Supplementary Appendix

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

Supplementary Appendix

CONTENTS

Neutralization of SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants

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Supplementary Methods

Vaccinated and hospitalized/ICU patient cohorts

Two-dose vaccinated health care worker (HCW) samples were collected under approved IRB protocols (2020H0228 and 2020H0527). Demographic information was self-reported, and all subjects provided informed consent. Sera were collected 3-4 weeks post-second vaccine dose for 15 HCWs (7 female and 8 male; median age 37; age range 31-56), which included 4 Moderna mRNA-1273 and 11 Pfizer/BioNTech BNT162b2 vaccinated HCWs.

Boosted HCW samples were additionally collected under the same IRB protocols. Demographic information was self-reported, and all subjects provided informed consent. Sera were collected 1-11 weeks post-homologous booster vaccine dose for 15 HCWs (7 female and 8 male; median age 37; age range 22-48), which included 4 Moderna mRNA-1273 and 11 Pfizer/BioNTech BNT162b2 vaccinated HCWs. Note that 12 HCWs had both post-second dose samples and post-booster dose samples analyzed.

Delta-wave ICU patient samples were collected under an approved IRB protocol (2020H0175). Demographic information was self-reported, and all subjects provided informed consent. 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 confirmed 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 the variant. In total, 5/18 patients were confirmed to have been infected with Delta. 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). Demographic information was self-reported, and all subjects provided informed consent. Sera were collected 1-8 days after hospitalization for 30 COVID-19 patients (11 female and 19 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, 7 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

All cell lines were maintained in 5% CO_2 and at 37°C. HEK293T (ATCC CRL-11268, RRID: CVCL_1926) and HEK293T-ACE2 (BEI NR-52511, RRID: CVCL_A7UK) cells were maintained in DMEM (Gibco, 11965-092) supplemented with 10% FBS (Sigma, F1051) and 1% penicillin-streptomycin (HyClone, SV30010). CaLu-3 cells (RRID: CVCL_0609) were maintained in EMEM (ATCC 30-2003) supplemented with 10% FBS and 1% penicillin-streptomycin.

Plasmids

Pseudotyped virus was produced using a pNL4-3-inGluc lentivirus vector which contains a ΔENV HIV-1 backbone bearing a *Gaussia* luciferase reporter gene[1,2]. GenScript Biotech (Piscataway, NJ) produced and cloned SARS-CoV-2 spike constructs with N- and C-terminal flag tags using KpnI and BamHI restriction enzyme cloning into a pcDNA3.1 vector.

Pseudotyped lentivirus production and infectivity

Lentiviral pseudotypes were produced as previously described[3]. pNL4-3-inGluc and spike constructs were transfected into HEK-293T cells in a 2:1 ration using polyethylenimine transfection. Pseudotyped virus was harvested 48 and 72 hrs after transfection. Pseudotyped virus for each SARS-CoV-2 spike, produced in parallel, were used to infect target HEK293T-ACE2 or CaLu-3 cells. *Gaussia* luciferase activity was assessed 48 hrs after infection by combining *Gaussia* luciferase substrate (0.1M Tris pH 7.4, 0.3M sodium ascorbate, 10 μM coelenterazine) with cell culture media. Luminescence was immediately measured by a BioTek Cytation5 plate reader.

Virus neutralization assay

Pseudotyped lentivirus neutralization assays were performed as previously described[2–5]. HCW serum or hospitalized/ICU patient plasma was serially diluted. Equal amounts of infectious pseudotyped lentivirus bearing SARS-CoV-2 S protein were added to the diluted serum, resulting in final dilutions of 1:80, 1:320, 1:1280, 1:5120, 1:20480, and no serum control for HCW and

BA.1-wave samples. Delta-wave samples were treated with Triton X-100 to inactivate virus. To prevent Triton X-100 toxicity from impacting the assay, final dilutions for Delta-wave serum were 1:1280, 1:2560, 1:5120, 1:10240, and 1:20480. Virus and serum were incubated for 1 hr at 37°C and then added to HEK293T-ACE2 cells for infection by neutralized virus. Gaussia luciferase output by infected cells was measured 48 and 72 hrs after infection by taking 20 μL of infected cell culture media and adding 20 μL of Gaussia luciferase substrate. Luminescence was measured immediately using a BioTek Cytation5 plate reader. NT_{50} values were determined by least-squares-fit, non-linear regression in GraphPad Prism 9 (San Diego, CA). Heat maps with NT_{50} generated by GraphPad Prism 9.

