

Supplemental information

**Rapid, high throughput, automated detection
of SARS-CoV-2 neutralizing antibodies against
Wuhan-WT, delta and omicron BA1, BA2 spike trimers**

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Wuhan (Vaccine Strain) – WT DNA synthesized sequence:

ATGTTTCGTGTTCCCTGGTGCTGCTGCCTTTGGTTAGCAGCCAGTGCCTGAACC
TGACCACCAGAACCAGCTGCCACCTGCTTATACCAATAGCTTTACAAGAGGCGTGTACTACCCTGATAA
GGTGTTCAGAAGCTCGGTACTCCATCTACTCAGGACCTGTTCCCTGCCCTTCTTTCAGTAATGTGACTGG
TTCCACGCCATCCACGTGAGCGGCACCAACGGCACCAGAGGTTTCGACAATCCTGTGCTGCCTTTCAATG
ACGGCGTGTACTTTGCCAGCAGAAAAAGTCCAACATCATCAGAGGCTGGATCTTCGGCACCACCCTGGA
TTCTAAGACCCAAAGCTGCTTATCGTGAACAACGCCACAAATGTTGTGATCAAGGTGTGCGAATTTTCAG
TTCTGCAACGACCCCTTTCCTGGGAGTGTACTACCACAAGAACAACAAGAGCTGGATGGAATCTGAGTTCA
GAGTATATTCTTCTGTAACAACGTACCTTTGAGTACGTGAGCCAGCCGTTCCCTGATGGACCTCGAAGG
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CGGAGATTTCTTACTGCTGGCTGGACCGCCGGCCGCTGCCTACTACGTGGGCTACCTGCAGCTAGAACC
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AAACCAAGTGCACCCCTGAAGAGCTTACCCTGGAAAAGGGCATCTACCAGACGAGCAATTTTCAGAGTCCA
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ACCGCAAGATAGTACTACTATAACTACAAGCTTCCAGACTTACCAGGCTGTGTGATCGCCCTGGAACCT
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AATCATGCTGTGCTGCATGACAAGCTGTTGTTCTGTGCTGAAGGGCTGCTGTAGCTGTGGCTCTTGAGGA
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GGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCC GCCCTAA
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TATTTATGCAGAGGCCGAGGCCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTTGGA
GG

Amino acid sequence of spike protein of Wuhan strain:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSNGTNGTKRFDNPVLPF
NDGVYFASTEKSNIRGWIFGTTLDSKTQSLIVNNAITNVVIKVCDFQCNDFLGVYHKNKSWMESEFRVYSSANNCTFEYVS
QPFLMDLEGKQGNFKNLREFVFNIDGYFKIYSKHTPINLVRDLPPQFSALEPLVDLPIGINITRFQTLALHRSYLT PGDSSSGW
TAGAAAAYVGYLQPRFTLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFN
TRFASVYAWNRRKIRSNVADYSVLYNSASFSTFKCYGVSPTKLNLDLCTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDFFT
GCVIAWNSNNLDSKVGNNYNYLRLFRKSNLKPFFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFE
LLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLIILDITPCSFGGVSVITPGT
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SQSIIAYTMSLGAENSVAYSNNISAIPTNFTIISVTEILPVSMTKTSVDCTMYICGDSSTECNLLLQYGSFCTQLNRLALTGIAVEQ
DKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSPKSKRSFIEDLLFNKVTLDAGFIKQYGDCLGDIAARDLCAQKFNGLTV
LPPLLTDEMIAYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALG
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VLGQSKRVDFCGKYHLMSFPQSAPHGVVFLHVTVPAQEKNFTTAPAI CHDGHKAFHPREGV FVSNGTHWFVTQRNFYEPQIITTD
NTFVSGNCDVVIGIVNNTVYDLPQLPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELG
KYEQ-

