Supplementary Appendix

Supplement to: Evans JP, Qu P, Zeng C, et al. Neutralization of the SARS-CoV-2 deltacron and BA.3 variants. N Engl J Med. DOI: 10.1056/NEJMc2205019

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

Supplementary Appendix

CONTENTS

Supplementary Methods 3

Acknowledgements 6

Author Contributions 7

Figure S1. Variant SARS-CoV-2 Spike Constructs 8

Figure S2. Neutralization resistance to vaccinated and boosted HCW sera for the Omicron variant sub-lineages and the Delta variant 9

Figure S3. Neutralization resistance to Delta-wave and Omicron-wave patient sera for the Omicron variant sub-lineages and the Delta variant 10

Table S1. Demographic and sample collection information for HCW and COVID-19 patient cohorts 12

Supplementary References 13

Neutralization of the SARS-CoV-2 Deltacron and BA.3 Variants

John P. Evans, M.S.[1], Panke Qu, M.S.[1], Cong Zeng, Ph.D.[1], Yi-Min Zheng, M.D., M.S.[1], Claire Carlin, M.S.[1], Joseph S. Bednash, M.D.[1], Gerard Lozanski, M.D.[1], Rama Mallampalli, M.D.[1], Linda J. Saif, Ph.D.[1], Eugene M. Oltz, Ph.D.[1], Peter Mohler, Ph.D.[1], Richard J. Gumina, M.D., Ph.D.[1], Shan-Lu Liu, M.D., Ph.D.[1*]

[1]The Ohio State University, Columbus, OH, USA

[*]Corresponding author: Shan-Lu Liu, M.D., Ph.D., Center for Retroviral Research, Department of Veterinary Biosciences, Department of Microbial Infection and Immunity, and Infectious Diseases Institutes, The Ohio State University

1900 Coffey Road, Columbus, OH 43210; Telephone: (614) 292-8690;

Fax: (614) 292-6473; Email: liu.6244@osu.edu

Authors for Print Edition

John P. Evans, M.S., Panke Qu, M.S., and Shan-Lu Liu, M.D., Ph.D.; Center for Retroviral Research, Department of Veterinary Biosciences, and Infectious Diseases Institutes, The Ohio State University, Columbus, OH, USA, 43210

Supplementary Methods

Vaccinated and hospitalized/ICU patient cohorts

Vaccinated HCW samples were collected under approved IRB protocols (2020H0228 and 2020H0527). All subjects provided informed consent, and demographic information was self-reported. Due to urgent recruitment, age, race, and comorbidities were not considered during enrollment. Sera were collected 3-4 weeks post-second vaccine dose for 10 HCWs (4 female and 6 male; median age 37.5; age range 29-48), which included 3 Moderna mRNA-1273 and 7 Pfizer/BioNTech BNT162b2 vaccinated HCWs. Sera were additionally collected 1-11 weeks post homologous booster dose.

Delta-wave ICU patient samples were collected under an approved IRB protocol (2020H0175). All subjects provided informed consent, and demographic information was self-reported. Due to urgent recruitment, age, race, and comorbidities were not considered during enrollment. Plasma samples were collected 3 days after ICU admission for 18 Delta-wave patients (6 female and 12 male; median age 60; age range 22-87; 4 African American/Black non-Hispanic or Latino, 1 White Hispanic or Latino, and 13 White non-Hispanic or Latino). Where detectable, the variant of SARS-CoV-2 infecting the ICU patients was determined by viral RNA extraction on nasal swabs with QIAamp MinElute Virus Spin kit followed by RT-PCR (CDC N1 F: 5′-GACCCCAAAATCAGCGAAAT-3′; CDC N1 R: 5′-TCTGGTTACTGCCAGTTGAATCTG-3′; CDC N2 F: 5′-TTACAAACATTGGCCGCAAA-3′; CDC N2 R: 5′-GCGCGACATTCCGAAGAA-3′) and Sanger sequencing to identify types of variant with 5/18 patients being confirmed Delta cases. Additionally, these Delta-wave patients included 1 patient vaccinated with 1 dose of the Johnson & Johnson vaccine, 4 patients vaccinated with 2 doses of

the Pfizer/BioNTech BNT162b2 vaccine, and 1 patient vaccinated with 3 doses of the Moderna mRNA-1273 vaccine.