Quantification and Statistical Analysis

All statistical analysis was performed using GraphPad Prism 9 and are described in the figure legends. NT_{50} values were determined by least-squares fit non-linear regression in GraphPad Prism 9. Throughout, n refers to subject number and bars represent either ± standard deviation (**Fig S1 B and C**) or geometric means ± 95% confidence intervals (**Fig 1A-D, S2B and D, S3B and D**)**.** Generally, comparisons between multiple groups were made using a one-way repeated measures ANOVA with Bonferroni post-test (**Fig 1A-D, Fig S1B-C**) or two-way repeated measures ANOVA with Bonferroni post-test (**Fig S2B and D, Fig S3B and D**). Analysis of the influence of sex on neutralization capacity could not be performed due to small sample size—confounding variables including vaccination status, vaccine type, and time since vaccination would skew such an analysis. Infectivity was quantified using luminescence readings measured by a BioTek Cytation5 plate reader. Three readings were taken and averaged for each of the three replicates.

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Author Contributions

S.-L.L. conceived and directed the project. P.Q. performed most of the experiments. J.N.F, X.Z., J.P.E. assisted in experiments and contributed data processing and analyses. C.C., J.S.B., G.L., R.K.M., R.J.G. provided clinical samples. P.Q., J.N.F., J.P.E., and S.-L.L. wrote the paper. P.J.M. facilitated shipping of the original Omicron construct. Y.-M.Z, L.J.S., E.M.O., P.J.M., and R.J.G. provided insightful discussion and revision of the manuscript.

	2-dose HCWs (n=15)	3-dose HCWs (n=15)	Delta-Wave ICU Patients (n=18)	Omicron-Wave Non-ICU Patients (n=30)
Age in Years at Sample Collection [Median (Range)]	37 (31-56)	37 (22-48)	60 (22-87)	62 (28-78)
Gender [n (% of Total)]				
Male	8 (53.3%)	8 (53.3%)	12 (66.7%)	19 (63.3%)
Female	7 (46.7%)	7 (46.7%)	6 (33.3%)	11 (36.7%)
Sample Collection Window	Jan.2021-Mar. 2021,	Oct. 2021-Nov.2021	Aug. 2021-Dec. 2021	Feb. 2022-Mar. 2022
Type of Vaccine [n (% of Total)]				
2-dose Moderna	4 (36.4%)	na	na	4 (13.3%)
2-dose Pfizer	11 (63.6%)	na	4 (22.2%)	4 (13.3%)
3-dose Moderna	na	4 (36.4%)	1 (5.6%)	na
3-dose Pfizer	na	11(63.6%)	na	7 (23.3%)
1-dose J&J	na	na	1 (5.6%)	na
Sample Collection Timing [Median (Range)]				
Days post 1st dose for Recipients of one dose	na	na	141	na
Days post 2nd dose for Recipients of two doses	27 (22-31)	na	255 (204-254)	313 (149-367)
Days post 3rd dose for Recipients of three doses	na	18 (5-80)	12	158 (64-183)
Prior SARS-CoV-2 Infection Confirmed by PCR [n (% of Total)]	1 (6.7%)	2 (13.3%)	dnc	dnc

Table S1: Demographic and sample collection information of HCWs and Delta- or Omicron-wave patients. Summary information for the HCW sera samples collected post 2-dose and 3-dose of mRNA is shown. In addition, summary information of the Delta-wave ICU and Omicron-wave

non-ICU COVID-19 patients is also provided. na means “not applicable”, and dnc indicates “data not collected”.

