Correlating the differences in the receptor binding domain of SARS-CoV-2 spike variants on their interactions with human ACE2 receptor

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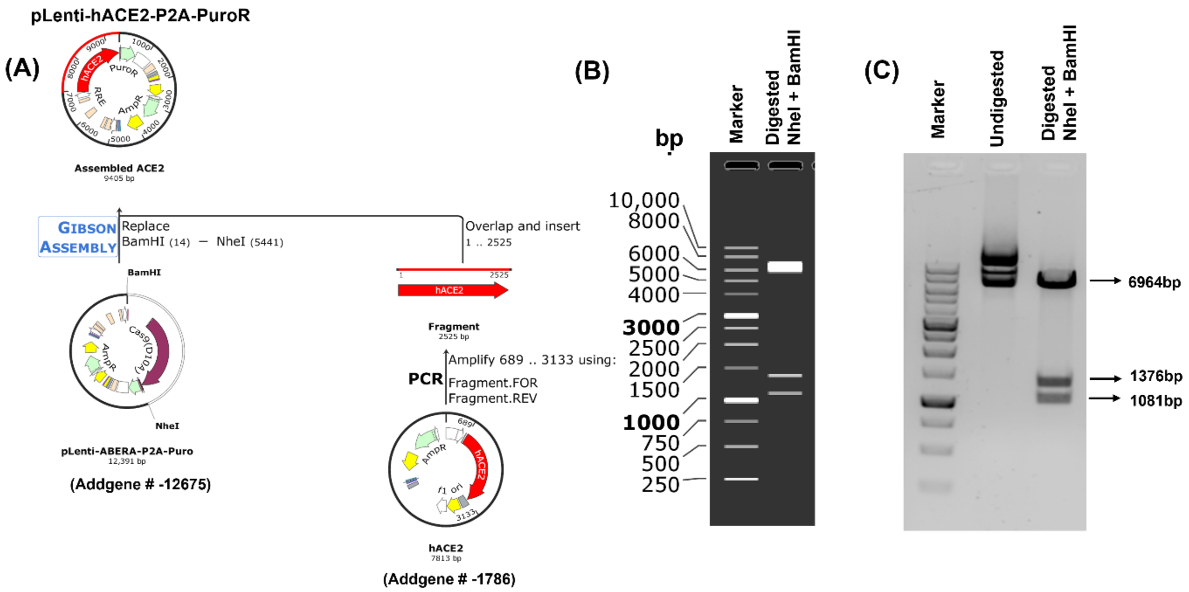


Figure S1: Cloning of hACE gene and characterization. Overview of molecular cloning of hACE2 gene into lentiviral plasmid by Gibbson assembly (A). The fragmentation of pLenti-hACE2-P2A-PuroR plasmid by restriction digestion (NheI and BamHI enzyme) was predicted insilico by Snapgene software (B) and confirmed by in vitro digestion (C).

