

Supplementary Material

Supplementary Tables

Table S1. MD structural model details

MD1		Residues	# Res Atoms
	S-plug (ACE2-like)	19-103 (85 res.)	4174
	Spike RBD domain	336-516 (181 res.)	60324
	Water molecules	20108	2
	Ions (Na ⁺)	2	2
MD2			
	S-plug2 (ACE2-like)	19-103 (85 res.)	4212
	Spike RBD domain	336-516 (181 res.)	61968
	Water molecules	20656	2
	Ions (Cl ⁻)	2	2

Table S2. Spike–ACE2 binding affinities measured by different studies

Protein coated/analyte	K _D (nM)	Coating method	Detection method	References
SARS-CoV-2 Spike/ACE2	14.7	Non-covalently immobilised to sensor chip	SPR	[1]
SARS-CoV-2 Spike/ACE2	1.2	Non-covalently immobilised to sensor chip	Blitz	[2]
ACE2/SARS-CoV-2 RBD	4.7	Covalently immobilised to sensor chip	SPR	[3]
SARS-CoV-2 RBD/ACE2	44.2	Covalently immobilised to sensor chip	SPR	[4]

Supplementary Figures

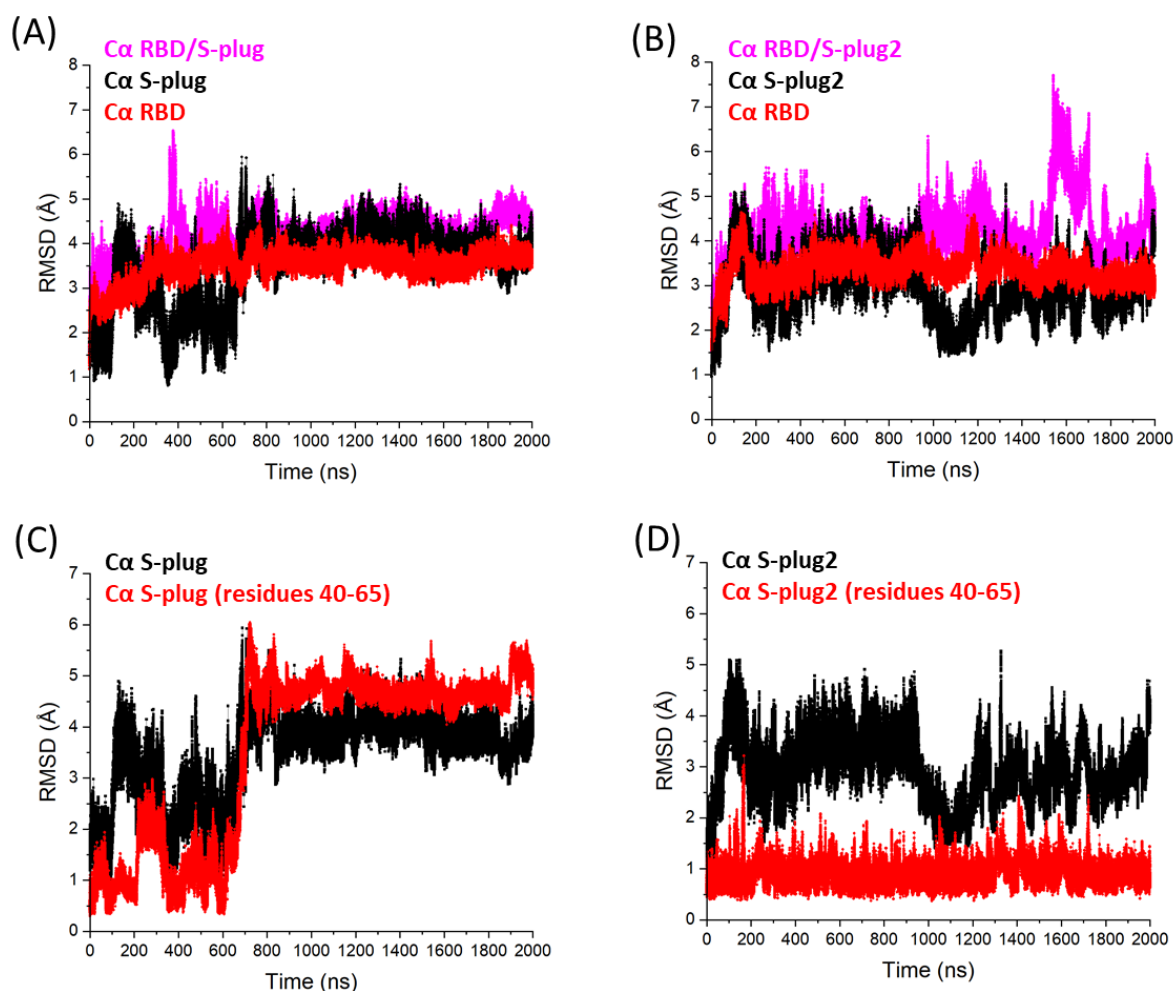


Figure S1. RMSD time evolution. (A) C^α RMSD values (MD simulation of S-plug/RBD) are reported in black, red and magenta for S-plug, RBD and the entire complex, respectively; (B) C^α RMSD values of (MD simulation of S-plug2/RBD) are reported in black, red and magenta for S-plug2, RBD and the entire complex, respectively; (C) C^α RMSD values computed for S-plug (black) and the region 40-65 C^α in the S-plug/RBD simulation; (D) C^α RMSD values computed for S-plug2 (black) and the region 40-65 C^α in the S-plug2/RBD simulation.

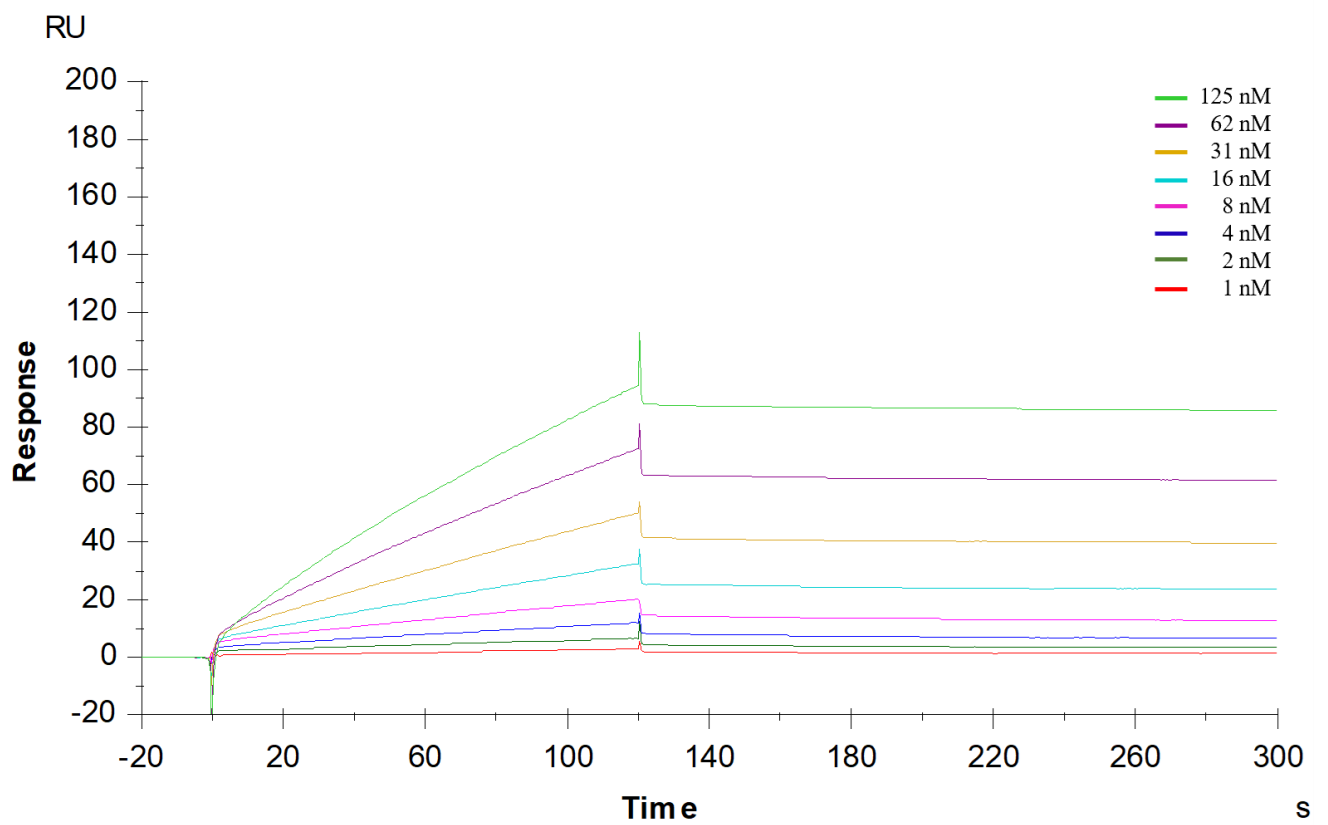


Figure S2. Surface Plasmon Resonance (SPR) analysis. Sensorgrams to evaluate the binding affinity of the host ACE2 domain to SARS-CoV-2 Spike S1 domain.

