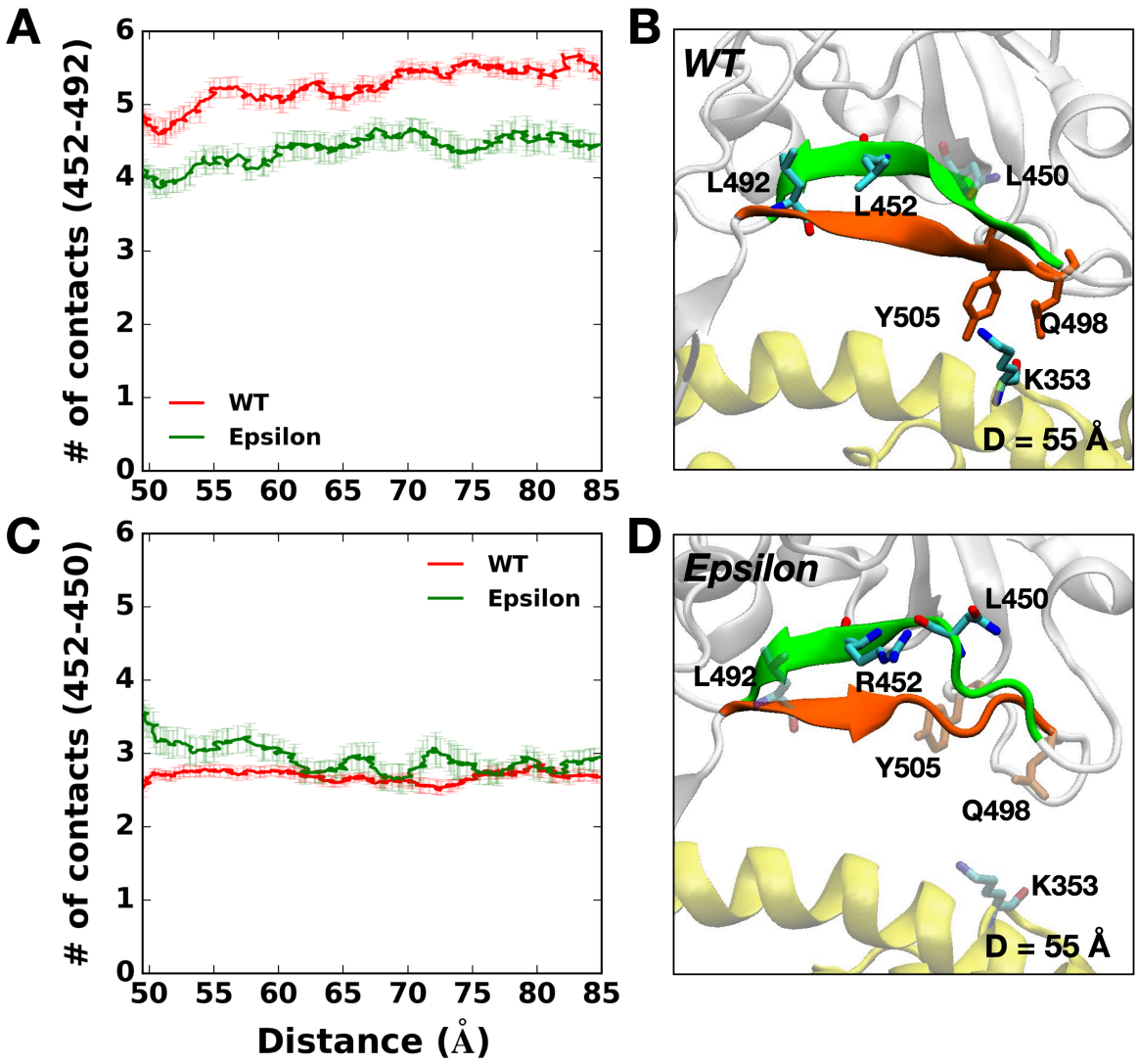
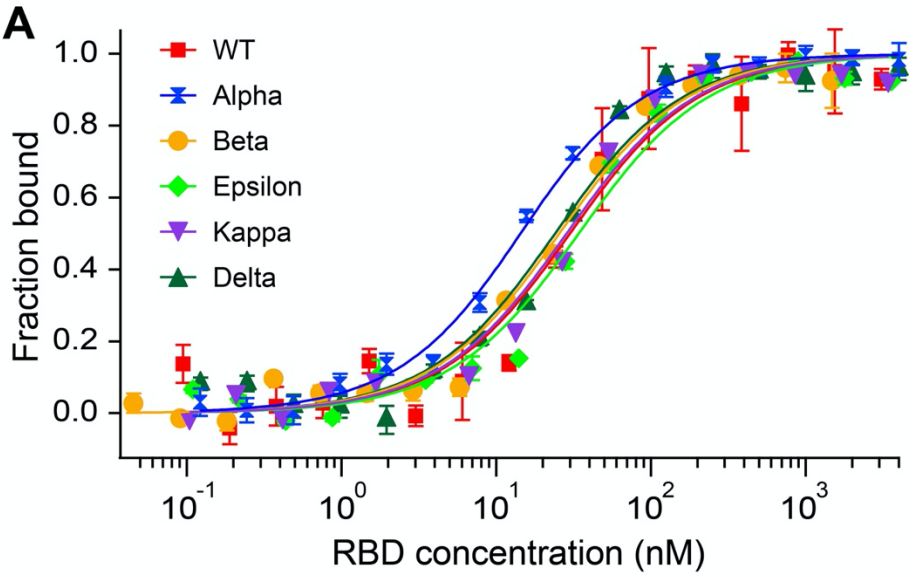


