Supplementary Methods

Clinical cohorts

Sera analyzed in this study was categorized into several cohorts. Boosted samples consisted of sera from individuals who had received three doses of monovalent, referred to as wild-type (WT), mRNA vaccines (either Moderna mRNA-1273 or Pfizer BNT162b2). Sera was also collected from individuals after a fourth monovalent mRNA vaccine (referred to as "4 shots WT"). Bivalent vaccine sera were collected from individuals who had received three monovalent mRNA vaccine doses followed by one dose of the Pfizer or Moderna bivalent vaccine targeting BA.4/BA.5 in addition to the ancestral strain. BA.4/BA.5 breakthrough sera was collected from individuals who had received monovalent mRNA vaccines followed by infection with Omicron sub-lineages BA.4 or BA.5. Samples were examined by anti-nucleoprotein (NP) ELISA to confirm status of prior SARS-CoV-2 infection.

A subset of sera analyzed in this study was collected at the University of Michigan through the Immunity-Associated with SARS-CoV-2 Study (IASO), an ongoing cohort study in Ann Arbor, Michigan that began in 2020[1]. All IASO participants provided written informed consent and serum samples were collected under the protocol approved by the Institutional Review Board of the University of Michigan Medical School.

A subset of vaccinee and breakthrough sera analyzed in this study was collected at Columbia University Irving Medical Center. All subjects provided written informed consent, and all serum collections were performed under protocols reviewed and approved by the Institutional Review Board of Columbia University.

Clinical information for the different study cohorts is summarized in **Table S1** with detailed information on each case provided in **Table S2**.

Cell lines

Vero-E6 cells (CRL-1586) and HEK293T cells (CRL-3216) were obtained from the American Type Culture Collection. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% penicillin-streptomycin in an atmosphere of 5% CO_2 at 37 °C.

SARS-CoV-2 spike plasmids

Plasmids encoding the spike (S) protein of SARS-CoV-2 variants D614G, BA.1, BA.2, BA.4/BA.5, BA.4.6, BA.2.75, SARS-CoV, GD-Pangolin, GX-Pangolin, and WIV1 were

previously constructed[2-7]. BA.2.75.2 spike were constructed with the QuikChange II XL site-directed mutagenesis kit according to the manufacturer's instructions (Agilent). The sequence of each construct was confirmed by Sanger sequencing prior to experimental use.

Pseudovirus production

Pseudotyped SARS-CoV-2 variants and other tested sarbecoviruses were generated in the background of vesicular stomatitis virus (VSV). The native VSV glycoprotein (G) was replaced with the S protein from each SARS-CoV-2 variant or other tested sarbecoviruses as previously described[8]. Briefly, HEK293T cells were transfected with plasmids encoding the appropriate S protein using 1 mg/mL of PEI. Transfected HEK293T cells were then cultured at 37 °C with 5% CO_2 for 24 hours. Cells were then infected with VSV-G pseudotyped ΔG-luciferase (G*ΔG-luciferase, Kerafast). After a two-hour incubation at 37 °C, infected HEK293T cells were washed three times before being cultured in fresh medium for another 24 hours under the same conditions. Supernatants were subsequently collected, centrifuged to remove precipitates, and aliquoted for storage at -80 °C. Prior to infection of target cells, the viral stock was incubated with 20% I1 hybridoma (anti-VSV-G) supernatant (ATCC; CRL-2700) for 1 h at 37°C to neutralize contaminating VSV-G pseudotyped ΔG-luciferase.

Pseudovirus neutralization

Before each neutralization assay, all pseudoviruses were titrated to equilibrate the viral input. Sera were heat-inactivated and all samples run in triplicate in 96-well plates. Sera were four-fold serially diluted in media starting at a 1:100 dilution. Pseudoviruses were added and the virus-sample mixture was incubated at 37 °C for 1 hour. Control wells only containing virus were included on all plates. Vero-E6 cells were then added at a density of 4×10^4 cells per well and plates were incubated at 37 °C with 5% CO_2 for 10 hours. Cells were then lysed and luciferase activity was measured using the Luciferase Assay System (Promega) and SoftMax Pro v.7.0.2 (Molecular Devices) according to instructions from both manufacturers.

Quantification and statistical analysis

The inhibitory dilution retaining 50% neutralization (ID_{50}) was obtained for each serum-virus combination using a five-parameter dose-response curve in GraphPad Prism v.9.2. Statistical significance between unpaired groups was evaluated using the two-tailed Mann-Whitney test in GraphPad Prism v.9.2. Levels of significance are denoted as follows: $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$.