Omicron-wave hospitalized patient samples were collected under an approved IRB (2020H0527). All subjects provided informed consent, and demographic information was self-reported. Due to urgent recruitment, age, race, and comorbidities were not considered during enrollment. Sera were collected 1-8 days after hospitalization for 31 COVID-19 patients (11 female and 20 male; median age 62; age range 28-78) admitted in late January and February of 2022. These included 15 unvaccinated patients. Additionally, 8 patients were vaccinated with two doses of the Pfizer/BioNTech BNT16b2 vaccine (n = 4) or Moderna mRNA-1273 vaccine (n = 4), and sample collection occurred 5-11 months (median 9 months) after 2nd vaccine dose. Finally, 8 patients were vaccinated with three doses of the Pfizer/BioNTech BNT162b2 vaccine and sample collection occurred 2-6 months (median 5 months) after booster vaccine administration.

Cell lines and maintenance

HEK293T (ATCC CRL-11268, CVCL_1926), HEK293T-ACE2 (BEI NR-52511) cells were maintained in DMEM (Gibco, 11965-092) supplemented with 10% FBS (Sigma, F1051) and 1% penicillin-streptomycin (HyClone, SV30010).

Plasmids

We utilized a previously reported pNL4-3-inGluc lentivirus vector which is based on ΔEnv HIV-1 and bears a *Gaussia* luciferase reporter gene that is expressed in virus target cells but not virus producing cells[1,2]. Additionally, SARS-CoV-2 variant spike constructs with N- and C-terminal flag tags were produced and cloned into a pcDNA3.1 vector by GenScript Biotech

(Piscataway, NJ) using KpnI and BamHI restriction enzyme cloning. The specific amino acid changes for the BA.3 construct were as follows: A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212, ins214EPE, S477N, T478K, E484A, Q493R, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, and N969K. The specific amino acid changes for the Deltacron construct were as follows: T19R, A27S, T95I, G142D, E156G, Δ157-158, Δ211, L212I, ins214EPE, 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, and L981F.

Pseudotyped lentivirus production and virus neutralization assay

Lentiviral pseudotypes were produced as previously reported[3]. Briefly, HEK293T cells were transfected with pNL4-3-inGluc and spike construct in a 2:1 ratio using polyethylenimine transfection. Virus was harvested 24, 48, and 72 hrs after transfection. Relative virus titers were determined by infection of HEK293T-ACE2 cells, and *Gaussia* luciferase activity was assessed 48 hrs after infection by combining cell culture media with *Gaussia* luciferase substrate (0.1 M Tris pH 7.4, 0.3 M sodium ascorbate, 10 μM coelenterazine). Luminescence was immediately measured by a BioTek Cytation5 plate reader.

Pseudotyped lentivirus neutralization assays were performed as previously described[2-4]. Briefly, HCW serum or patient sera was 4-fold serially diluted and equal amounts of infectious SARS-CoV-2 variant pseudotyped virus was added to the diluted serum. Final dilutions of 1:1280, 1:5120, 1:20480, and no serum control were used for Delta-wave ICU patient plasma to avoid Triton X-100 toxicity, while final dilutions of 1:80, 1:320, 1:1280, 1:5120, 1:20480, and no serum control were used for the HCWs and Omicron-wave patients. Virus and serum were incubated for

1 hr at 37°C and then transferred to HEK293T-ACE2 cells for infection. *Gaussia* luciferase activity was determined 48 and 72 hrs after infection by combining 20 μL or cell culture media with 20 μL of *Gaussia* luciferase substrate. Luminescence was immediately measure by a BioTek Cytation5 plate reader. NT_{50} values were determined by least-squares-fit, non-linear regression in GraphPad Prism 5 (San Diego, CA) and presented as geometric means.

Statistics

Statistical analysis was performed in GraphPad Prism 9. Statistical analysis was performed using log_{10} transformed NT_{50} values to better approximate normality. Comparisons between multiple groups were made using a one-way repeated measures ANOVA with Bonferroni post-test (Fig. 1A-D, Fig. S2A and C, and Fig. S3A and D) or two-way repeated measures ANOVA with Bonferroni post-test (Fig. S3C and F).

Acknowledgements

We thank the NIH AIDS Reagent Program and BEI Resources for supplying important reagents that made this work possible. We thank the Clinical Research Center/Center for Clinical Research Management of The Ohio State University Wexner Medical Center and The Ohio State University College of Medicine in Columbus, Ohio, specifically Francesca Madiai, Dina McGowan, Breona Edwards, Evan Long, and Trina Wemlinger, for logistics, collection and processing of samples. In addition, we thank Sarah Karow, Madison So, Preston So, Elizabeth Schwartz, Daniela Farkas, and Finny Johns in the clinical trials team of The Ohio State University for sample collection and other supports.

