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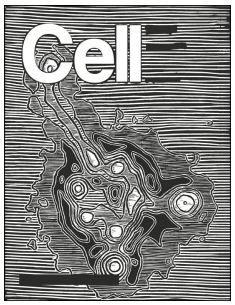

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PII: S0092-8674(22)00329-4

DOI: https://doi.org/10.1016/j.cell.2022.03.019

Reference: CELL 12407

To appear in: *Cell*

Received Date: 26 January 2022

Revised Date: 15 February 2022

Accepted Date: 14 March 2022

Please cite this article as: Servellita, V., Syed, A.M., Morris, M.K., Brazer, N., Saldhi, P., Garcia-Knight, M., Sreekumar, B., Khalid, M.M., Ciling, A., Chen, P.-Y., Kumar, G.R., Gliwa, A.S., Nguyen, J., Sotomayor-Gonzalez, A., Zhang, Y., Frias, E., Prostko, J., Hackett Jr., J., Andino, R., Wadford, D.A., Hanson, C., Doudna, J., Ott, M., Chiu, C.Y., Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants, *Cell* (2022), doi: https://doi.org/10.1016/j.cell.2022.03.019.

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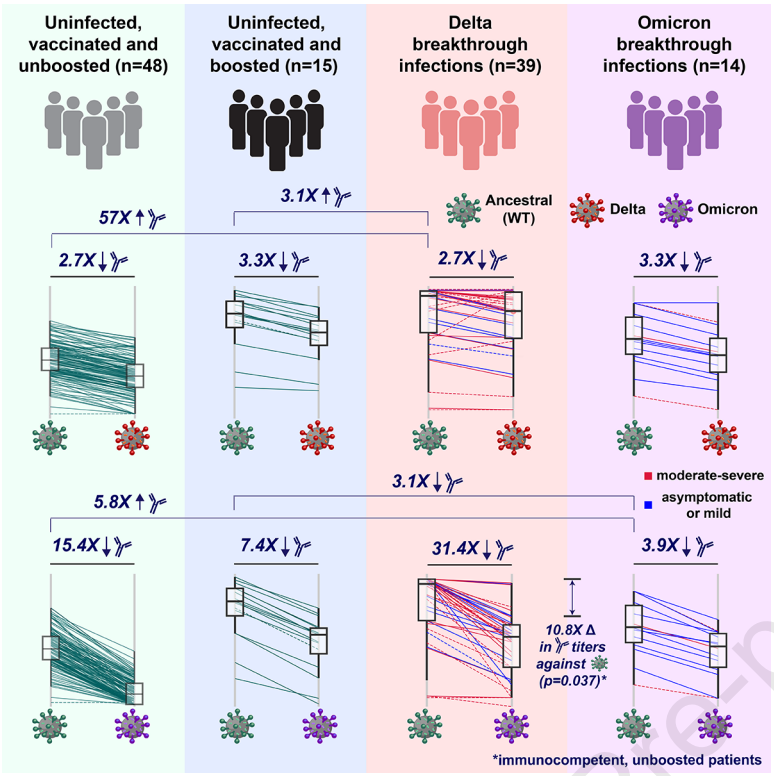

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Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants

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Key words: SARS-CoV-2, COVID-19, antibody neutralization, Omicron variant, B.1.1.529, Delta variant, B.1.617.2, breakthrough infection, boosted breakthrough infection, variant severity, virus-like particle (VLP), quantitative antibody assay, variant of concern, pseudovirus infectivity studies, humoral immunity

