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Supplementary appendix

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Supplementary Methods

1. Cloning Strategy

RNA from a patient sample was isolated using a GeneJet Viral RNA extraction kit (ThermoFisher Scientific), and cDNA was generated using Superscript IV (Invitrogen) using Random Hexamer primers, per the manufacturers' instructions. An amplicon encompassing the entire spike was first amplified from cDNA using Q5 High Fidelity 2x Master Mix (New England Biolabs), and a second-round PCR was used to amplify a sub-region of the spike (with codons corresponding to amino acid positions 43 to 1000) incorporating all of the Omicron variant reference mutations. This second round also introduced Gibson assembly overhangs, exploiting regions of existing homology between the codon-optimized parent plasmid and the codon-native spike, in order to maximize overhang length while still keeping primer length under 35bp, allowing for overnight primer synthesis. The N-terminal and C-terminal flanking regions of the parent plasmid were similarly amplified. The three PCR products were cloned by Gibson Assembly into a restriction-enzyme (NheI and XbaI) digested, codon-optimized SARS-CoV-2 Spike expression vector (in pcDNA3.1) harbouring a mutation that introduces a stop codon that truncates the last 19 amino acids of the cytoplasmic tail (facilitating efficient incorporation onto lentiviral particles). The resulting spike-encoding expression vector was confirmed by sequencing to encode an amino acid sequence identical to that of the Omicron consensus. A visual depiction of the cloning strategy is available in Supplementary Figure 1.

Primers (5'-3')

Spike PCR:

First round (primers from [1]):

SARSCoV1200_22_LEFT GTGATGTTCTTGTTAACAACTAAACGAACA

SARSCoV1200_24_RIGHT ATGAGGTGCTGACTGAGGGAAG

Second round:

FWD_N_term_sample CAAGGTGTTCAGATCCTCAGTTTTACATTCAACTC

REV_C_term_sample TCTGCAGTCTGCCTGTGATCAACCTATCAATTTGC

N-terminus flank PCR:

Fwd_CMV_plasmid ACGCAAATGGGCGGTAGGCGTG

REV_N_term_plasmid TGAATGTAAAACTGAGGATCTGAACACCTTGTCGG

C-terminus flank PCR:

FWD_C_term_plasmid AAATTGATAGGTTGATCACAGGCAGACTGCAGAGC

Rev_plasmid TGGCAACTAGAAGGCACAGTCGAG

Cloned Omicron Spike coding sequence (with 19AA CT truncation)

Native codon Omicron insert Codon optimized parent sequence (no AA differences to Omicron reference)