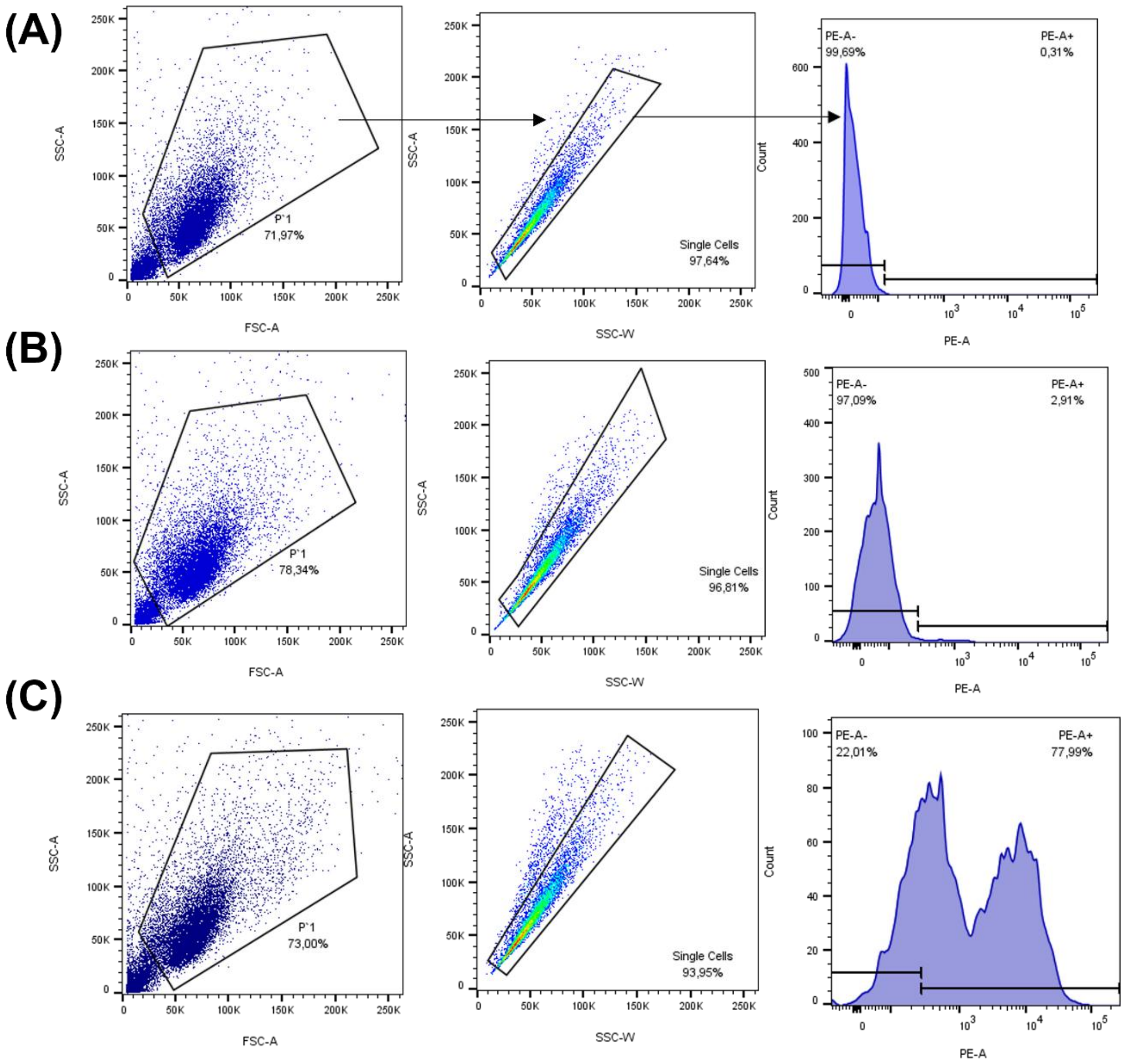


Figure S2: Gating strategy of Flow cytometric evaluation. P1 Shows Population of the 293T-hACE2 cells. To discriminate the doublets, single cells were gated from P1 Population. PE+ve cells were gated from the singlet population. Unstained (A), Histograms for Isotype controls for Streptavidin-PE (B), Wild spike bound cells (C). The PE positive was gated against isotype as a control.