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Summary

Virus-like particle (VLP) and live virus assays were used to investigate neutralizing immunity against Delta and Omicron SARS-CoV-2 variants in 259 samples from 128 vaccinated individuals. Following Delta breakthrough infection, titers against WT rose 57-fold and 3.1-fold compared to uninfected boosted and unboosted individuals, respectively, versus only a 5.8-fold increase and 3.1-fold decrease for Omicron breakthrough infection. Among immunocompetent, unboosted patients, Delta breakthrough infections induced 10.8-fold higher titers against WT compared to Omicron (p=0.037). Decreased antibody responses in Omicron breakthrough infections relative to Delta were potentially related to a higher proportion of asymptomatic or mild breakthrough infections (55.0% versus 28.6%, respectively), which exhibited 12.3-fold lower titers against WT compared to moderate-severe infections (p=0.020). Following either Delta or Omicron breakthrough infection, limited variant-specific cross-neutralizing immunity was observed. These results suggest that Omicron breakthrough infections are less immunogenic than Delta, thus providing reduced protection against reinfection or infection from future variants.

(149 words)

Introduction

Variants of concern have emerged throughout the COVID-19 (Coronavirus Disease 2019) pandemic, causing multiple waves of infection (Dyson et al. 2021). The Omicron (B.1.1.529) variant has been shown to be highly transmissible with decreased susceptibility to therapeutic monoclonal antibodies and neutralizing antibodies conferred by vaccination or prior infection (Flemming 2022; VanBlargan et al. 2022). These characteristics are likely due to more than 30 mutations in the spike protein (Cao et al. 2021). Omicron has spread to become the predominant circulating lineage worldwide as of February 2022 amidst lower background levels of Delta (B.1.617.2) variant infection (Gangavarapu et al., 2020). The surge in Omicron led to a temporary reinstatement of public health interventions to reduce transmission and a renewed focus on vaccination efforts, although evidence to date suggests that Omicron causes less severe disease than other

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) variants (Wolter et al. 2022; Davies et al. 2022).

The development of neutralizing antibody responses in Delta and Omicron breakthrough infections remains largely unexplored. Here we evaluated neutralizing antibody titers against Delta, Omicron, and ancestral WA-1 wild-type (WT) viruses in fully vaccinated individuals, some of whom were boosted and/or subsequently developed a SARS-CoV-2 breakthrough infection. Neutralization was assessed using two independent assays that incorporated either SARS-CoV-2 virus-like particles (VLP) containing all the Omicron mutations in the spike, nucleocapsid, matrix, and fusion structural proteins (Syed et al. 2021; Syed et al. 2022) or live viruses (Servellita et al. 2022). We also correlated neutralization results with quantitative spike antibody levels and investigated relationships between neutralizing antibody titers and infecting variant or clinical severity associated with the breakthrough infection.

Results

Neutralizing antibody levels in vaccinated individuals wane over time and are reduced against the Delta and Omicron variants

VLP and live virus neutralization assays were performed in parallel on 143 plasma samples collected from 68 subjects enrolled in a prospectively enrolled longitudinal cohort (the UMPIRE, or "UCSF eMPloyee and community Immune REsponse study"), 15 (22.1%) of whom had received a booster and none of whom were previously infected **(Table S1)**. We chose available samples from the earliest and most recent time points collected from each subject ≥14 days after the last vaccine dose for neutralization testing. Sample collection dates for fully vaccinated, unboosted individuals (n=48) ranged from 14 to 305 days (median = 91 days) following completion of the primary series of 2 doses for an mRNA vaccine (BNT162b2 from Pfizer or mRNA-1273 from Moderna) or 1 dose of the adenovirus vector vaccine (Ad26.COV2.S from Johnson and Johnson); for boosted individuals (n=15), collection dates ranged from 2 to 74 days (median = 23 days) following the booster

dose. Overall, median neutralizing antibody titers were 2.5-fold lower using live viruses compared to VLPs **(Figure S1).**