ATGTTCGTGTTTCTGGTGCTGCTGCCTCTGGTGTCCAGCCAGTGTGTGAACCTGACCACCAGAACACAGCTGCCTCCAGCCTACAC
CAACAGCTTTACCAGAGGCGTGTACTACCCCGACAAGGTGTTCAGATCCTCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTT
CTTTTCCAATGTTACTTGGTTCCATGTTATCTCTGGGACCAATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGG
TGTTTATTTTGCTTCCATTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCCAGTCCCTACT
TATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTTTGTAATGATCCATTTTTGGACCACAAAAACAACAA
AAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCT
TGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTTAAGAATATTGATGGTTATTTTAAAATATATTCTAAGCACA
CGCCTATTATAGTGCGTGAGCCAGAAGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCAATAGGTATTAAC
ATCACTAGGTTTCAAACTTTACTTGCTTTACATAGAAGTTATTTGACTCCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCA
GCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAACCATTACAGATGCTGTAGACTGTGC
ACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTGTAGAAAAAGGAATCTATCAAACTTCTAACTTTAGAGTCC
AACCAACAGAATCTATTGTTAGATTTCCTAATATTACAAACTTGTGCCCTTTTGATGAAGTTTTTAACGCCACCAGATTTGCATCTG
TTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATAATCTCGCACCATTTTTCACTTTTAAGT
GTTATGGAGTGTCTCCTACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCA
GACAAATCGCTCCAGGGCAAACTGGAAATATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGG
AATTCTAACAAGCTTGATTCTAAGGTTAGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTTGA
GAGAGATATTTCAACTGAAATCTATCAGGCCGGTAACAAACCTTGTAATGGTGTTGCAGGTTTTAATTGTTACTTTCCTTTACGAT
CATATAGTTTCCGACCCACTTATGGTGTTGGTCACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACTTCTACATGCACCAGCA
ACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAAATGTGTCAATTTCAACTTCAATGGTTTAAAAGGCACAGGTGT
TCTTACTGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGCTGTCCGTGATCCAC
AGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGTCAGTGTTATAACACCAGGAACAAATACTTCTAACCAGGTT
GCTGTTCTTTATCAGGGTGTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTTTATTCT
ACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGGCTGAATATGTCAACAACTCATATGAGTGTGACATACCCAT
TGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAAGTCTCATCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCT
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CAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGATTGTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTT
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TTTTTGCACAAGTCAAACAAATTTACAAAACACCACCAATTAAATATTTTGGTGGTTTTAATTTTTCACAAATATTACCAGATCCAT
CAAAACCAAGCAAGAGGTCATTTATTGAAGATCTACTTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGG
TGATTGCCTTGGTGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAAAGGCCTTACTGTTTTGCCACCTTTGCTCACAGA
TGAAATGATTGCTCAATACACTTCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAA
TACCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACCAAAAATTGATTGCC
AACCAATTTAATAGTGCTATTGGCAAAATTCAAGACTCACTTTCTTCCACAGCAAGTGCACTTGGAAAACTTCAAGATGTGGTCAA
CCATAATGCACAAGCTTTAAACACGCTTGTTAAACAACTTAGCTCCAAATTTGGTGCAATTTCAAGTGTTTTAAATGATATCTTTTC
ACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGATAGGTTGATCACAGGCAGACTGCAGAGCCTCCAGACATACGTGACCCAG
CAGCTGATCAGAGCCGCCGAGATTAGAGCCTCTGCCAATCTGGCCGCCACCAAGATGTCTGAGTGTGTGCTGGGCCAGAGCAAGA
GAGTGGACTTTTGCGGCAAGGGCTACCACCTGATGAGCTTCCCTCAGTCTGCCCCTCACGGCGTGGTGTTTCTGCACGTGACATAC
GTTCCCGCTCAAGAGAAGAATTTCACCACCGCTCCAGCCATCTGCCACGACGGCAAAGCCCACTTTCCTAGAGAAGGCGTGTTCG
TGTCCAACGGCACCCATTGGTTCGTGACACAGCGGAACTTCTACGAGCCCCAGATCATCACCACCGACAACACCTTCGTGTCTGG
CAACTGCGACGTCGTGATCGGCATTGTGAACAATACCGTGTACGACCCTCTGCAGCCCGAGCTGGACAGCTTCAAAGAGGAACTG
GACAAGTACTTTAAGAACCACACAAGCCCCGACGTGGACCTGGGCGATATCAGCGGAATCAATGCCAGCGTCGTGAACATCCAG
AAAGAGATCGACCGGCTGAACGAGGTGGCCAAGAATCTGAACGAGAGCCTGATCGACCTGCAAGAACTGGGGAAGTACGAGCA
GTACATCAAGTGGCCCTGGTACATCTGGCTGGGCTTTATCGCCGGACTGATTGCCATCGTGATGGTCACAATCATGCTGTGTTGCA
TGACCAGCTGCTGTAGCTGCCTGAAGGGCTGTTGTAGCTGTGGCAGCTGCTGCTAG

2. Monoclonal Antibody Production

Antibody sequences were extracted from deposited RCSB entries and codon optimized (using a human germline-aware codon optimization strategy), then synthesized as gene fragments and cloned into pTWIST transient expression vectors by Gibson assembly or restriction cloning (NotI, BamHI). Cloned plasmids were verified by sanger sequencing.