Delta Strain (B.1.617.2) DNA sequence synthesized

ATGTTTCGTGTTCCCTGGTGCTTCTGCCACTGGTGTCTAGCCAGTGCCTGAACTCAGAA
CCAGGACCCAGCTGCCCCCTGCCTACACCAATTCCTTTACCAGAGGCGTCTACTACCCGATAAGGTGTT
CAGAAGCTCCGTGCTGCACAGCACACAAGACCTGTTCTCGCCATTCTTCAGCAATGTGACATGGTTCCAC
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CTGCCAATAATTGCACATTTGAGTACGTGAGCCAGCCTTCTCTGATGGACCTGGAAGGCAAGCAAGGCAA
CTTCAAGAACCTGCGGGAGTTTCGTGTTCAAGAACATCGACGGCTACTTCAAGATCTACAGCAAGCACACC
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GTGGCCAAGAACCTGAACGAGAGCCTGATCGACTTGCAGGAGCTGGGCAAATACGAGCAG

Amino acid sequence of spike protein of Delta strain:

MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS
GNTGTRKFRDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLI VNNATNVVIKVECFQFCNDPFLD
VYHKNKNSWMESGVYSSANNCTFEYVSQPF LMDLEKQGNFKNLREFVFKNIDGYFKIYSKHTP
INLVRDL PQGFSALEPLVDLP IGINITRFQTLALHRSYLT PGDSSSSGWTA GAAAYVYG
LQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFP
NITNLCPFGVEFNATR FASVYAWNRKRISNCVADYSVLVNSASFSTFKCYGVSPTKLN
DLCFNTVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGC VIAWNSNLD
SKVGGNYNYR YRLFRKSNLKPFERD ISTEIYQAGSKPCNGVEGFNCFYPLQSYGFQ
PTNGVGYQYPRVVVLSFELL HAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVL
TESNKKFLPFQQFGRDIADTTDAVRDPQTL EILDITPCSFGGVSVITPGTNT
SNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSY
ECDIPIGAGICASYQTQ TNSRGSASSVASQ SIIAYTMSLGAENSVAYSNN
SIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDS TECSNLLLQYGSFCTQLN
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LLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLP PLLTDEMIAQYTS
ALLAGTITSGWTFGAGPALQI PPFMQMAYRFNGIGVTQNVLYENQKLIANQFNSA
IGKIQDLSSTPSALGKL QNVVNQNAQALNTLVKQLSSNFGAISSVLNDILSR
LDPPEAEVQIDRLITGRQLSLQTYVTQQLIRAAEIRASANLAATKMSECVL
GQSKRVDFCGKGYHLSMFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICH
DGKAHF PREGV FVSNGTHWVFVTRNFYEPQIITDNT FVSGNCDVVI
GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLDGDISGINASV
VNIQKEIDRLNEVAKNLNESLIDLQELGKY EQ-

Omicron-BA1 Nucleotide sequence:

atgttcgtgttccctggtgttattgcctctggtttctagccaatgtgtcaacctcaccacacggaccagctgcctcctgcctacac
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cttgggacggagcggacatcaccaccaccaccatcactgataatga

Amino acid sequence of spike protein of BA1 strain:

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRFDNPVLPFND
GVYFASIEKSNIIIRGWIIFGTTLDSKTQSLIVNNATNVVIVKCEFFQCNDFLDHKNKSWMESEFRVYSSANNCTFEYVVSQPFM
DLEKQGNFKNLREVFVKNIIDGYFKIYSKHTPIIVREPDLDPQGFSALEPLVDLPIGINITRFQTLALHRSYLTGDDSSSGWTAG
AAAYVGYLQPRFTLLKYNENGTITDAVDCALDPLSEKTKLSTFVEKGIYQTSNFRVQPTESIVRFPNITNLCPFDEVFNATRF
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Amino acid sequence of spike protein of BA2 strain:

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FigS1:

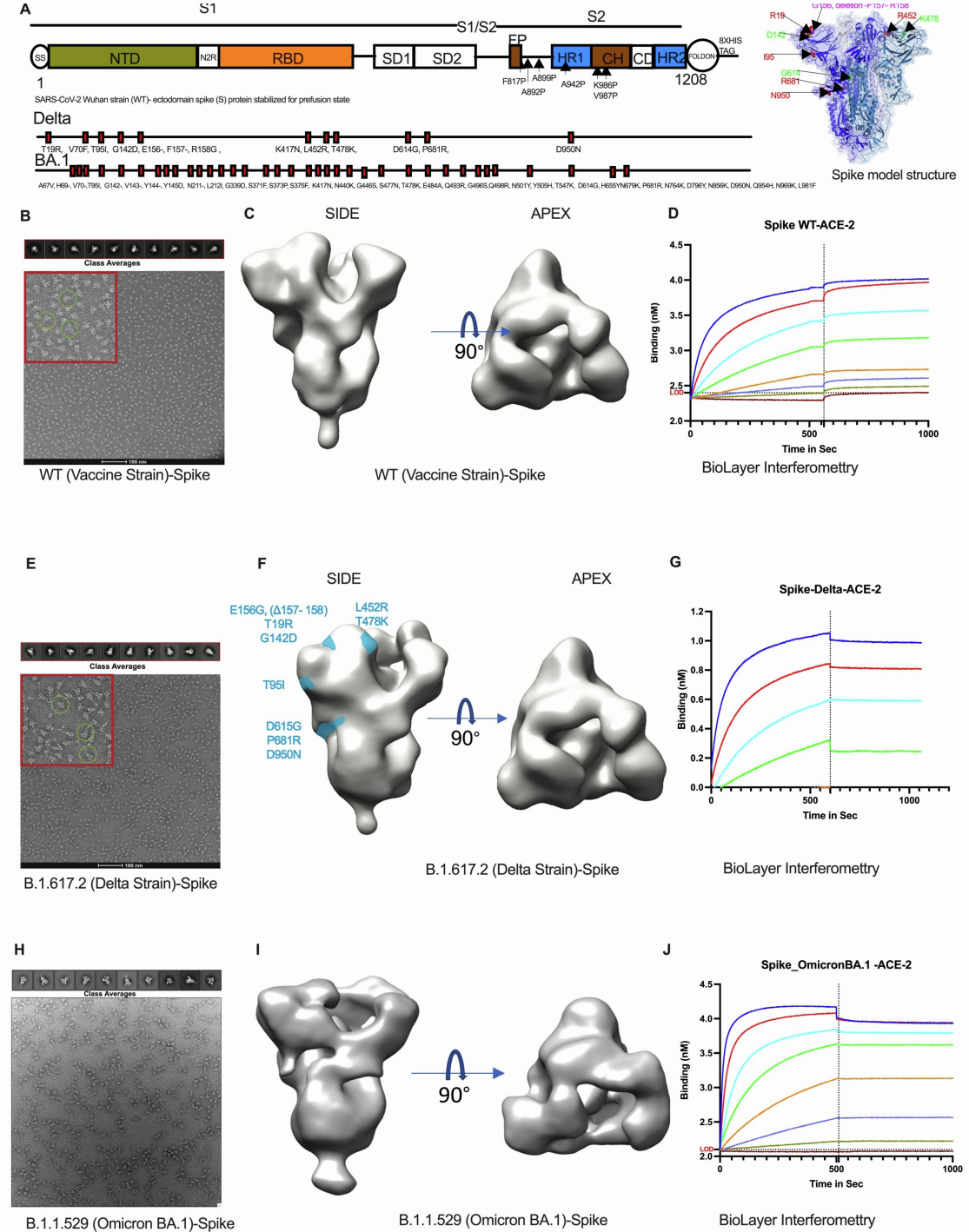
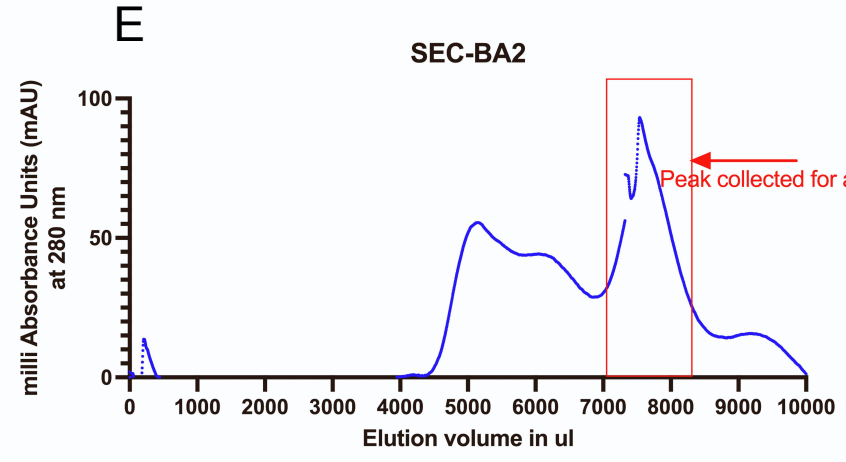
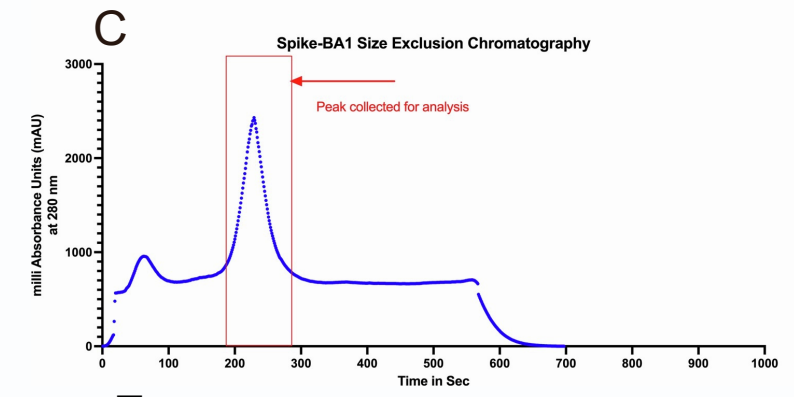