In unboosted vaccinated individuals, median VLP neutralizing antibody titers to Delta and Omicron relative to WT virus, expressed as NT50 ("neutralization titers 50"), or titers that neutralized 50% of VLP activity, were reduced 2.7-fold (262 → 96) and 15.4-fold (262 → 17), respectively **(Figure 1A and B, left).** In comparison, live virus neutralization titers against Delta and Omicron were reduced at least 3.0-fold (120 → <40) **(Figure 1A and 1B, right)**, with the lower fold reduction for Omicron accounted for by the higher limit of detection (LOD) for the live virus (NT50 = 40) compared to VLP neutralization (NT50 = 10) assay. Using VLPs, the proportion of individuals with neutralizing antibodies against Omicron above an NT50 cutoff of 40 was ~20%, as compared to ~80% and ~95% for Delta and WT, respectively **(Figure 1C, left).** The corresponding proportions using live viruses were ~5%, ~45%, and ~75% for Omicron, Delta, and WT, respectively **(Figure 1C, right).** In boosted individuals, VLP titers against WT were 18-fold higher (4,727 versus 262) than in unboosted individuals **(Figure 1A, B, D, and E, left),** and decreases in titers against Delta and Omicron relative to WT were more modest at 3.3-fold and 7.4-fold, respectively **(Figure 1D and E, left).** The increase in VLP neutralization titers corresponded to >93% of boosted individuals having neutralizing antibodies against all 3 lineages above an NT50 cutoff of 40 **(Figure 1F, left).** In contrast, live virus neutralization titers in boosted individuals showed 21.4-fold lower titers (1,475 → 69) against Omicron relative to WT **(Figure 1E, right)**, with only ~62% of boosted individuals having neutralizing antibodies against Omicron **(Figure 1F, right).** Following vaccination, longitudinal median VLP neutralization titers against WT decreased by 93% (14-fold, 2,043 → 146), with relative decreases in titers against Delta and Omicron ranging from 2.9-4.7-fold and 12.2-43.5-fold, respectively, compared to WT **(Figure 1G).**

Breakthrough infection increases neutralizing antibody levels against WT and variant-specific immunity

To investigate neutralizing antibody responses and the extent of cross-neutralizing immunity, we analyzed plasma samples from 60 patients with confirmed SARS-CoV-2 breakthrough infections **(Table S1)**. Of the 60 cases, 28 and 20 were found to be associated with Delta and Omicron breakthrough infections, respectively, by viral whole-genome sequencing. For the remaining 12 cases, we were unable to confirm the lineage because of a lack of respiratory swab sample or insufficient viral genome coverage for definitive identification. Of the 12 cases, 11 were presumptively identified as Delta breakthrough cases because they were collected between July 30 and December 1, 2021, during a period when Delta accounted for 97.1 – 99.6% of the circulating lineages in California (CDPH 2022), and one sample was identified as presumptive Omicron since it was collected on January 10, 2022, when Omicron was the dominant lineage in California (97% of cases) (CDPH 2022). The 20 Omicron cases identified were of the BA.1 lineage. Of the 60 breakthrough cases, 34 (56.7%) were classified as moderate-severe COVID-19, 13 (21.7%) were boosted, and 14 (23.3%) were immunocompromised **(Table S1)**. The number of days between sample collection and symptom onset or PCR test positivity, whichever was earlier, ranged from 1 to 55 days (median = 14 days).

Using VLP assays, we found that patients with Delta breakthrough infections (n=39), 5 of whom were boosted, had higher median VLP neutralization titers against WT of 57-fold (14,835 versus 262) and 3.1-fold (14,835 versus 4,727) compared to those from unboosted and boosted individuals, respectively **(Figure 1A and B, left and Figure 2A).** In addition, neutralization titers against Delta rose to the same level as WT in the live virus assay **(Figure 2B, left).** Cross-neutralizing activity against Omicron was also observed but was limited as the 31.4-fold and >46.8-fold reductions in Omicron neutralization relative to WT for the VLP and live assays **(Figure 2A and B, middle)**, respectively, were comparable to those seen in uninfected, unboosted individuals (33.3-43.5-fold reductions) **(Figure 1G, 14-30 days and 30-60 days).** The proportion of Delta breakthrough individuals with neutralizing antibodies against Omicron above an NT50 cutoff of 40 was calculated at ~75% and ~43% for the VLP and live virus assays, respectively **(Figure 2A and B, right)**