Expi293 cells were transfected with plasmids encoding for the heavy and light chain at 1µg/mL (i.e. 0.5µg/mL each) according to the manufacturer's instructions (ThermoFisher). Dense Expi293 cultures (day 5-7 post transfection) were

centrifuged at 300xg for 5 minutes to pellet the cells. Supernatant was filtered using Steriflip® 0.22 µm (Merck) filter units. Expi supernatant was directly loaded onto Protein G Agarose (Pierce) gravity columns, washed twice with PBS and eluted using Protein G Elution Buffer (Pierce). The eluted fractions were immediately neutralized with 1M TRIS-Buffer (pH = 8) to physiological pH. Absorption at 280 nm was quantified by Nanodrop™ 2000c to determine protein containing fractions. These fractions were then pooled and buffer exchanged using SnakeSkin™ dialysis tubing (10 MWCO, Pierce) followed by further dialysis and concentration using Amicon Ultra-4 10kDa centrifugal units (Merck).

3. AlphaFold2 Omicron Model

AlphaFold2[2], implemented in ColabFold[3], was used to construct models of two overlapping Omicron spike fragments, with residues 1 to 698, (founder coordinates) and 590 to 1147, using "Templates=true", and without Amber relaxation. The amino acid sequence used was identical to that which we cloned into our PSV plasmid. These two fragment models were pieced together using their overlap (in Chimera 1.15), and this was found to adopt the conformation that matched the "RBD-down" protomer in PDB: 7MJJ[4], which was used to contextualize the visualization in figure 1.

4. Monoclonal Antibody Escape Mutations

The RBD from the AlphaFold2 model was docked into a crystal complex for each mab. Sites that were mutated in Omicron, and proximal to the mAb/RBD interface, were selected, and cross-referenced with deep mutational scanning data, and visualized in dms-view[5].

PDBs used: 6XDG[6] (REGN-10933 and REGN-10987), 7KMG[7] (LY-CoV555) 7C01[8] (LY-CoV016), 6WPS[9] (S309).

Deep Mutational Scanning data from:

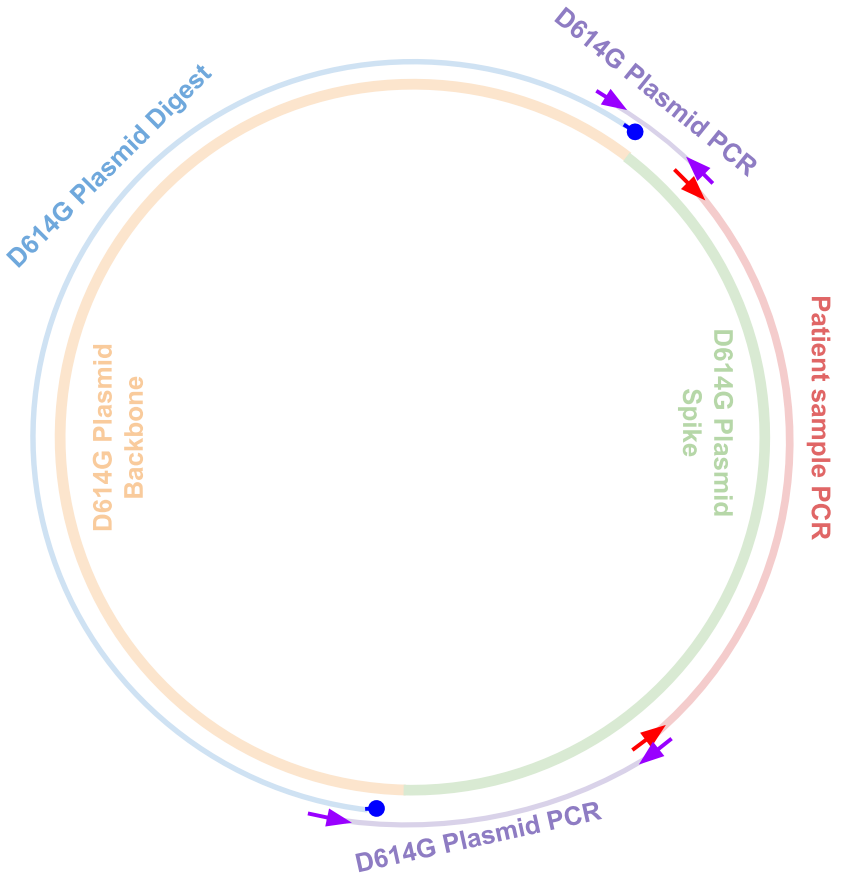
https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_clinical_Abs/ [10]

https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_LY-CoV555/ [11]

https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_Vir_mAbs/ [12]