Figure S4. The number of heavy atom contacts (A) between RBD residues 452 and 492 and (C) between RBD residues 452 and 450. WT contains L452, and it is mutated to R452 in Epsilon variant. (B) L452 in one β-sheet colored in green interacts with L492 in another β-sheet presented by orange, and such interactions maintain stable secondary structures. (D) R452 of Epsilon variant located in a β-strand colored in green tends to interact with L450 in the same β-strand, having less stable secondary RBD structures by shortening the β-strands. Residues interacting with each other are represented by solid sticks, and those that lost their interaction are shown as transparent sticks.

A



B

RBD variants	K_d (nM)	Standard deviation (± nM)
WT	27.5	4.8
Alpha	11.8	0.8
Beta	23.1	2.4
Epsilon	31.7	3.9
Kappa	26.0	3.2
Delta	21.5	2.9

Figure S5. (A) Microscale thermophoresis (MST) analysis of the interaction between ACE2 and six different RBD variants. Error bars represent standard deviations from three to six individual repeat measurements. The binding affinities were determined by fitting the data with the 'K_d' model of the MO Affinity software. (B) Affinities of ACE2 binding to RBD variants detected by MST. The MST responses were fitted to the 1:1 binding model. The K_d rates are shown as fit ± one standard deviation.

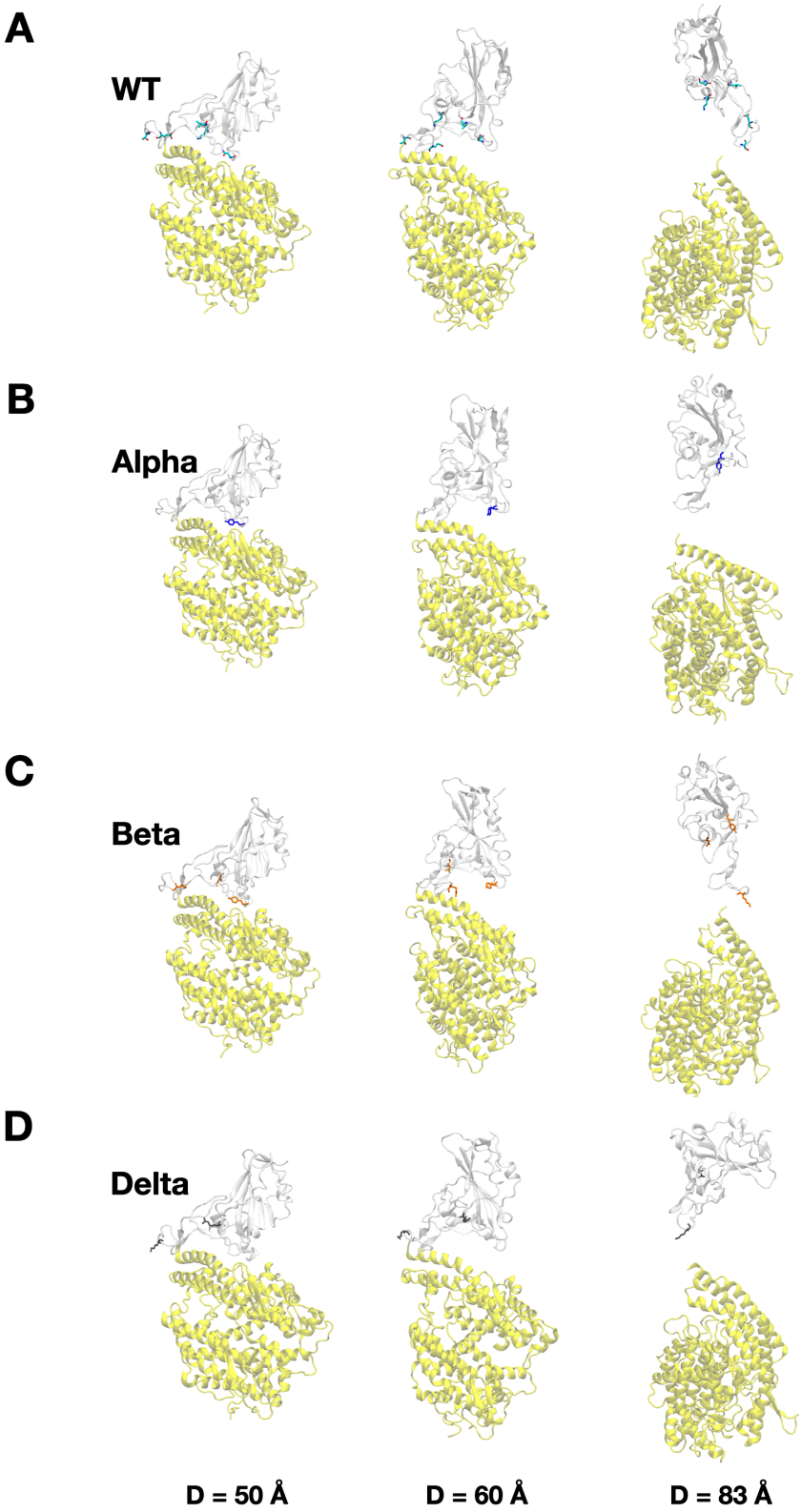


Figure S6. Separation process between ACE2 and RBD variants (A) WT, (B) Alpha, (C) Beta, and (D) Delta at D = 50 Å (initial structures), 60 Å (during the pulling processes), and 83 Å (after dissociations), respectively. The color scheme is the same as in **Figure 1A, B**.

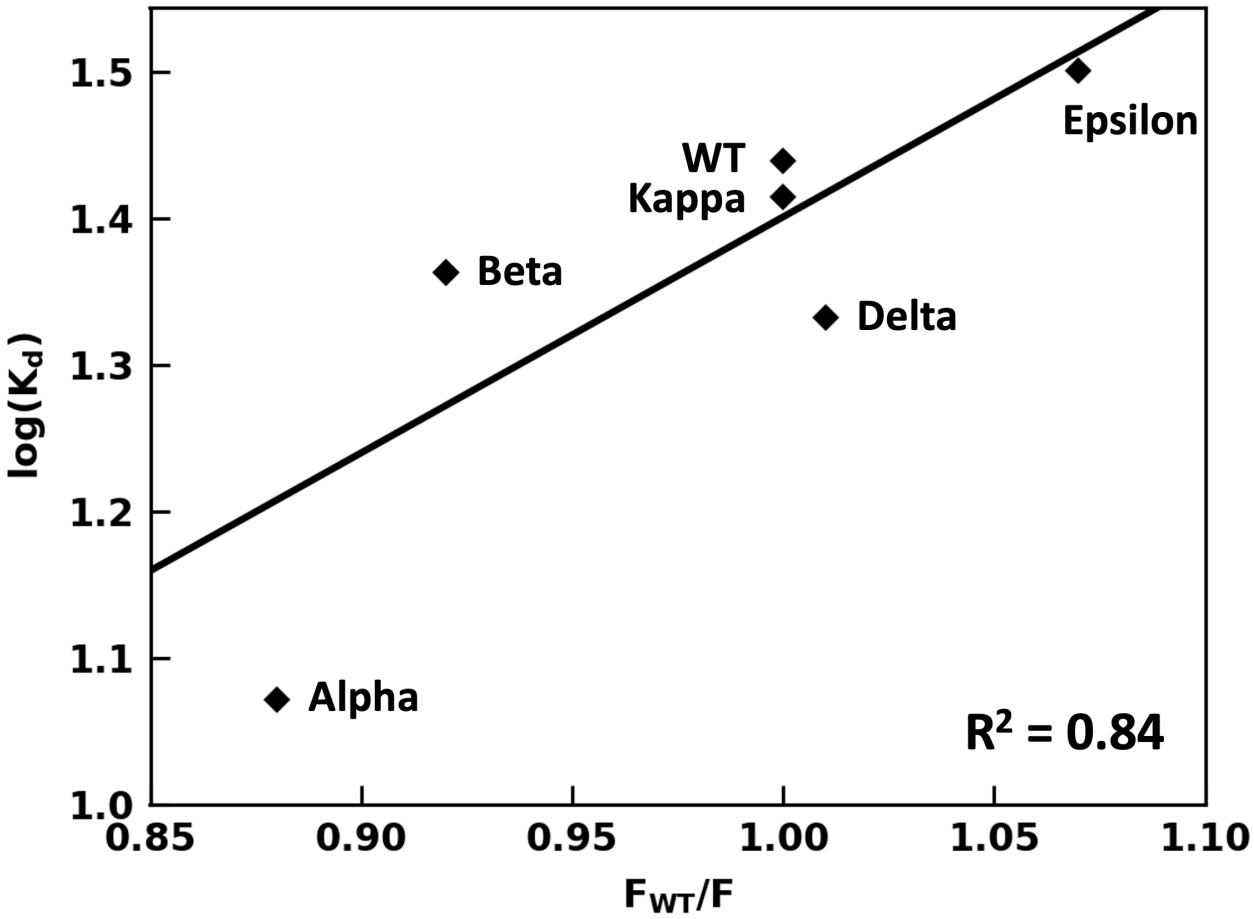


Figure S7. Linear regression on the F_{WT}/F and $\log(K_d)$. The coefficient of determination (R^2 = 0.84) indicates a good correlation between our MST experiments and SMD simulations.