Among the 21 total Omicron breakthrough infections in the study, plasma samples from 14 cases, 4 in boosted individuals, were available for both VLP and live virus neutralization studies. In contrast to Delta,

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Omicron breakthrough infections exhibited much smaller increases in neutralizing titers against WT, 5.8-fold (1,524 versus 262) compared to unboosted individuals and to about one-third of the titers achieved from boosting (1,524 versus 4,727) **(Figure 1A and C, left and 2C).** Neutralizing titers against Omicron in Omicron breakthrough individuals were 3.9-6.6-fold lower than WT **(Figure 2C and D, middle).** Omicron breakthrough infection resulted in ~85% **(Figure 2C, right)** and ~65% **(Figure 2D, right)** of individuals having neutralizing antibodies against Omicron above an NT50 cutoff of 40 for the VLP and live virus assays, respectively, approaching the proportion of those having neutralizing antibodies to Delta (~85% for both assays) **(Figure 2C and D, right).** In contrast, cross-neutralization against Delta in Omicron breakthrough infections was limited, with 3.3-fold and 2.2-fold reductions in titers for the VLP and live assays **(Figure 2C and D, left)**, respectively, comparable to those observed previously in uninfected vaccinated individuals (2.7-3.0 fold) **(Figure 1A).** Thus, for both Delta and Omicron breakthrough infections, the extent of conferred cross-neutralizing immunity beyond an increase in neutralization titers against WT was limited.

Next, a head-to-head comparison of neutralization titers from Omicron and Delta breakthrough infections was performed using available samples collected 4 to 32 days after symptom onset or PCR test positivity (n=55, 35 Delta and 20 Omicron out of 60 total breakthrough infections) (**Figure 3).** The cohorts were largely comparable, exhibiting no significant differences with respect to advanced age, sex, disease severity, immune status, and collection date relative to time of symptom onset or PCR test positivity **(Table 1).** Kernel density plots showed that available samples from Omicron breakthrough infections were collected a median 4 days earlier than Delta. These differences were not significant (p=0.34-0.38), and the distribution of Omicron cases was skewed toward later time points **(Figure 3A and B, left).** A significantly higher proportion of patients in the Omicron cohort had received a booster (**Table 1**, 40.0% versus 14.3%, p=0.048), which was expected given the later surge of Omicron (Gangavarapu, et al., 2020) and the higher level of antibody evasion associated with Omicron relative to Delta (Laurie, et al, 2022; Liu, et al., 2022).

Delta breakthrough infections resulted in 3.5-fold (19,806 versus 5,682, p=0.76) higher neutralization titers against WT compared to Omicron **(Figure 3A, middle).** This difference was not significant, likely

because of potential confounding factors such as immunocompromised state (**Table 1**, p=0.059) and having received a booster dose (**Table 1**, p=0.048). When only immunocompetent, unboosted patients were included in the analysis, Delta breakthrough infections had 10.8-fold (20,481 versus 1,905, p=0.037) higher neutralization titers against WT compared to Omicron **(Figure 3B, middle).** For both Delta and Omicron breakthrough infections, a rise in neutralization titers occurred typically within 7 days after symptom onset or PCR test positivity **(Figure 3C)**. The rate of rise in immunocompromised, unboosted patients was 1.4-fold higher (β, or slope coefficient of 551 versus 389) for Delta breakthrough infections compared to Omicron (**Figure 3C, insets).**

Increased clinical severity of the breakthrough infection is associated with higher neutralizing antibody titers

Visual inspection of the antibody neutralization plots revealed generally higher titers in moderate-severe compared to asymptomatic or mild infections, regardless of the infecting variant **(Figure 3A and B, middle).** Moderate-severe breakthrough infections from Delta or Omicron were found to elicit 5.0-fold higher neutralizing antibody titers (20,121 versus 3,982, p=0.20) compared to asymptomatic or mild infections **(Figure 3A, right).** When considering only the subset of immunocompetent, unboosted patients (**Figure 3B, right),** there were 12.3-fold higher neutralizing antibody titers against WT (20,481 versus 1,671, p=0.020).

