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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Ramanathan M, Ferguson ID, Miao W, Khavari PA. SARS-CoV-2 B.1.1.7 and B.1.351 spike variants bind human ACE2 with increased affinity. *Lancet Infect Dis* 2021; published online May 19. https://doi.org/10.1016/S1473-3099(21)00262-0.

Materials and Methods

Recombinant Proteins
Human ACE2 protein was purchased from Sino Biological (SinoBiological, catalog#10108-H05H) and re-suspended in PBS-T to obtain 4.54uM stock concentration. Recombinant Spike Receptor Binding Domains were ordered from BEI resources. Protein quality was determined using a Tycho NT.6 (**Figures S4 & S5**) (NanoTemper Technologies). Human hnRNPC was purchased from Abnova (catalog# H00003183P01S.)

Microscale Thermophoresis
Spike RBD proteins were labeled using Monolith His-Tag Labeling Kit RED-tris-NTA (NanoTemper Technologies) following manufacturer protocol at 2:1 protein to dye ratio. Briefly, 100 uL of 200nM Spike RBD (Hu-1 or B.1.1.7 or B.1.351 RBD) was mixed with 100 uL of 100nM dye for 30 minutes at room temperature, followed by centrifugation for 10 minutes at 15000g at 4C. A series of sixteen 1:1 dilutions of hACE2 were prepared, with 2.27uM the highest concentration. Each dilution of hACE2 was mixed with one volume of Spike labeling reaction mix prior to incubation for 5 minutes at room temperature. Mixed samples were loaded into Monolith NT.115 Capillaries (NanoTemper Technologies). As a specificity control, hnRNPC was utilized instead of hACE2; MST showed no binding (**Figure S6**). MST was performed using a Monolith NT.115 instrument (NanoTemper Technologies) at room temperature, with 60-80% excitation power and Medium MST power.

Statistical Analyses
All statistical analyses were performed with GraphPad Prism 8.4 (Graphpad Software Inc.). Student's t-test was performed to compare the binding affinity of SCoV2 and variants with human ACE2 protein.

Acknowledgements
The following reagent was produced under HHSN272201400008C and obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, Hu-1 with C-Terminal Histidine Tag, Recombinant from HEK293F Cells, NR-52366. The following reagents were obtained through BEI Resources, NIAID, NIH: Spike Glycoprotein Receptor Binding Domain (RBD) from SARS-Related Coronavirus 2, South Africa Variant (NR-54005) and United Kingdom Variant (NR-54004) with C-Terminal Histidine Tag, Recombinant from HEK293 Cells. This work was supported by the USVA Office of Research and Development I01BX00140908 (P.A.K.), NIH CA142635, AR45192, AR076965 and HG007919 (P.A.K).

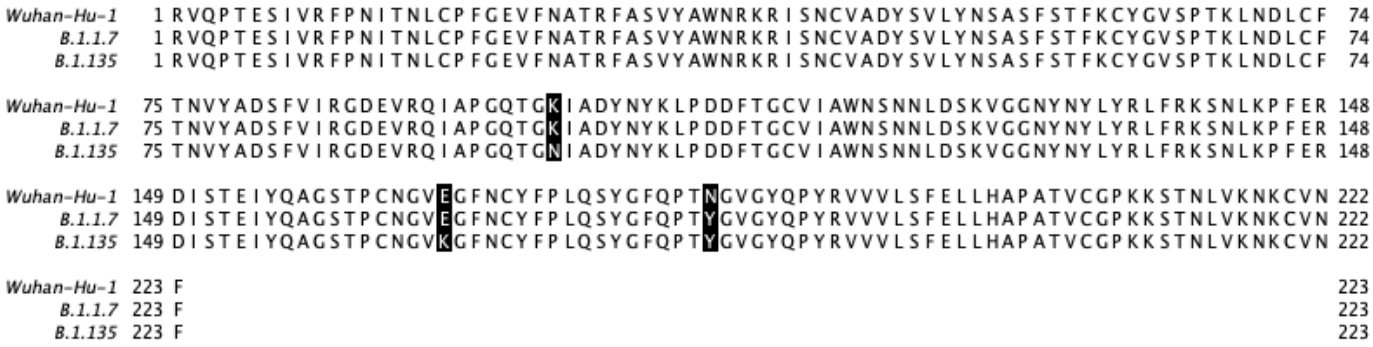

Figure S1: Sequence and alignment of RBD proteins assayed. The sequence of the parental Hu-1 strain is used as SCoV2 RBD sequence. Mutations seen in B.1.1.7 and B.1.351 are highlighted.