Author Contributions

S.-L.L. conceived and directed the project. J.P.E. contributed the majority of the experimental work, data processing, and drafting of the manuscript. P. Q., C.Z., and Y.-M. Z. aided in experimental work and provided valuable discussion. C.C., J.S.B., G.L., R.M., R.J.G. provided clinical samples. J.P.E. and S.-L.L. wrote the paper. P.M. facilitated shipping of the Omicron construct. L.J.S., E.M.O., P.M., and R.J.G. provided insightful discussion and revision of the manuscript.

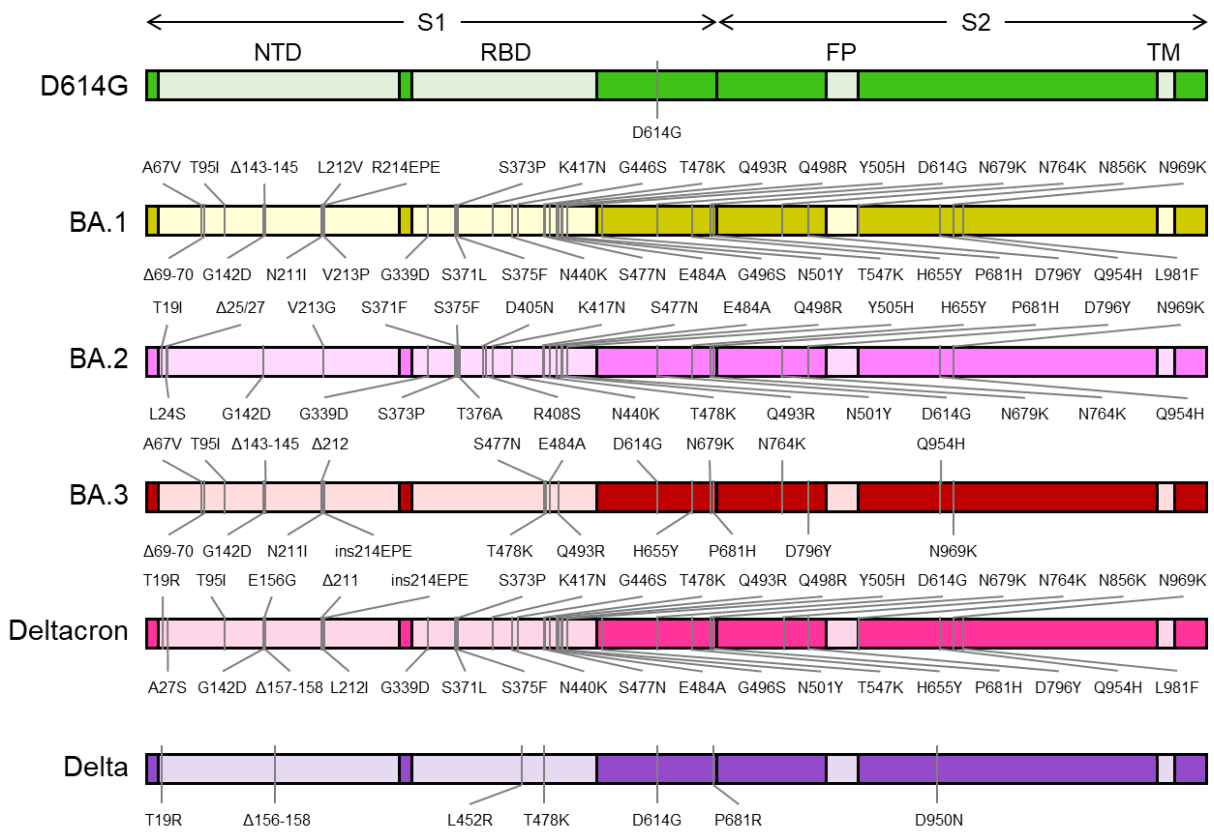

Figure S1: Variant SARS-CoV-2 Spike Constructs. Shown is a schematic of the SARS-CoV-2 spike constructs used for pseudotyped lentivirus production. The S1 and S2 subunits as well as the N-Terminal Domain (NTD), Receptor Binding Domain (RBD), Fusion Peptide (FP), and Transmembrane Domain (TM) are indicated. The specific constellation of mutations used for each variant is also indicated.

