Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

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Comparable Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2 Variants

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Supplementary Methods

Study population

A specimen biorepository at Beth Israel Deaconess Medical Center (BIDMC) obtained samples from individuals who received a SARS-CoV-2 vaccine and/or had a history of SARS-CoV-2 infection. The BIDMC institutional review board approved this study (2020P000361). All participants provided informed consent. Demographic and clinical characteristics were obtained by self-report and review of the electronic medical record. For the group of individuals receiving BNT162b2, participants were excluded if they had a known history of SARS-CoV-2 infection or evidence of prior infection by SARS-CoV-2 Nucleocapsid IgG detection (MesoScale Discovery Cat No: K15437U-2).[1,2] Participants were also excluded if they received other COVID-19 vaccines or received immunosuppressive medications.

Pseudovirus neutralizing antibody assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were used to measure pseudovirus neutralizing antibodies. In brief, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene) and spike protein expressing pcDNA3.1-SARS-CoV-2 SΔCT were co-transfected into HEK293T cells (ATCC CRL_3216) with lipofectamine 2000 (ThermoFisher Scientific). Pseudoviruses of SARS-CoV-2 variants were generated by using WA1/2020 strain (Wuhan/WIV04/2019, GISAID accession ID: EPI_ISL_402124), Omicron B.1.1.529 BA.1 (GISAID ID: EPI_ISL_7358094.2), or BA.2 (GSAID ID: EPI_ISL_6795834.2). The supernatants containing the pseudotype viruses were

collected 48h after transfection; pseudotype viruses were purified by filtration with 0.45-μm filter. To determine the neutralization activity of human serum, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 2×10^4 cells per well overnight. Three-fold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction (NT50) in relative light units was observed relative to the average of the virus control wells.

Statistical analysis

Descriptive statistics were calculated using GraphPad Prism 8.4.3, (GraphPad Software, San Diego, California). Data are presented as median with interquartile range (IQR) or number (percent).

Table S1. Characteristics of study population

	BNT162b2 (3 dose) N=24	COVID-19 Infection N=8
Age (years), median (range)	34 (23-69)	37 (23-60)
Sex at birth, female	21 (88)	6 (75)
Race		
White	20 (83)	6 (75)
Asian	2 (8)	0
Black	1 (4)	0
More than one race	1 (4)	0
Unknown/Other*	0	2 (25)
Ethnicity		
Hispanic or Latino	2 (8)	1 (13)
Non-Hispanic	22 (88)	6 (75)
Other	1 (4)	1 (13)
Medical condition		
Obese (BMI ≥30 kg/m2)	1 (4)	2 (25)
Hypertension	2 (8)	2 (25)
Diabetes	2 (8)	3 (50)
Immunosuppression	0	1 (13)
Pregnant	1 (4)	3 (50)
COVID-19 vaccination history		
BNT162b2 (3 doses)	24 (100)	2 (25)
mRNA-1273 (3 doses)	0	2 (25)
BNT162b2 (2 doses)	0	2 (25)
Ad26.COV2.S (1 dose)/mRNA-1273 (1 dose)	0	1 (13)
Unvaccinated	0	1 (13)
COVID-19 disease severity (NIH scale)		
Asymptomatic	N/A	1 (13)
Mild	N/A	5 (63)
Moderate	N/A	0
Severe	N/A	2 (25)
Critical	N/A	0
Days from primary vaccine series to booster vaccine**, median (IQR)	254 (248-258)	268 (244-295); n=5
Days from last vaccine dose to positive PCR test, median (IQR)	N/A	113 (27-219)
Days from positive PCR test to sampling, median (IQR)	N/A	14 (5-22)

Data displayed as median (range or interquartile range, IQR) and n (%). BMI, body mass index; NIH, National Institutes of Health; PCR, polymerase chain reaction for SARS-CoV-2; pregnant designation reflects time of sampling. *Self-identified race as Brazilian and Colombian. **Time from 2nd mRNA vaccine to 3rd vaccine or time from single Ad26.COV2.S vaccine to 2nd vaccine.

Table S2. Vaccine and infection characteristics of SARS-CoV-2 infected participants

Patient	Sex at birth	Age	Medical condition	COVID-19 vaccine history (primary/boost)	Days from last dose to PCR test	Days from PCR test to sampling	COVID-19 disease severity (NIH scale)
1	Female	35	Pregnant	mRNA-1273/mRNA-1273	33	13	Mild
2	Female	24	None	BNT162b2/BNT162b2	121	22	Mild
3	Male	39	None	Ad26.COV2.S/mRNA-1273	26	15	Mild
4	Female	50	HTN, DM	mRNA-1273/mRNA-1273	27	21	Asymptomatic
5	Male	60	DM, leukemia	BNT162b2/No 3rd Boost	219	9	Severe
6	Female	34	Pregnant, DM	BNT162b2/No 3rd Boost	230	2	Mild
7*	Female	23	Pregnant	None	N/A	4	Severe
8	Female	59	HTN, RA	BNT162b2/BNT162b2	113	35	Mild

PCR, polymerase chain reaction for SARS-CoV-2
NIH, National Institutes of Health
HTN, hypertension
DM, diabetes mellitus
Pregnant designation reflects time of sampling
*This unvaccinated individual had no detectable neutralizing antibody titers at the time of sampling as shown in Fig. 1C.

Table S3. Demographics of COVID-19 infected individuals in Boston compared with study participants

Boston residents through 2/8/22 *N=692,958*		*Study participants* *N=32*
Age (years)	**No. (%)**	
5-11	41,936 (6)	Age of study participants are generally representative of the broader population
12-15	22,518 (3)	
16-19	42,189 (6)	
20-29	157,447 (23)	
30-49	203,018 (29)	
50-64	105,032 (15)	
65-74	47,470 (7)	
75+	33,362 (5)	
Sex at birth		
Female	360,229 (52)	Female study participants are over-represented
Male	332,729 (48)	
Non-binary/not reported	2,095 (0.3)	
Race/Ethnicity		
American Indian/Alaska Native	1,452 (0)	The small sample size of study participants does not adequately represent the racial and ethnic diversity of the region; a variety of chronic medical conditions are represented
Asian	69,263 (10)	
Black	154,464 (22)	
Hispanic	138,847 (20)	
More than one race or ethnicity	15,540 (2)	
Non-Hispanic/Pacific Islander	400 (0)	
White	307,173 (44)	
Other/Unknown^	50,289 (7)	
COVID-19 vaccination status		
Unvaccinated	116,885 (17)	Vaccination regimens of participants were generally representative of the broader population
Partially vaccinated	90,780 (13)	
Fully vaccinated (>14d from final dose)	485,293 (70)	
Received a booster dose	240,740 (35)	

Data obtained from Massachusetts Department of Public Health publically available data.[3]

^Other includes responses that do not fit into the listed categories, including no response or "prefer not to say." Unknown includes records from selected providers whose software does not allow them to collect race and ethnicity data.

Supplementary References

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