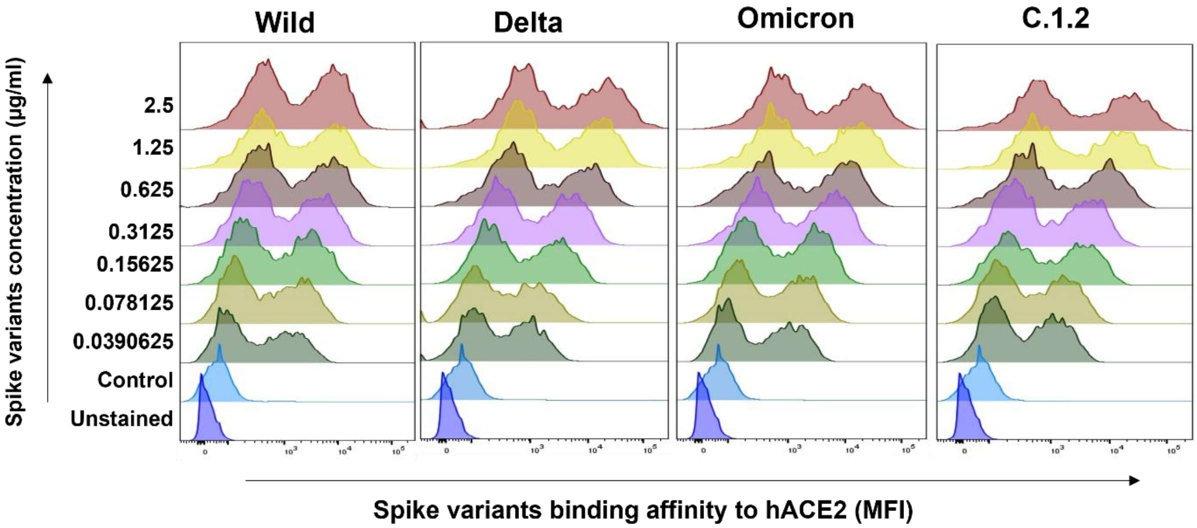
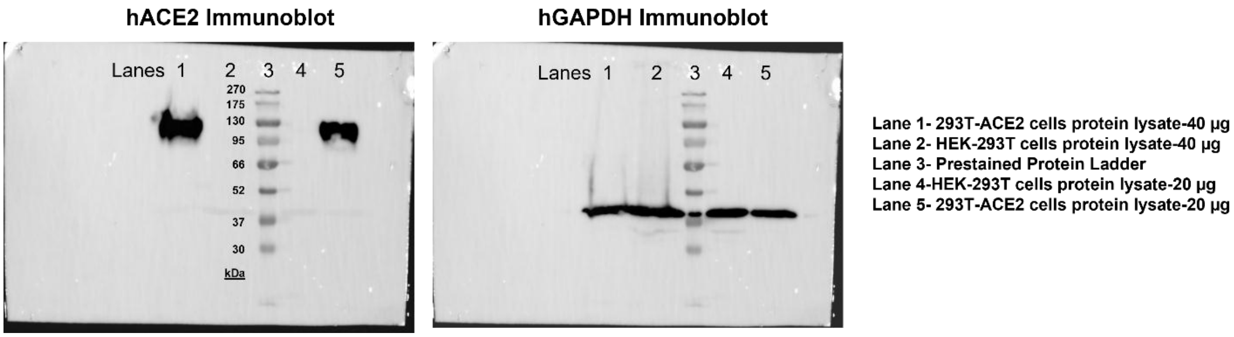


Figure S3. Histogram plots show the level of spike variants binding to dimeric hACE2 receptor present on surface of the cells at different concentrations, generated by FlowJo software. Unstained-cells only, Control-cells + streptavidin−PE conjugate.



Lane 1- 293T-ACE2 cells protein lysate-40 µg
Lane 2- HEK-293T cells protein lysate-40 µg
Lane 3- Prestained Protein Ladder
Lane 4-HEK-293T cells protein lysate-20 µg
Lane 5- 293T-ACE2 cells protein lysate-20 µg

Figure S4. Whole blot images of western blot, performed with 20 µg and 40 µg of protein lysate from HEK-293T and 293T-ACE2 cell lines, and prestained protein ladder (Bio-Helix, #PMB01-0500).

Table S1. Primer List

Primer name	**Primer sequence 5'- 3' direction**
Human ACE2 cloning	F-CCAGAACACAGGTGTCGTGACGCGGGATCCGCCACCATGTCAAGCTCTTCCTGGCTCC
	R- GAAGTTGGTGGCGCCGCTGCCGCTAGCAAAGGAGGTCTGAACATCATCAGTGTTTTG
Human ACE2	F- GGGATCAGAGATCGGAAGAAGAAA
	R- AGGAGGTCTGAACATCATCAGTG
Human GAPDH	F- CTGGGCTACACTGAGCACC
	R- AAGTGGTCGTTGAGGGCAATG