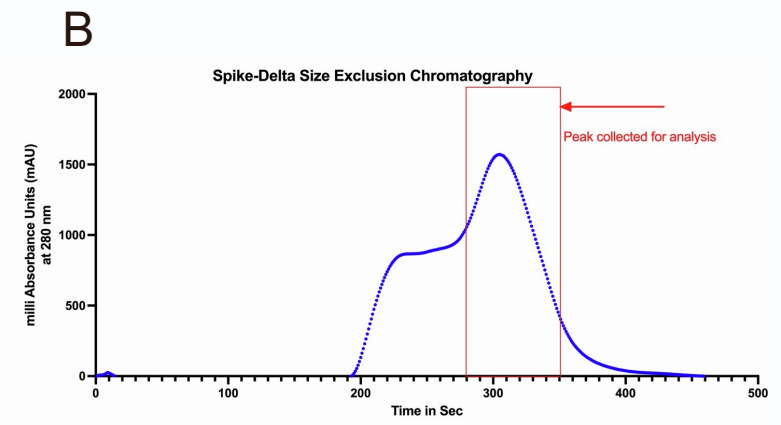
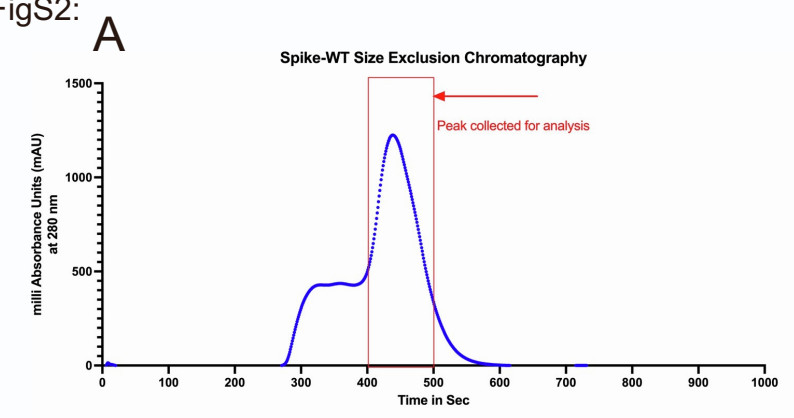