Supplementary Figures

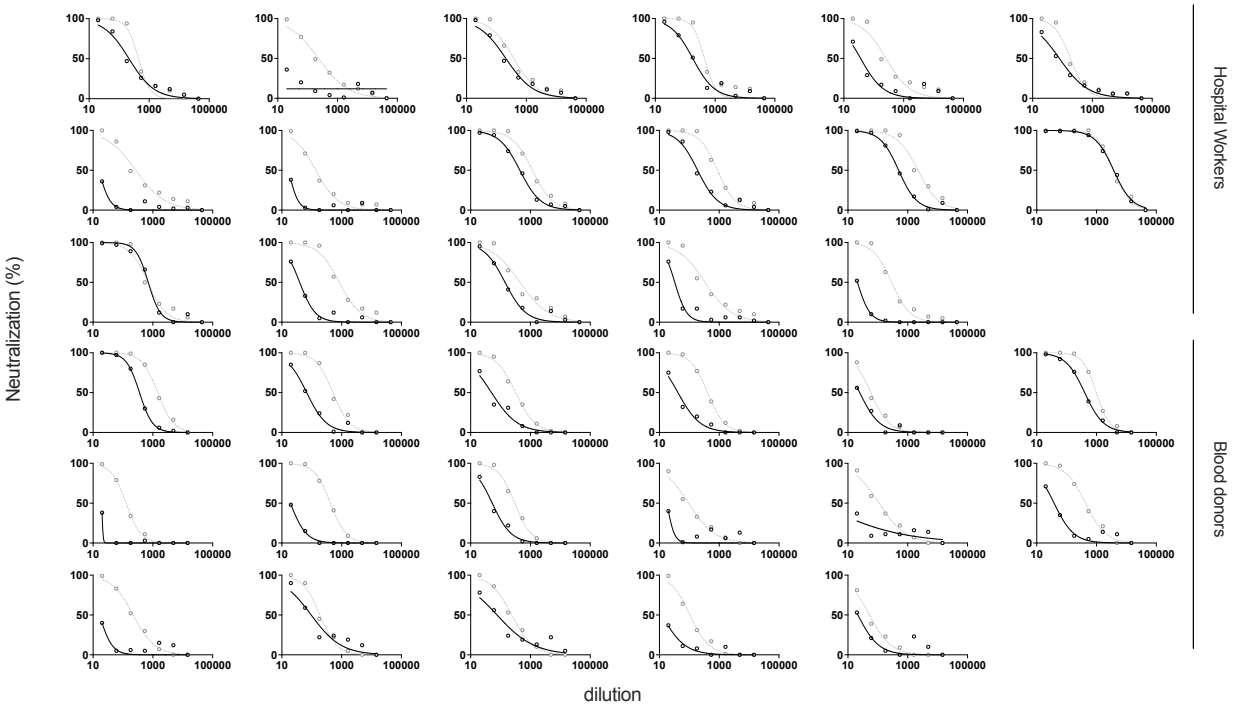
A



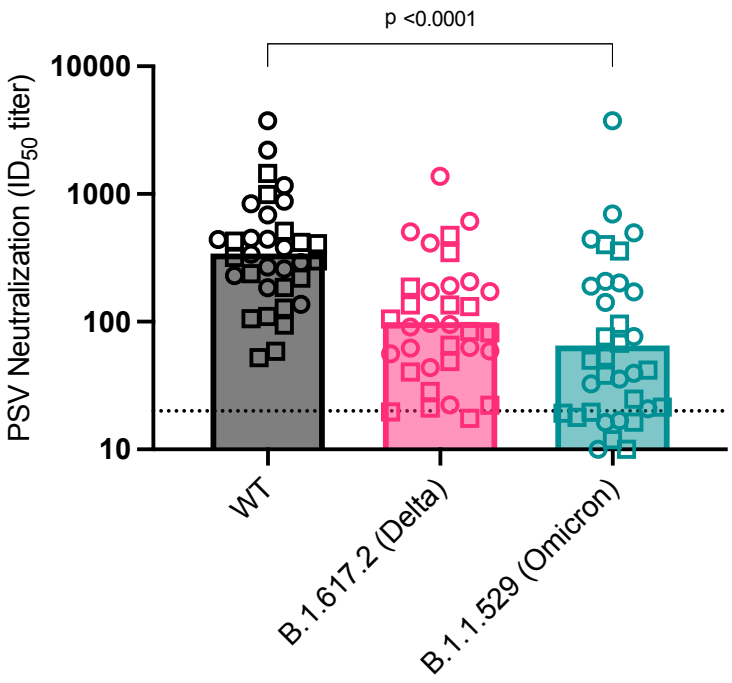
B

```
Primer: FWD_N_term_sample          5'- caaggtgttcagatcctcagttttacattcaactc - 3'
            Omicron Sample tttattaccctgacaaagttttcagatcctcagttttacattcaactcaggacttgtt
             D614G Plasmid tgtactaccccgacaaggtgttcagatccagcgtgctgcactctaccaggacctgtt
Primer: REV_N_term_plasmid (Rev Comp)      3'- ccgacaaggtgttcagatcctcagttttacattca - 5'
```

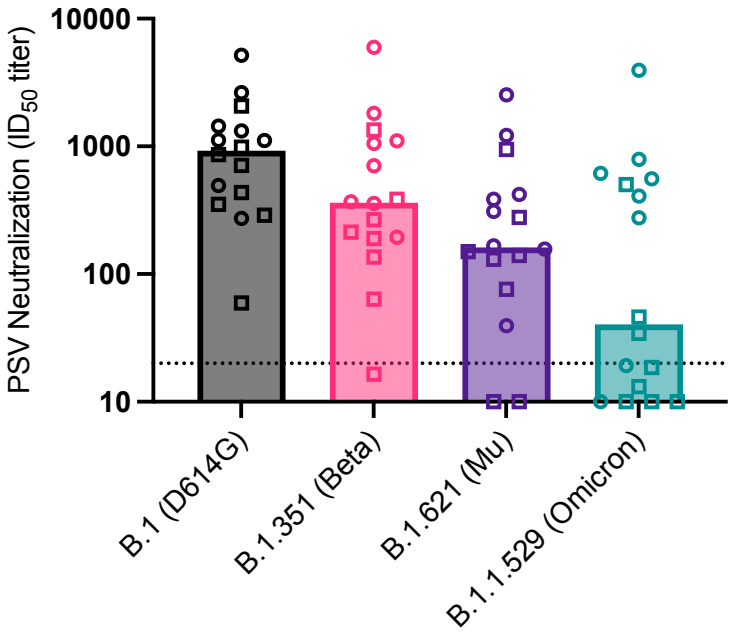
Supplementary Figure 1. Cloning strategy. A depicts the 4-fragment gibson assembly, combining a digested D614G plasmid backbone, two PCR fragments that incorporate the N terminus and C terminus of the codon optimized D614G spike, and the Omicron spike amplified from a patient sample. **B** shows an example of the junction between the codon optimized D614G plasmid and the Omicron sample sequence. Differences between codon optimized D614G and codon native Omicron are in red, and a coincidentally long region of nucleotide identity (**bold**) was exploited to ensure i) sufficient 3' identity for PCR amplification, ii) sufficient 5' overhangs for gibson assembly, and iii) primer lengths less than or equal to 35bp, which was required for overnight synthesis.