Acknowledgements

This study was supported by funding from the NIH SARS-CoV-2 Assessment of Viral Evolution (SAVE) Program as well as the NIH, NIAID contract number 75N93019C00051 (to A.G.). We thank David Manthei, Emily Stoneman, Victoria Blanc, Pamela Bennett-Baker, Savanna Sneeringer, Lauren Warsinske, Theresa Kowalski-Dobson, Alyssa Meyers, Zijin Chu, Hailey Kuiken, Lonnie Barnes, Ashley Eckard, Kathleen Lindsey, Dawson Davis, Aaron Rico, Casey Juntila, Daniel Raymond, Mayurika Patel, and Nivea Vydiswaran from the IASO study team for providing serum samples.

Author Contributions

L.L. and D.D.H. conceived the study. Q.W. and L.L. performed experiments and analyzed data. Q.W. managed the project. A.B., R.V., C.G. and A.G. collected serum samples. Q.W., A.B., L.L., and D.D.H. analyzed the results and wrote the manuscript. L.L. and D.D.H. directed and supervised the project. All authors reviewed and approved of the manuscript.

Declaration of Interests

D.D.H. is a co-founder of TaiMed Biologics and RenBio, consultant to WuXi Biologics and Brii Biosciences, and board director for Vicarious Surgical. Aubree Gordon serves on a scientific advisory board for Janssen Pharmaceuticals. Other authors declare no competing interests.

Table S1. Summary of clinical cohorts

Characteristic	3 shots WT (N=14)	BA.4/BA.5 breakthrough (N=20)	4 shots WT (N=19)	3 shots WT + bivalent (N=21)
Sex — no. (%)				
Female	6 (42.9%)	17 (85.0%)	17 (89.5%)	16 (76.2%)
Male	8 (57.1%)	3 (15.0%)	2 (10.5%)	5 (23.8%)
Mean Age (range) — years	52.1 (26, 71)	44.4 (24, 69)	55.3 (48, 63)	36.4 (23, 49)
Mean days post vaccination or infection (range)	39.2 (14, 90)	31.8 (15, 75)	24.0 (20, 36)	26.4 (23, 30)
First and Second Vaccine Type — no. (%)				
Pfizer (BNT162b2)	12 (85.7%)	17 (85.0%)	18 (94.7%)	17 (81.0%)
Moderna (mRNA-1273)	2 (14.3%)	3 (15.0%)	1 (5.3%)	4 (19.0%)
Third Vaccine Type — no. (%)				
Pfizer (BNT162b2)	11 (78.6%)	15 (75.0%)	18 (94.7%)	13 (61.9%)
Moderna (mRNA-1273)	3 (21.4%)	5 (25.0%)	1 (5.3%)	8 (38.1%)
Fourth Vaccine Type (monovalent or *bivalent) — no. (%)				
Pfizer	-	10 (50.0%)	18 (94.7%)	*9 (42.9%)
Moderna	-	0 (0.0%)	1 (5.3%)	*12 (57.1%)