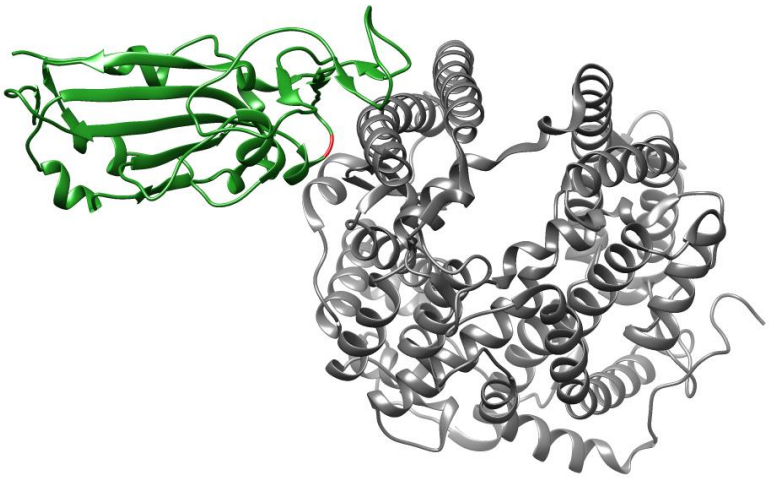

Figure S2: Structure of the SARS-CoV-2 RBD (PDB: 6M0J) with B.1.1.7 mutation (N501Y) on RBD colored red. Coloring schema: ACE2 (grey), receptor-binding domain (green)

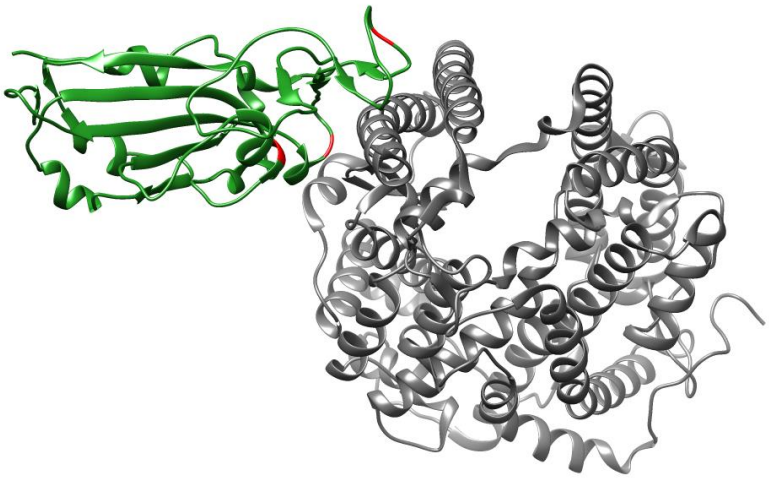

Figure S3: Structure of the SARS-CoV-2 RBD (PDB: 6M0J) with B.1.351 mutation (K417N, E484K, N501Y) on RBD colored red. Coloring schema: ACE2 (grey), receptor-binding domain (green)