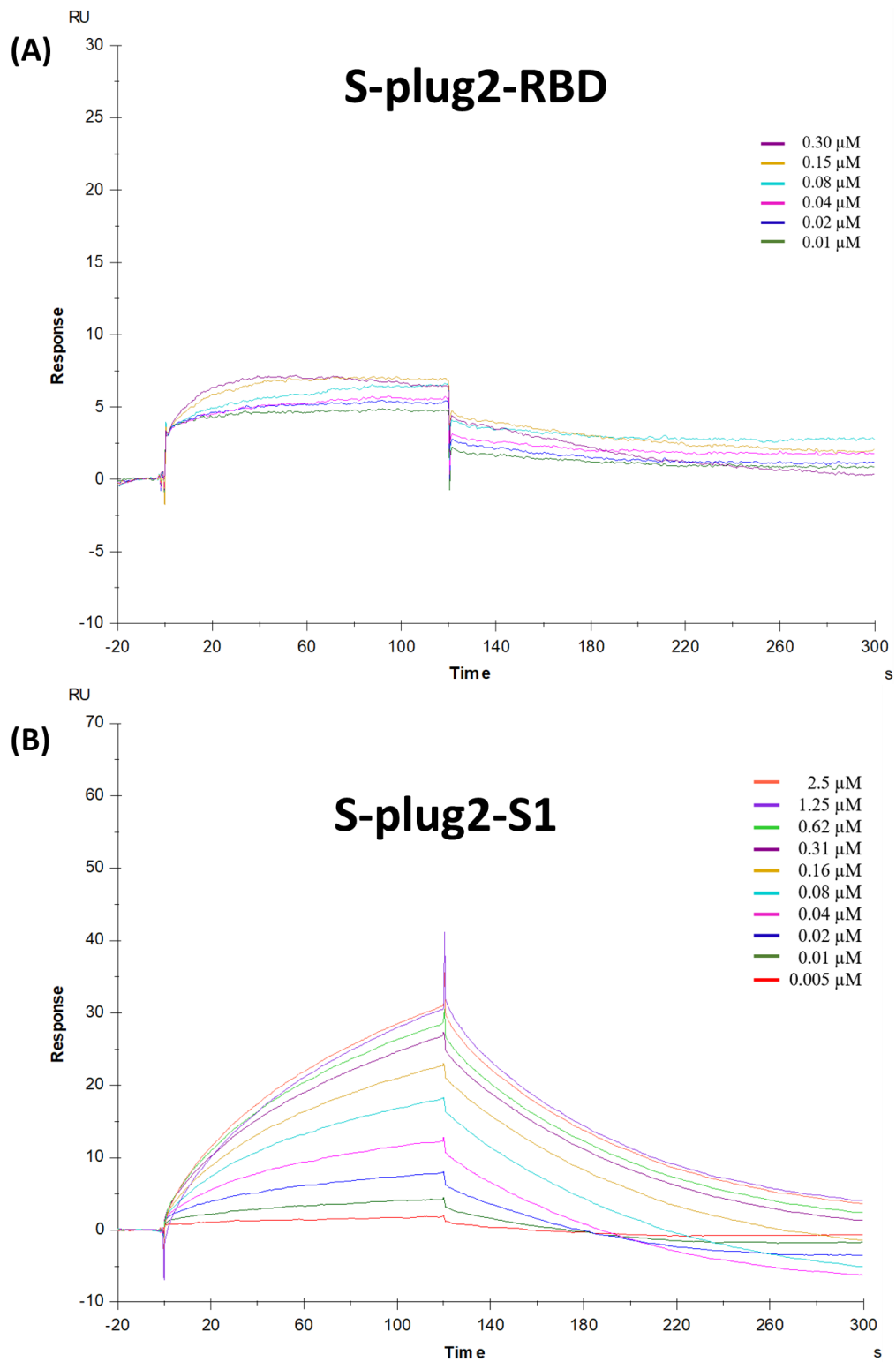


Figure S3. Surface Plasmon Resonance (SPR) analysis. Sensorgrams to evaluate the binding affinity of S-plug2 to (A) Spike RDB domain and (B) Spike S1 domain.

Supplementary References

1. Wrapp, D.; Wang, N.; Corbett, K.S.; Goldsmith, J.A.; Hsieh, C.L.; Abiona, O.; Graham, B.S.; McLellan, J.S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **2020**, *367*, 1260-1263, doi:10.1126/science.abb2507.
2. Walls, A.C.; Park, Y.J.; Tortorici, M.A.; Wall, A.; McGuire, A.T.; Veesler, D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **2020**, *181*, 281-292 e286, doi:10.1016/j.cell.2020.02.058.
3. Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L., et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **2020**, 10.1038/s41586-020-2180-5, doi:10.1038/s41586-020-2180-5.
4. Shang, J.; Ye, G.; Shi, K.; Wan, Y.; Luo, C.; Aihara, H.; Geng, Q.; Auerbach, A.; Li, F. Structural basis of receptor recognition by SARS-CoV-2. *Nature* **2020**, *581*, 221-224, doi:10.1038/s41586-020-2179-y.