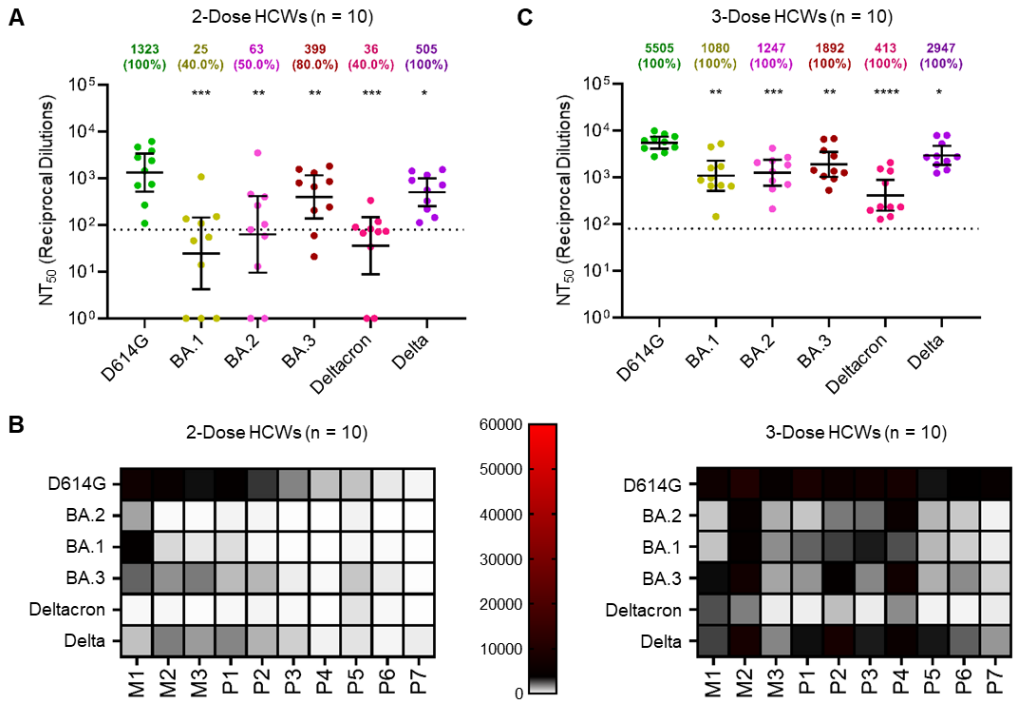

Figure S2: Neutralization resistance to vaccinated and boosted HCW sera for the Omicron variant sub-lineages and the Delta variant. (**A**) nAb titers against D614G, BA.1, BA.2, BA.3, Deltacron, and Delta pseudotyped virus are displayed for 10 HCW samples following 2 mRNA vaccine doses. (**B**) Heatmaps of nAb titers against HCWs following 2 or 3 mRNA vaccine doses and identified as "M" for Moderna mRNA-1273 vaccinated or "P" for Pfizer/BioNTech BNT162b2 vaccinated. (**C**) nAb titers are displayed for 10 HCW samples following 3 mRNA vaccine doses. Geometric mean NT_{50} values are displayed at the top of plots along with the percentage of patients with nAb titers above the limit of detection indicated by the dotted lines ($NT_{50} = 80$). Bars represent geometric means with 95% confidence interval. Statistical significance relative to D614G was determined by one-way (A and C) repeated measures ANOVA with Bonferroni's multiple testing correction. P-values are represented as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Data on BA.1, BA.2, and Delta is reproduced from our prior study[5] for convenience.