Fig. 1 Binding of SARS-CoV-2 Spike RBD with Human ACE2

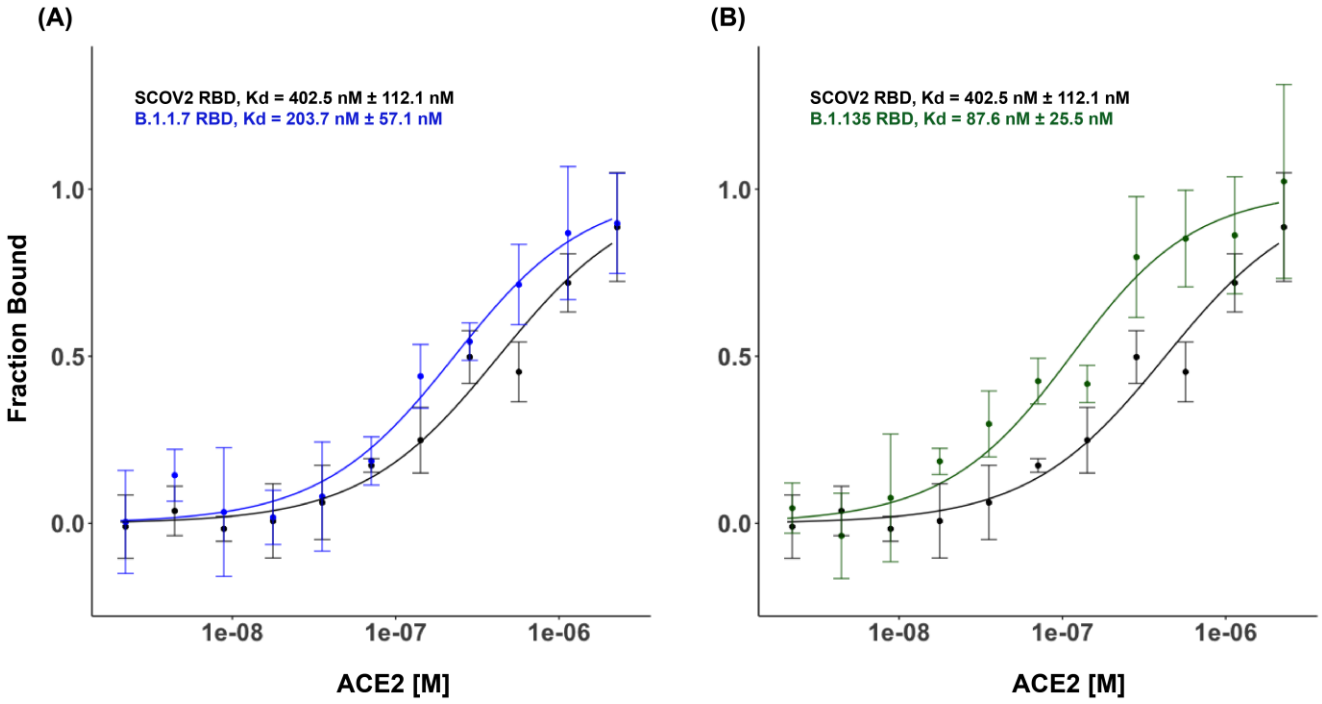

Fig. 1. Microscale thermophoresis (MST) with 100nM labeled Spike RBD (**A**) Binding curve of SCoV2 and B.1.1.7 RBD with a titration series of human ACE2 (n=3 series of independent replicates)
(**B**) Binding curve of SCoV2 and B.1.351 RBD with a titration series of human ACE2 (n=3 series of independent replicates).
MST data are mean ± SD. Error bars=SD