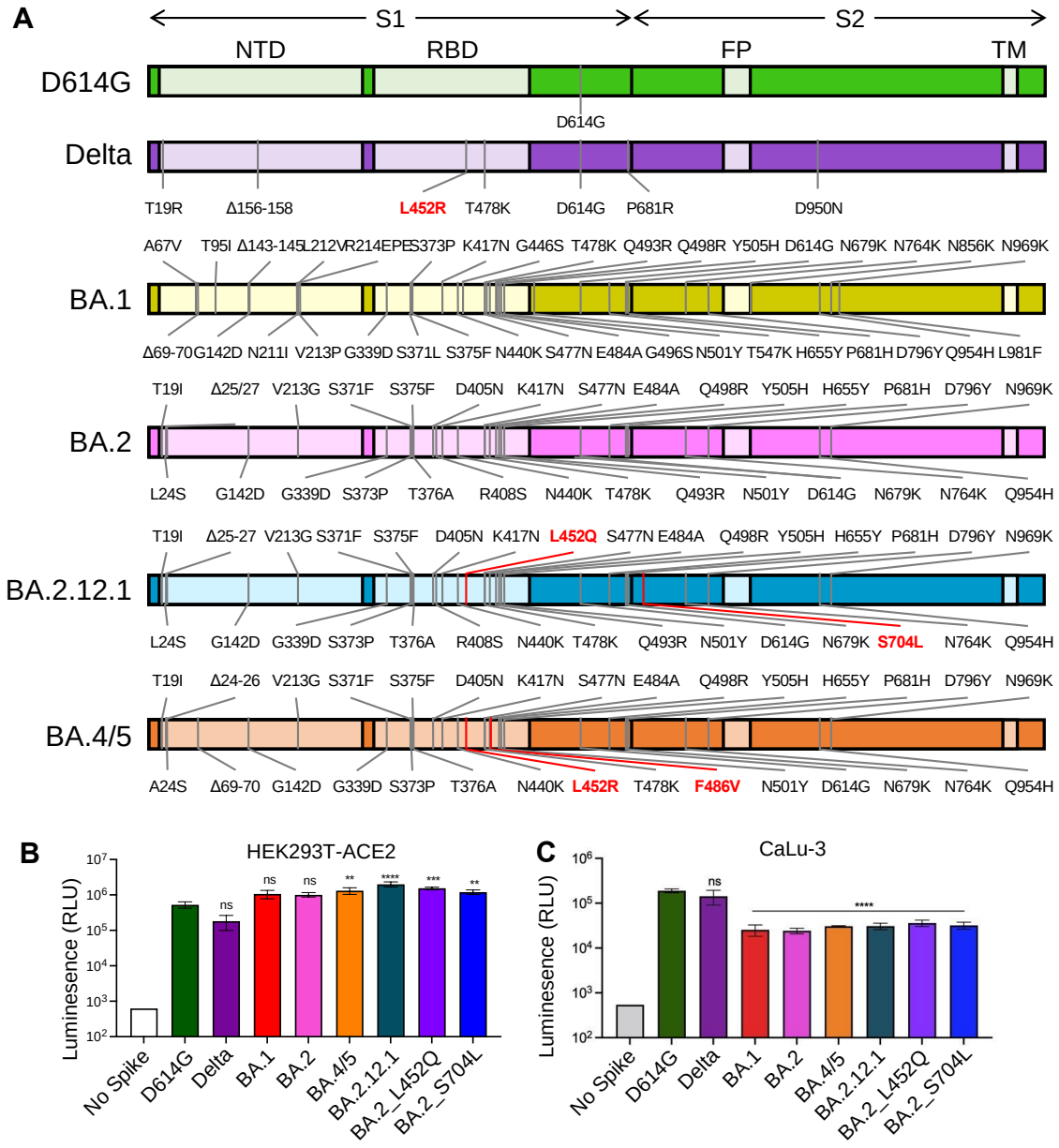

Figure S1. BA.4/5 and BA.2.12.1 subvariants are more infectious in HEK293T-ACE2 cells but exhibit comparably low infectivity in CaLu-3 cells relative to BA.1 and BA.2. (A) Schematic of SARS-CoV-2 variant spike proteins used for pseudotyping HIV-1 lentiviral vector

and neutralization assays. **(B)** Infectivity of pseudotyped viruses in HEK293T cells stably expressing ACE2 (HEK293T-ACE2). **(C)** Infectivity of pseudotyped lentivirus in human lung epithelia-derived CaLu-3 cells. Bars in **(B)** and **(C)** represent means ± standard deviation, and significance is determined by one-way repeated measures ANOVA with Bonferroni's multiple testing correction. Results of at least 3 independent experiments are averaged and shown. P-values are represented as **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, ns, not significant.