Figure S1. Development and validation of SARS-CoV-2 vaccine strain (Wuhan-WT), Delta, and Omicron BA.1 variant prefusion ectodomain spike targets for use in blockade of ACE-2 binding assay (BoAb). (A) SARS-CoV-2 spike (S) ectodomain proteins of WT (Wuhan strain), Delta (B.1.167.2) and Omicron BA.1 (B.1.1.529) for structural characterization and assay development, and a model construct of Delta S protein. (B, E and H) Raw negative stain electron micrographs for purified vaccine strain (B), delta variant (E), and Omicron variant (H) trimers. 2D class averages of various trimer orientations derived from the raw micrographs are shown in the upper panel and used for the 3D reconstruction shown in Panels C, F and I. (C,F,I) 3D reconstruction from negative stain electron microscopy class averages of our purified trimeric ectodomain of WT, Delta and Omicron BA.1. (D,G, and J) Biolayer interferometry analysis of each spike variant binding to an immobilized recombinant ACE-2 IgG Fc-chimera, the biotinylated form of which serves as the detector in our BoAb assay.

FigS2:



D WT Mw Delta BA1

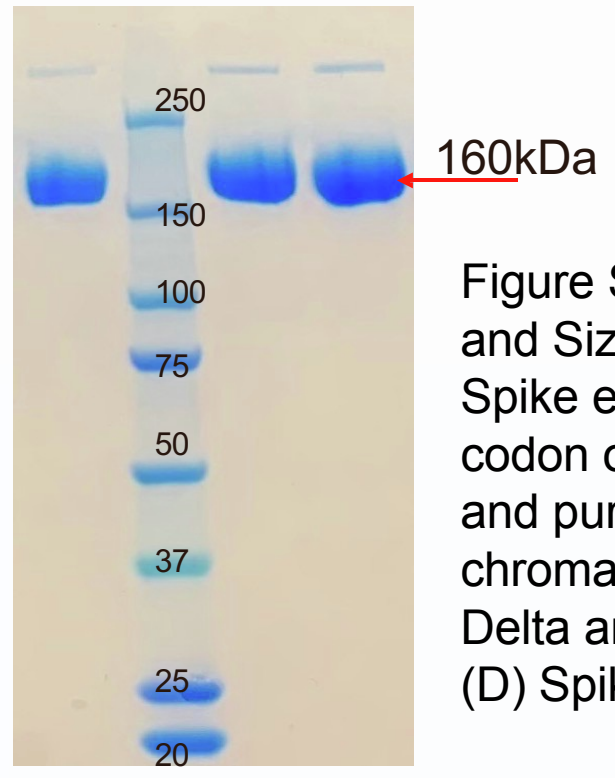


Figure S2: SARS-CoV-2 Spike protein purification and Size Exclusion Chromatographs. SARS-CoV-2 Spike ectodomain (1-1208 amino acids) human codon optimized plasmid transfected into 293F cells and purified the protein by affinity column chromatography, chromatograms A for WT, B for Delta and C and E for Omicron BA1 and BA2. (D) Spike protein Confirmation by SDS-PAGE.

FigS3:

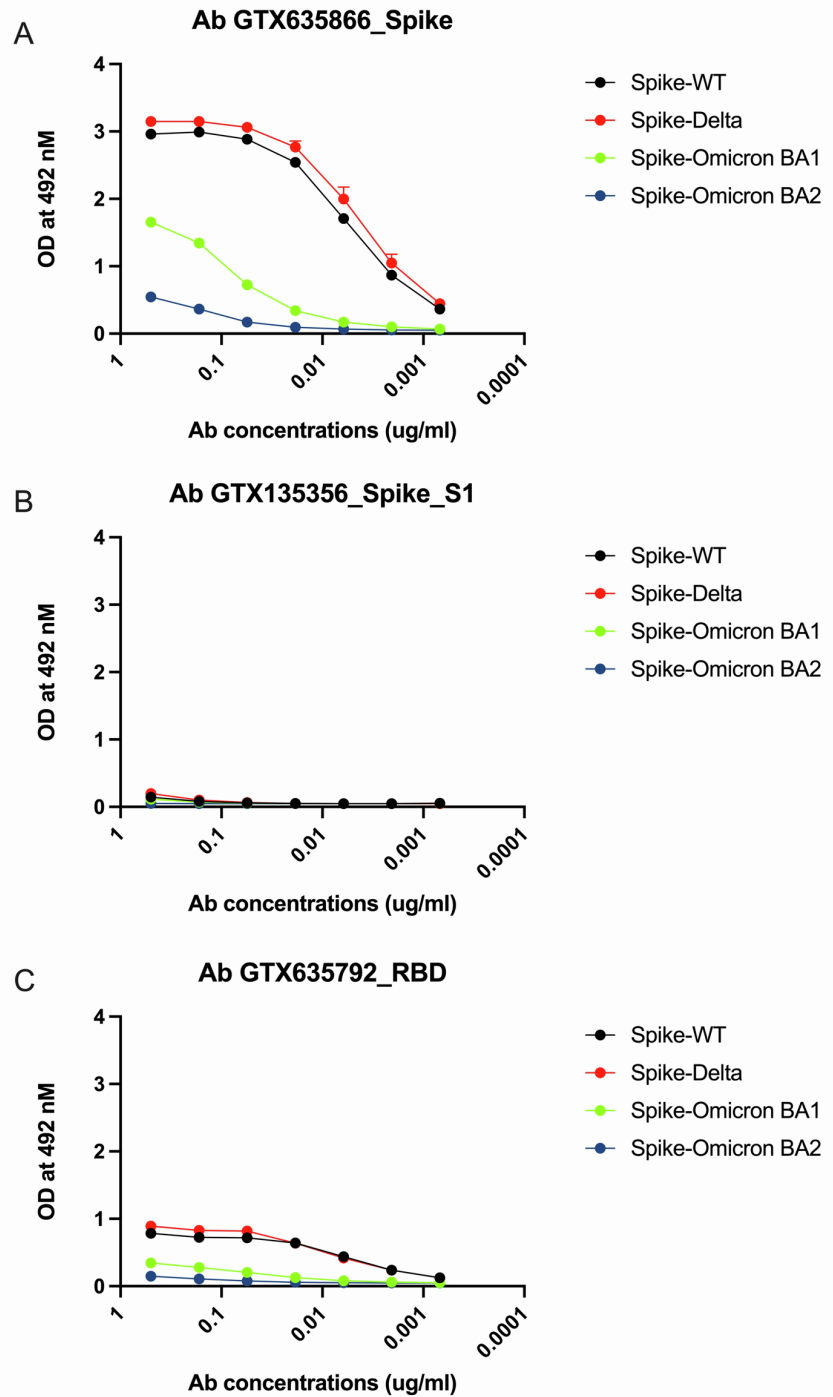


Figure S3. Binding properties of Spike variants of SARS-CoV-2. ELISA performed with 3-fold dilutions of selected monoclonal antibodies. (A) GTX635866 (GenTex) which targets the prefusion state of spike protein. This antibody shows very strong binding to Delta and Wuhan-WT and average to lower binding against Omicron variants BA1 and BA2, respectively. (B) GTX135356 (GenTex) which targets the S1 subunit of spike protein. This antibody shows very weak binding to all spike variants tested. (C) GTX635792 (GenTex) which targets the RBD of spike protein, and shows moderate binding to Delta and Wuhan-WT, but significantly lower binding to Omicron variants BA1 and BA2.