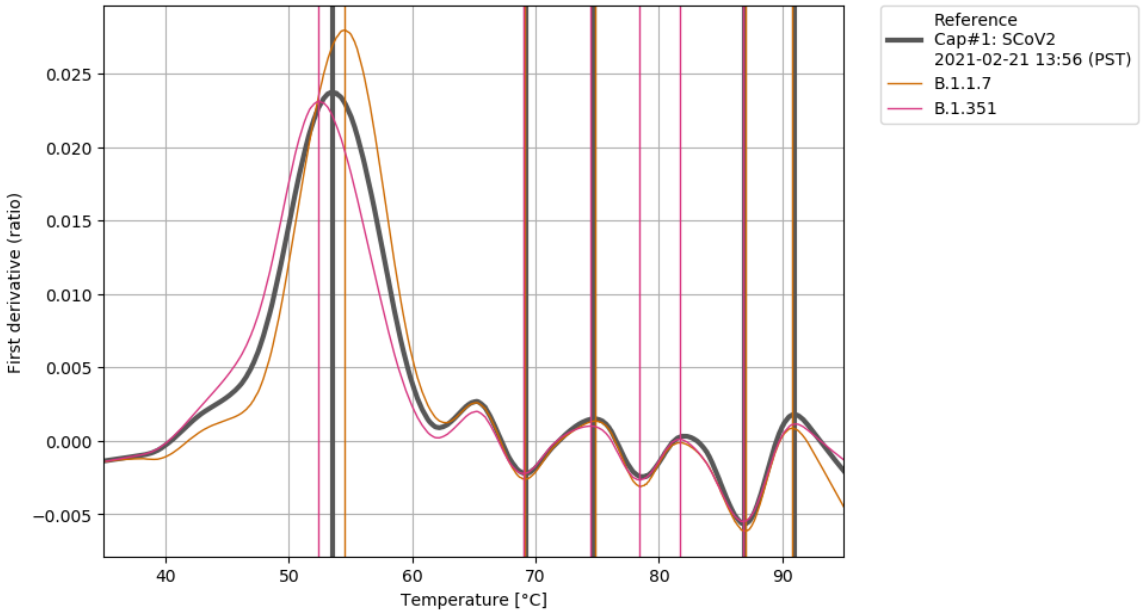

Figure S4: Protein quality of RBD proteins assayed. Tycho NT. 6 (Nanotemper) was used to measure changes in intrinsic fluorescence at 330 nm and 350 nm with increasing temperature. The fluorescence signals were plotted as a first derivative of the 350 nm/330 nm ratio.