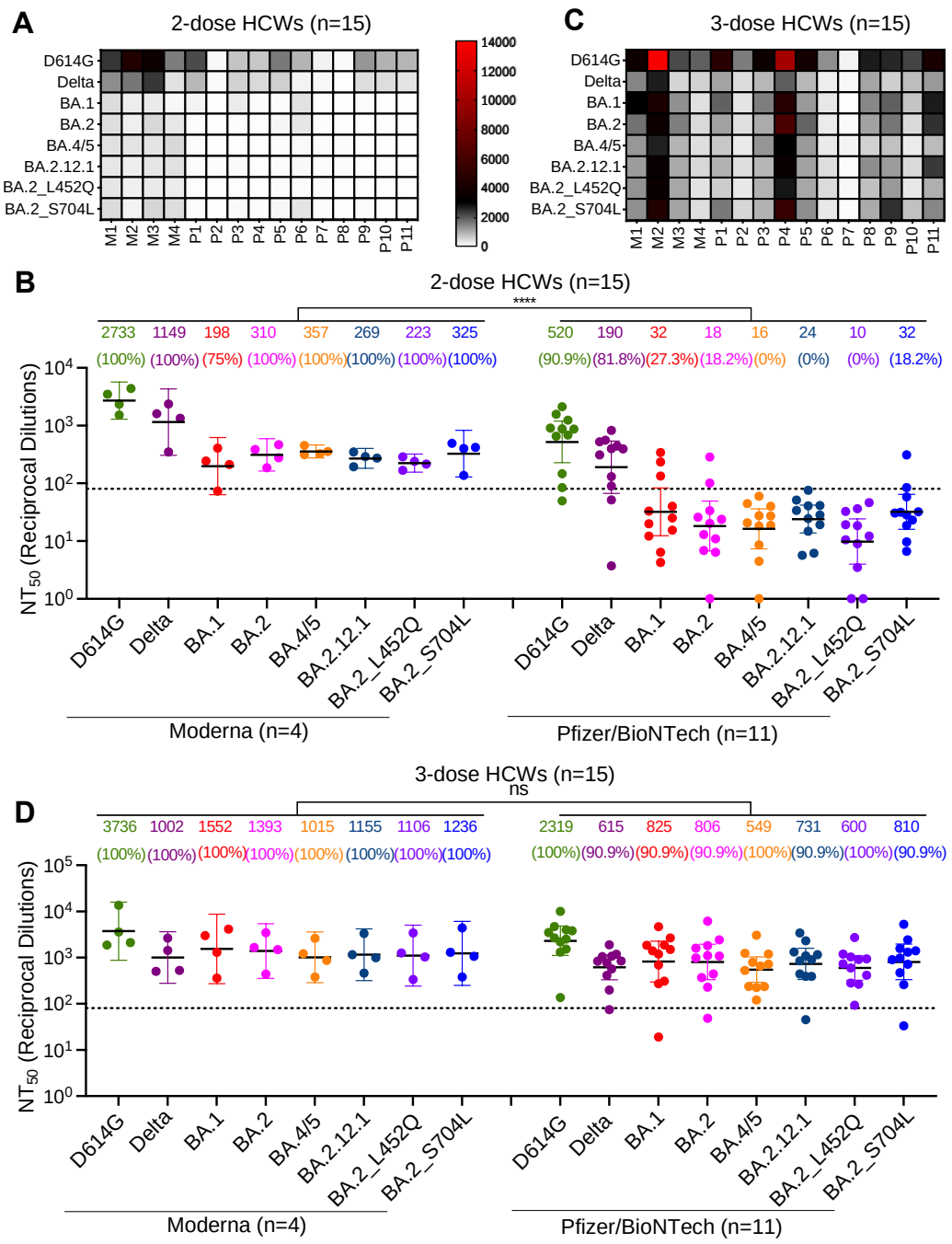

Figure S2. Heterogeneity of neutralization resistance by two-dose and 3-dose HCWs based on individual immunity and vaccine type. (A) Heatmap depicting NT_{50} values of HCWs that received 2-doses of either Moderna mRNA-1273 (“M”) or Pfizer/BioNTech BNT162b2 (“P”) vaccines; corresponding to data in Fig. 1B. **(B)** NT_{50} values for HCWs who received either two

doses of Moderna mRNA-1273 (n=4) or Pfizer/BioNTech BNT162b2 (n=11) plotted by vaccine type. **(C)** Heatmap depicting NT_{50} values of HCWs that received 3-doses of either Moderna or Pfizer mRNA vaccines; corresponding to data in Fig. 1C. **(D)** NT_{50} values for HCWs who received a homologous booster of Moderna (n=4) or Pfizer (n=11) vaccines plotted by vaccine type. Geometric mean NT_{50} values are depicted at the top of graphs **(B and D)** along with the percentage of subjects with NT_{50} values above the limit of detection (NT_{50} = 80, dotted line) . Bars represent geometric mean ± 95% confidence interval and significance was determined by two-way repeated measures ANOVA with Bonferroni's multiple testing correction. P values are represented as ****$p < 0.0001$, ns, not significant.