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Sample ID	Vaccine type and infected strain	Days post-vaccination or *infection	Documented COVID-19	Age	Gender
3 shots WT					
Q1	mRNA-1273/mRNA-1273/mRNA-1273	29	No	66	Female
Q2	BNT162b2/BNT162b2/BNT162b2	30	No	68	Male
Q3	BNT162b2/BNT162b2/BNT162b2	14	No	64	Female
Q4	BNT162b2/BNT162b2/BNT162b2	34	No	55	Male
Q5	BNT162b2/BNT162b2/BNT162b2	34	No	45	Male
Q6	BNT162b2/BNT162b2/BNT162b2	15	No	50	Female
Q7	BNT162b2/BNT162b2/BNT162b2	15	No	48	Female
Q8	BNT162b2/BNT162b2/BNT162b2	29	No	71	Male
Q9	BNT162b2/BNT162b2/BNT162b2	90	No	59	Male
Q10	BNT162b2/BNT162b2/BNT162b2	33	No	45	Male
Q11	BNT162b2/BNT162b2/BNT162b2	87	No	66	Female
Q12	BNT162b2/BNT162b2/BNT162b2	84	No	26	Male
Q13	mRNA-1273/mRNA-1273/mRNA-1273	23	No	28	Female
Q15	BNT162b2/BNT162b2/mRNA-1273	32	No	39	Male
BA.4/BA.5 breakthrough					
Q71	mRNA-1273/mRNA-1273/BNT162b2/BA.5.2.1	*29	Yes	29	Female
Q77	BNT162b2/BNT162b2/BNT162b2/BA.5	*22	Yes	61	Female
Q79	mRNA-1273/mRNA-1273/mRNA-1273/BA.5	*15	Yes	28	Female
Q80	mRNA-1273/mRNA-1273/mRNA-1273/BA.5	*21	Yes	24	Female
Q81	BNT162b2/BNT162b2/BNT162b2/BA.5	*75	Yes	35	Female
Q82	BNT162b2/BNT162b2/mRNA-1273/BA.5	*63	Yes	46	Female
Q83	BNT162b2/BNT162b2/BNT162b2/BA.5	*28	Yes	55	Male
Q84	BNT162b2/BNT162b2/BNT162b2/BA.5	*17	Yes	57	Female
UM-85	BNT162b2/BNT162b2/BNT162b2/BA.5	*29	Yes	44	Female
UM-86	BNT162b2/BNT162b2/mRNA-1273/BA.5	*29	Yes	36	Female
UM-87	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*31	Yes	54	Female
UM-88	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*28	Yes	69	Male
UM-89	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*42	Yes	44	Male
UM-90	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*28	Yes	41	Female
UM-91	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*28	Yes	44	Female
UM-92	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*31	Yes	29	Female
UM-93	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*29	Yes	48	Female
UM-94	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*29	Yes	49	Female
UM-95	BNT162b2/BNT162b2/mRNA-1273/BNT162b2/BA.5	*28	Yes	37	Female
UM-96	BNT162b2/BNT162b2/BNT162b2/BNT162b2/BA.5	*33	Yes	58	Female
4 shots WT					
UM-65	BNT162b2/BNT162b2/BNT162b2/BNT162b2	24	No	52	Female
UM-66	BNT162b2/BNT162b2/BNT162b2/BNT162b2	20	No	57	Female
UM-67	BNT162b2/BNT162b2/BNT162b2/BNT162b2	20	No	61	Female
UM-68	mRNA-1273/mRNA-1273/mRNA-1273/mRNA-1273	22	No	48	Female
UM-69	BNT162b2/BNT162b2/BNT162b2/BNT162b2	23	No	50	Female
UM-70	BNT162b2/BNT162b2/BNT162b2/BNT162b2	22	No	50	Female
UM-71	BNT162b2/BNT162b2/BNT162b2/BNT162b2	20	No	58	Female
UM-72	BNT162b2/BNT162b2/BNT162b2/BNT162b2	26	No	56	Female
UM-73	BNT162b2/BNT162b2/BNT162b2/BNT162b2	29	No	63	Female
UM-74	BNT162b2/BNT162b2/BNT162b2/BNT162b2	25	No	58	Female
UM-75	BNT162b2/BNT162b2/BNT162b2/BNT162b2	21	No	62	Male
UM-76	BNT162b2/BNT162b2/BNT162b2/BNT162b2	26	No	54	Female
UM-77	BNT162b2/BNT162b2/BNT162b2/BNT162b2	23	No	53	Male
UM-78	BNT162b2/BNT162b2/BNT162b2/BNT162b2	21	No	55	Female
UM-79	BNT162b2/BNT162b2/BNT162b2/BNT162b2	23	No	59	Female
UM-80	BNT162b2/BNT162b2/BNT162b2/BNT162b2	21	No	49	Female
UM-81	BNT162b2/BNT162b2/BNT162b2/BNT162b2	27	No	57	Female
UM-82	BNT162b2/BNT162b2/BNT162b2/BNT162b2	27	No	55	Female
Q97	BNT162b2/BNT162b2/BNT162b2/BNT162b2	36	No	53	Female
3 shots WT + bivalent					
UM-36	BNT162b2/BNT162b2/BNT162b2/Moderna Bivalent	24	No	38	Female

Sample ID	Vaccine type and infected strain	Days post-vaccination or *infection	Documented COVID-19	Age	Gender
UM-37	BNT162b2/BNT162b2/BNT162b2/Moderna Bivalent	27	No	42	Female
UM-39	mRNA-1273//mRNA-1273/mRNA-1273/Moderna Bivalent	24	No	36	Male
UM-40	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	25	No	37	Female
UM-41	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	24	No	36	Male
UM-43	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	25	No	49	Female
UM-44	BNT162b2/BNT162b2/BNT162b2/Moderna Bivalent	25	No	37	Female
UM-47	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	26	No	45	Male
UM-48	BNT162b2/BNT162b2/mRNA-1273/Moderna Bivalent	26	No	43	Female
UM-51	mRNA-1273/mRNA-1273/mRNA-1273/Moderna Bivalent	29	No	32	Female
UM-52	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	23	No	43	Female
UM-53	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	26	No	43	Female
UM-54	BNT162b2/BNT162b2/mRNA-1273/Moderna Bivalent	29	No	38	Female
UM-55	BNT162b2/BNT162b2/BNT162b2/Moderna Bivalent	28	No	38	Female
UM-56	BNT162b2/BNT162b2/mRNA-1273/Moderna Bivalent	27	No	36	Female
UM-60	BNT162b2/BNT162b2/BNT162b2/Moderna Bivalent	30	No	24	Female
Q101	mRNA-1273/mRNA-1273/mRNA-1273/Moderna Bivalent	30	No	32	Female
Q102	BNT162b2/BNT162b2/mRNA-1273/Moderna Bivalent	23	No	39	Male
Q103	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	30	No	26	Female
Q104	mRNA-1273/mRNA-1273/mRNA-1273/Pfizer Bivalent	30	No	27	Female
Q105	BNT162b2/BNT162b2/BNT162b2/Pfizer Bivalent	23	No	23	Male

Supplementary References

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