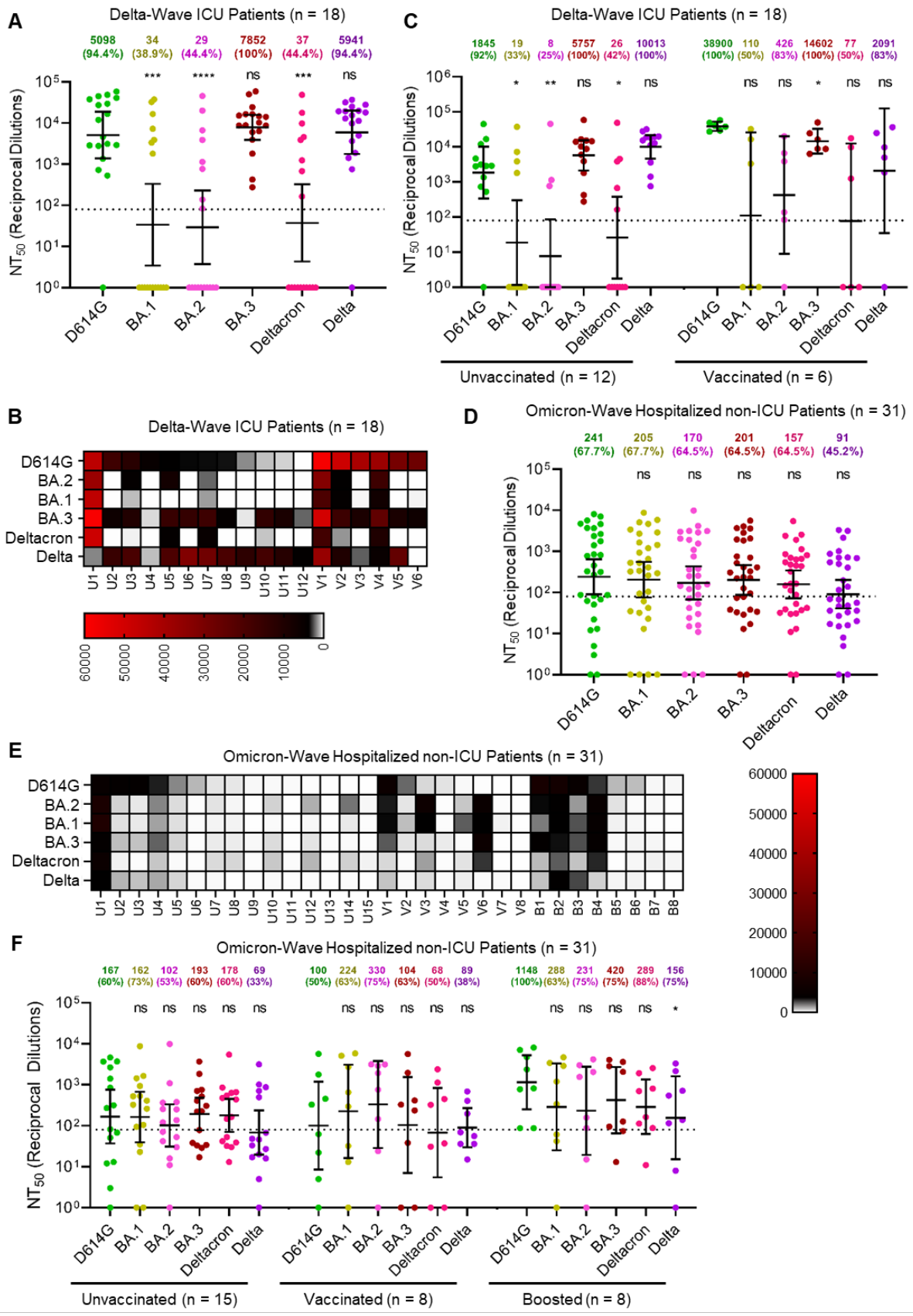
A
Delta-Wave ICU Patients (n = 18)
5098 (94.4%)
34 (38.9%)
29 (44.4%)
7852 (100%)
37 (44.4%)
5941 (94.4%)
***
****
ns
***
ns
NT50 (Reciprocal Dilutions)
D614G
BA.1
BA.2
BA.3
Deltacron
Delta
C
Delta-Wave ICU Patients (n = 18)
1845 (92%)
19 (33%)
8 (25%)
5757 (100%)
26 (42%)
10013 (100%)
38900 (100%)
110 (50%)
426 (83%)
14602 (100%)
77 (50%)
2091 (83%)
Unvaccinated (n = 12)
Vaccinated (n = 6)
B
Delta-Wave ICU Patients (n = 18)
D
Omicron-Wave Hospitalized non-ICU Patients (n = 31)
241 (67.7%)
205 (67.7%)
170 (64.5%)
201 (64.5%)
157 (64.5%)
91 (45.2%)
E
Omicron-Wave Hospitalized non-ICU Patients (n = 31)
F
Omicron-Wave Hospitalized non-ICU Patients (n = 31)
167 (60%)
162 (73%)
102 (53%)
193 (60%)
178 (60%)
69 (33%)
100 (50%)
224 (63%)
330 (75%)
104 (63%)
68 (50%)
89 (38%)
1148 (100%)
288 (63%)
231 (75%)
420 (75%)
289 (88%)
156 (75%)
Unvaccinated (n = 15)
Vaccinated (n = 8)
Boosted (n = 8)