FigS4:

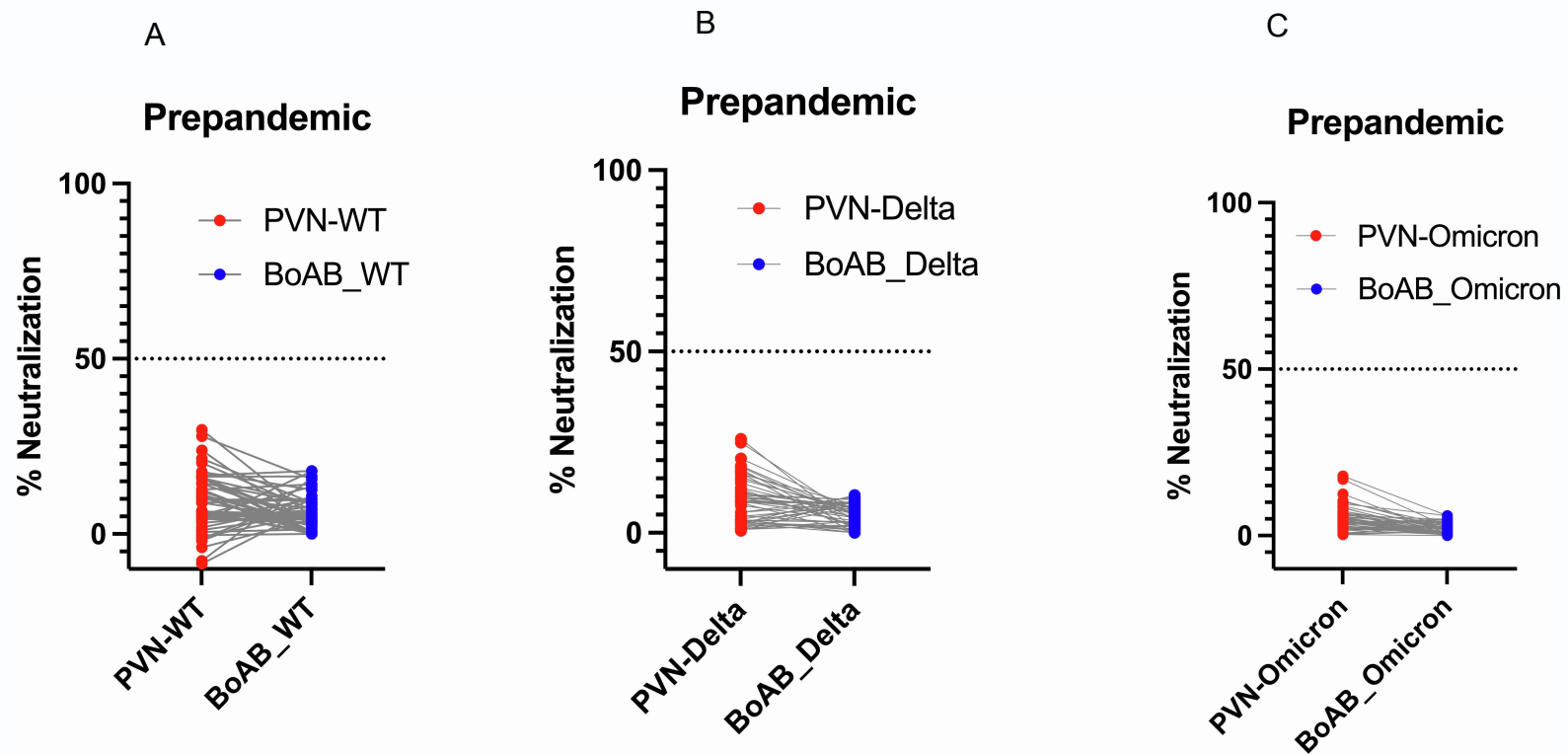
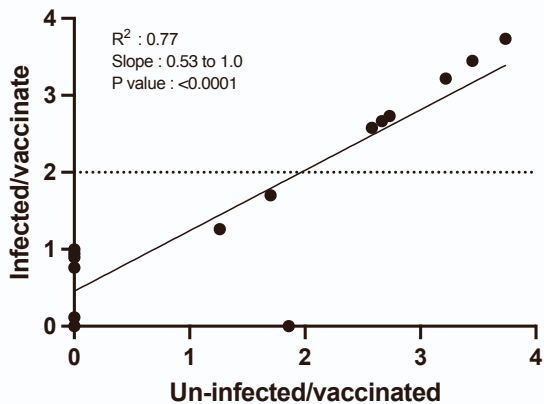