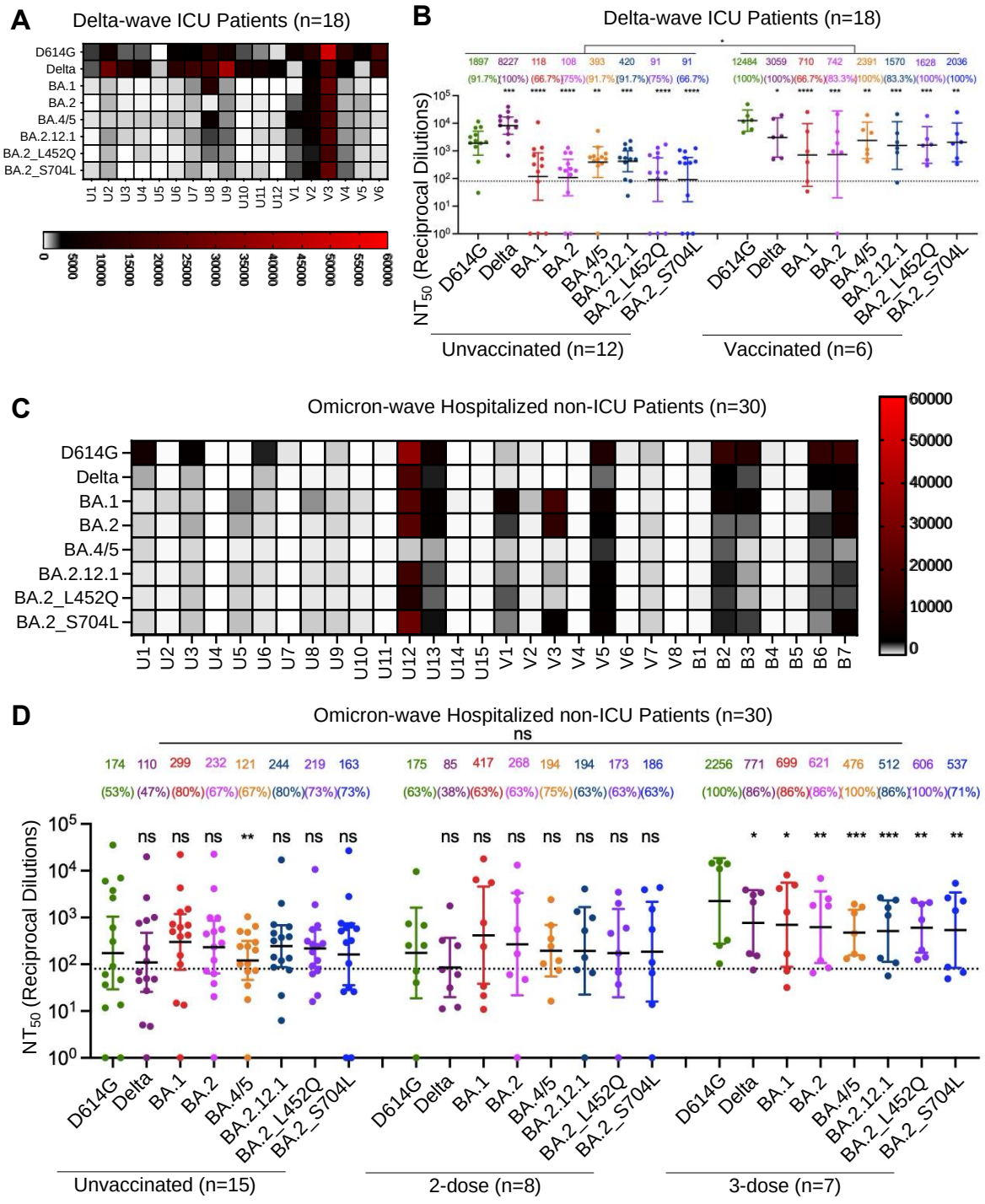

Figure S3. Vaccinated Delta-wave sera more strongly neutralizes BA.4/5 and BA.2.12.1 compared to BA.1 and BA.2. (A) A heatmap depicting individual patient NT_{50} values from Delta

wave patients. Patients are identified by "U" for unvaccinated and "V" for vaccinated; corresponding to data in Fig. 1D. **(B)** NT_{50} data from **Fig. 1C** is plotted by vaccination status with unvaccinated (n=12) and vaccinated (n=6) individuals. **(C)** A heatmap showing patient NT_{50} values against each variant for BA.1-wave patients. Patients are identified by "U" and "V" as described for **(A)** and "B" for 3-dose boosted samples; corresponding to data in Fig. 1E. **(D)** NT_{50} data from **Fig 1D** is plotted by vaccination status with unvaccinated (n=15), 2-dose (n=8), and 3-dose (n=7) individuals depicted. Geometric mean NT_{50} values are displayed at the top of plots **(B and D)** along with the percentage of patients with NT_{50} values above the limit of detection (NT_{50} = 80, dotted line); bars represent geometric mean ± 95% confidence interval and significance was determined by two-way 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, ns, not significant.

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