Figure S3: Neutralization resistance to Delta-wave and Omicron-wave patient sera for the Omicron variant sub-lineages and the Delta variant. (**A**) nAb titers against D614G, BA.1, BA.2, BA.3, Deltacron, and Delta pseudotyped virus are displayed for 18 ICU patient samples collected during the Delta-wave of the pandemic. (**B**) Heatmaps of nAb titers against Delta-wave ICU patients identified as "U" for unvaccinated and "V" for vaccinated. (**C**) Delta-wave patient nAb titers are divided by vaccination status. (**D**) nAb titers are displayed for 31 hospitalized non-ICU patients collected during the Omicron-wave of the pandemic. (**E**) Heatmaps of nAb titers against Omicron-wave hospitalized non-ICU patients identified as "U" for unvaccinated, "V" for vaccinated, and "B" for vaccinated and boosted. (**F**) Omicron-wave patient nAb titers are divided by vaccination status. Geometric mean NT_{50} values are displayed at the top of plots along with the percentage of patients with nAb titers above the limit of detection indicated by the dotted line ($NT_{50} = 80$). Bars represent geometric means with 95% confidence interval. Statistical significance was determined by one-way (A and D) or two-way (C and F) repeated measures ANOVA with Bonferroni's multiple testing correction. P-values are represented as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, and ns for not significant. Data on BA.1, BA.2, and Delta is reproduced from our prior study[5] for convenience.

	Vaccinated HCW (n = 10)	Delta-Wave ICU Patients (n = 18)	Omicron-Wave Hospitalized Non-ICU Patients (n = 31)
Sex [n (% of Total)]			
Female	4 (40.0%)	6 (33.3%)	11 (35.5%)
Male	6 (60.0%)	12 (66.6%)	20 (64.5%)
Age in Years at Sample Collection [Median (Range)]	37.5 (29-48)	60 (22-87)	62 (28-78)
Sample Collection Window	Jan. 2021 - March 2021, Oct. 2021 - Nov. 2021	Aug. 2021 - Dec. 2021	Feb. 2022- March 2022
Vacc Type [n (% of Total)]			
Moderna 2-Dose	3 (30.0%)	na	4 (12.9%)
Moderna 3-Dose	3 (30.0%)	1	na
Pfizer 2-Dose	7 (70.0%)	4 (22.2%)	4 (12.9%)
Pfizer 3-Dose	7 (70.0%)	na	8 (25.8%)
J&J 1-Dose	na	1	na
Sample Collection Timing [Median (Range)]			
Days Post 1st Dose for Recipients of 1 Dose	na	141	na
Days Post 2nd Dose for Recipients of 2 Doses	26.5 (22-28)	255 (204-254)	274.5 (149-328)
Days Post 3rd Dose for Recipients of 3 Doses	15 (7-80)	12	158 (64-183)
Prior COVID-19 Confirmed by PCR [n (% of Total)]	1 (10%)	dnc	dnc

Table S1: Demographic and sample collection information for HCW and COVID-19 patient cohorts. Displayed are summary data for the HCW samples collected post-second mRNA vaccine dose and post booster mRNA vaccine dose. Additionally, summary information is provided for the Delta-Wave and Omicron-Wave COVID-19 patients. Where it appears, na indicates “not applicable” and dnc indicates “data not collected”.

Supplementary References

1. Goerke AR, Loening AM, Gambhir SS, Swartz JR. Cell-free metabolic engineering promotes high-level production of bioactive Gaussia princeps luciferase. Metab Eng 2008;10:187-200.

2. Zeng C, Evans JP, Pearson R, et al. Neutralizing antibody against SARS-CoV-2 spike in COVID-19 patients, health care workers, and convalescent plasma donors. JCI Insight 2020;5.

3. Evans JP, Zeng C, Carlin C, et al. Neutralizing antibody responses elicited by SARS-CoV-2 mRNA vaccination wane over time and are boosted by breakthrough infection. Sci Transl Med 2022;14:eabn8057.

4. Zeng C, Evans JP, Faraone JN, et al. Neutralization of SARS-CoV-2 Variants of Concern Harboring Q677H. mBio 2021;12:e0251021.

5. Evans JP, Zeng C, Qu P, et al. Neutralization of SARS-CoV-2 Omicron sub-lineages BA.1, BA.1.1, and BA.2. Cell Host & Microbe 2022; In Press.