Figure S4: Comparison of BoAB and PVN assay on Prepandemic samples against SARS-CoV-2 variants. Pre-pandemic samples (n=50) tested against SARS-CoV-2 wuhan WT (A), Delta (B) and Omicron (C) variants in the pseudovirus neutralization assay and BOAB assay.

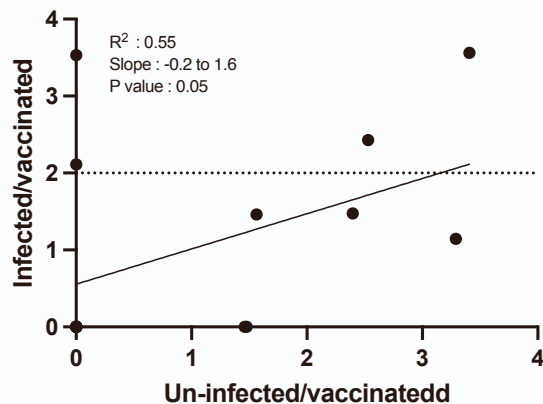
Fig.S5.

Figure S5: Correlation of Neutralizing antibodies between Un-infected/vaccinated vs Infected/vaccinated in BoAB assay and PVN assay.

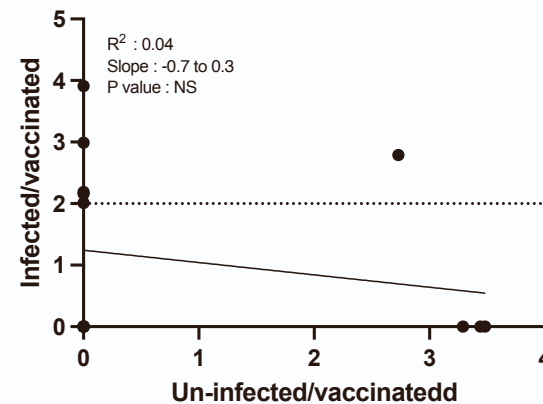
Wuhan-WT-BoAB



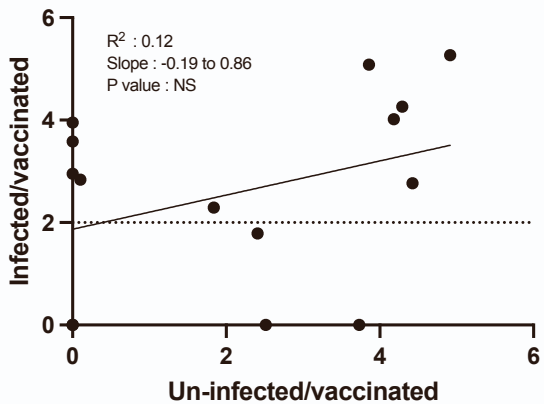
Delta-BoAB



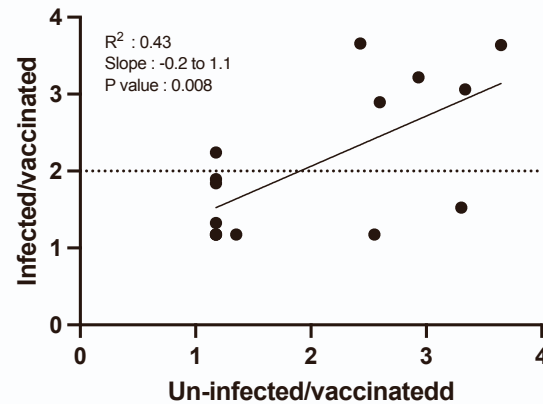
Omicron-BoAB



Wuhan-WT-PVN



Delta-PVN



Omicron-PVN