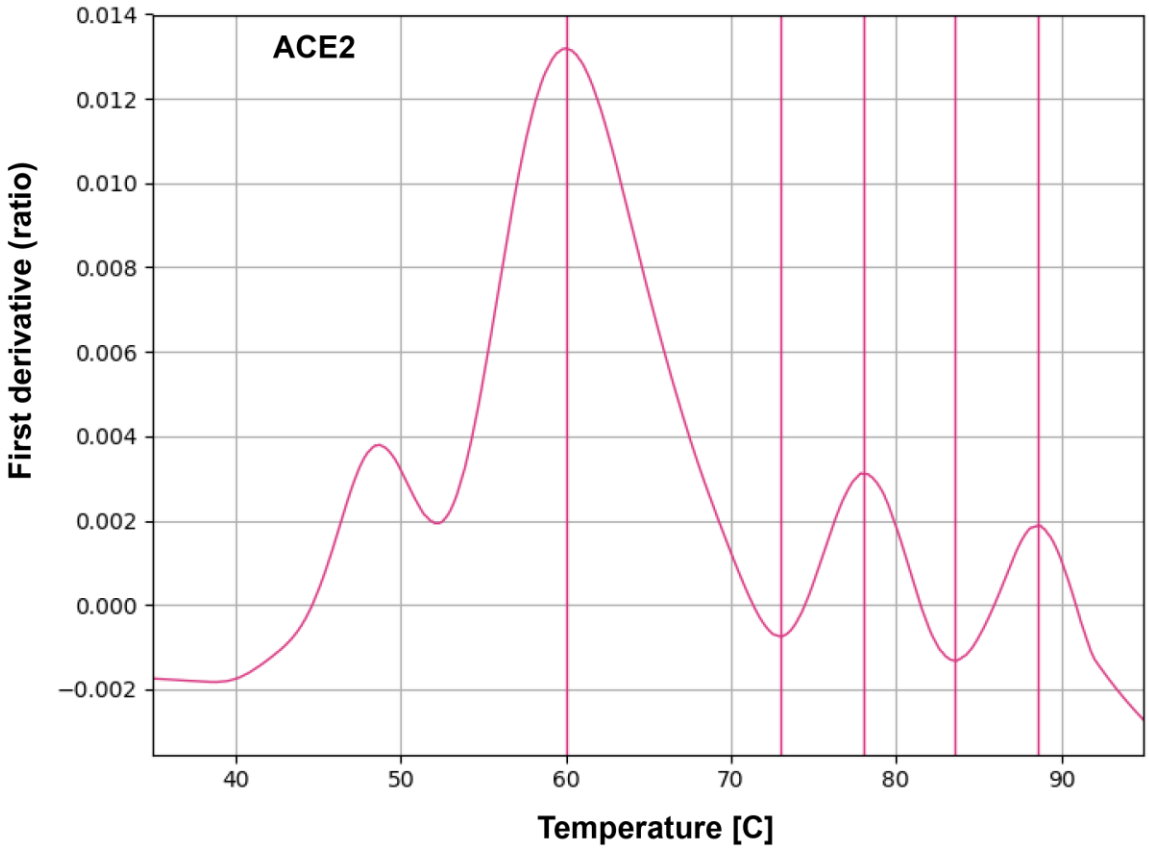

Figure S5: Protein quality of ACE2. Tycho NT. 6 (Nanotemper) was used to measure changes in intrinsic fluorescence at 330 nm and 350 nm with increasing temperature. The fluorescence signals were plotted as a first derivative of the 350 nm/330 nm ratio.

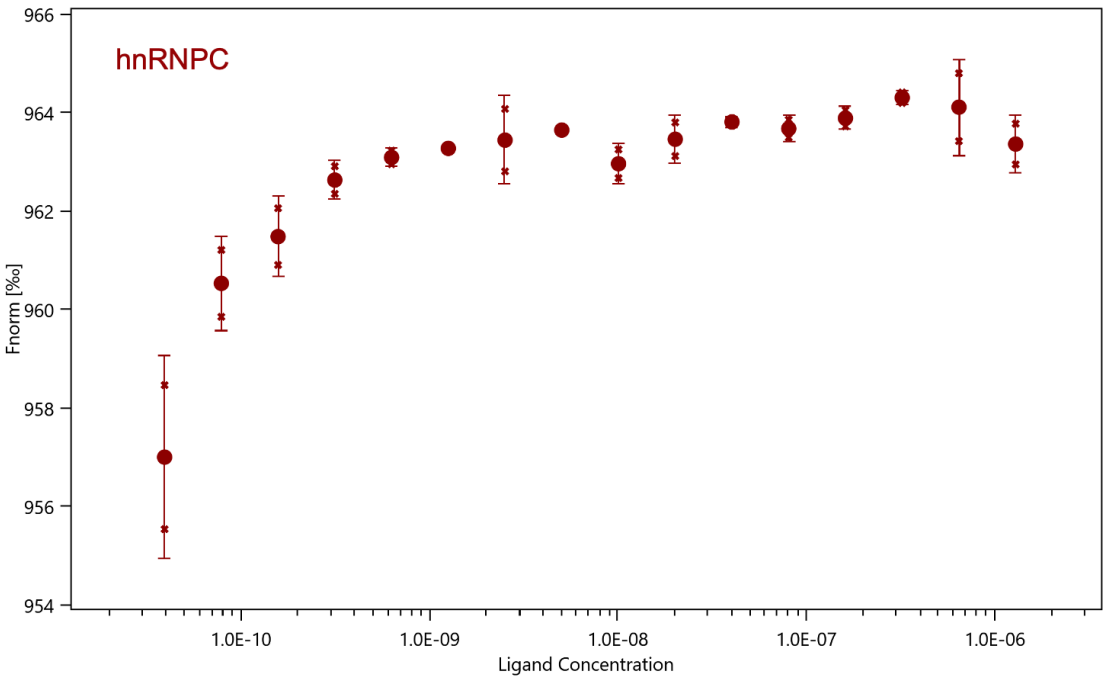

Figure S6: Microscale thermophoresis with 100nM labeled Spike RBD. SCoV2 and hnRNPC with a titration series of human hnRNPC protein shows no binding, confirming SCoV2 does not interact non-specifically (n=2 series of independent replicates).