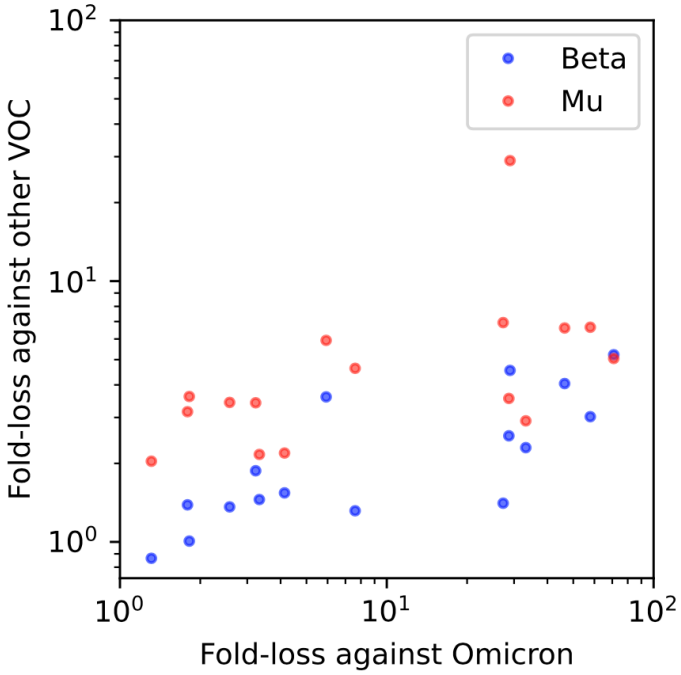
Supplementary Figure 2. Neutralization curves. Neutralization curves for 17 sera each from the HW and BD cohorts against WT (grey) and Omicron/B.1.1.529 (black).



Supplementary Figure 3. Neutralizing activity compared to Delta. Neutralizing activity of sera from blood donors (BD) (squares) and Hospital Workers (HW) (circles) against WT, Delta, and Omicron.



Supplementary Figure 4. Neutralizing activity compared to Beta and Mu. Neutralizing activity of sera from a subset of BD (squares) and HW (circles) against B.1, Beta, Mu, and Omicron, from an independent assay replicate. Sample selection was biased towards samples that showed extreme maintenance or loss against Omicron. For the subset of samples included in both runs, Omicron titers were reproducible (rho = 0.96, calculated from ID50s in the log domain).



Supplementary Figure 5. Correlations. For the subset of samples run against D614G, Omicron, Beta, and Mu, the fold-loss against Omicron (defined as D614G IC50/Omicron ID50) was more strongly correlated with fold-loss against Beta (rho = 0.78, log domain) than Mu (rho = 0.57, log domain).

Supplementary Tables

Supplementary Table 1. List of mutations in the Omicron spike evaluated here, relative to Wu-Hu-1.

A67V
H69-
V70-
T95I
G142D
V143-
Y144-
Y145-
N211-
L212I
+214EPE
G339D
S371L
S373P
S375F
K417N
N440K
G446S
S477N
T478K
E484A
Q493R
G496S
Q498R
N501Y
Y505H
T547K
D614G
H655Y
N679K
P681H
N764K
D796Y
N856K
Q954H
N969K
L981F

Supplementary Table 2. Neutralizing ID_{50} titers for pooled vaccine standard reagents.

	Founder ID_{50}	Delta ID_{50}	Omicron ID_{50}
Pfizer	415	155	<20
Moderna	321	143	<20
J&J	385	178	57

Supplementary Table 3. Heterogeneity in fold-changes. Table shows statistics for the heterogeneity in the difference in $\log_{10}$ neutralization titers (which is the logarithm of the Omicron/WT fold-change). The BD and HW groups have similar standard deviations, indicating that the degree of heterogeneity is similar between these groups. Also shown are Shapiro-Wilk test statistics and P values for the BD and HW cohort, failing to reject the null hypothesis that these are normally distributed, indicating that the fold changes themselves are consistent with a log-normal distribution.

	BD	HW
N	40	17
Mean	0.9152	0.675
Std. Deviation	0.411	0.4382
Lower 95% CI of mean	0.7838	0.4497
Upper 95% CI of mean	1.047	0.9003
Shapiro-Wilk W	0.9895	0.9476
Shapiro-Wilk P value	0.9676	0.4202

References

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