Quantitative spike antibody assays show decreased correlation with and are less predictive of neutralizing activity against the Delta and Omicron variants

We compared VLP and live virus neutralization with results from a commercial FDA Emergency Use Authorization (EUA) authorized spike IgG quantitative assay that measures levels of antibodies against the WT (WA-1) RBD region of the SARS-CoV-2 spike protein **(Figure 4).** The results showed that neutralization and quantitative antibody titers rise in tandem ($p < 1.7x10^{-15}$ for all comparisons), although there was decreased correlation of neutralization and quantitative antibody titers with Omicron (Spearman's ρ=0.49-0.75) and Delta

(ρ=0.83-0.88) relative to WT (ρ=0.91-0.93). Of note, many cases of Delta breakthrough infection with low to moderate levels of spike IgG antibody failed to neutralize Omicron **(Figure 4B, bottom row).** Quantitative spike IgG titers of 10^3–10^4 **(Figure 4B, middle row)** and >10^5 **(Figure 4B, bottom row)** reliably predicted Delta and Omicron neutralization, respectively.

Discussion

Here we used VLP and live virus neutralization assays to investigate neutralizing antibody responses in 128 vaccinated individuals, both boosted and unboosted, and after Delta and Omicron vaccine breakthrough infections. Our results suggest that vaccine boosting and/or breakthrough infections confer broad hybrid immunity by increasing neutralizing antibody titers against WT to levels comparable to those achieved shortly after completion of a primary vaccine series and prior to waning, with higher relative immunity against the infecting variant. Notably, Delta-specific titers in Delta breakthrough infections rose to become comparable to levels against WT, while Omicron-specific titers in Omicron breakthrough infections rose to become comparable to levels against Delta. We also found that the magnitude of increase in neutralization titers against WT is greater with Delta than with Omicron breakthrough infections (10.8-fold, p=0.037) and for infections that are more clinically severe (12.3-fold, p=0.020).

Our results are consistent with those from studies by Wratil, et al (2022) and Walls, et al. (2022) that examined neutralizing responses in Delta and Omicron breakthrough infections (n=31) and Delta breakthrough infections (n=15), respectively, and found robust increases in antibody titers to WT and cross-neutralization of other variants. Interestingly, the study by Wratil, et al. (2022) also found that sera from Delta breakthrough infections cross-neutralized Omicron less well. Another study by Khan, et al. (2021) investigated the role that cross-neutralizing immunity plays in Omicron breakthrough infections. The investigators reported that sera from patients with Omicron breakthrough infections enhanced Delta virus neutralization to a limited extent (4.4-fold), but that immunity elicited against the specific infecting variant (Omicron) was higher (17.4-fold).

A few other published studies have looked at the effect of boosting on neutralization of Omicron. Pseudovirus studies from Laurie, et al. (2022) and Liu, et al. (2022) reported 4 to 8-fold and mean 6-fold reductions in neutralization titers, respectively, against Omicron in boosted individuals. These reductions are comparable to the 7.4-fold reduction that we observed using the VLP assay. However, these modest reductions are likely offset by the substantial increase in neutralizing antibody titers against WT conferred by the booster dose that we observed in the current study, which also has been reported by Gruell, et al. (2022). Taken together, these results indicate that booster immunization provides robust neutralizing immunity against the Omicron variant and highlight the importance of vaccine boosters in enhancing immunity to both existing and novel variants.

Our findings have implications regarding the likelihood that Omicron infections will provide mass immunization on the population level against SARS-CoV-2. Widespread infections from Omicron globally in both vaccinated and unvaccinated persons have been reported, although Omicron has been shown to cause milder disease with reduced risk of hospitalization and death relative to prior lineages (Wolter et al., 2022). In addition, epidemiologic data to date suggest that Omicron has outcompeted more pathogenic circulating variants such as Delta (Gangavarapu et al., 2020). These observations raise the prospect that Omicron may be a harbinger of the end of the pandemic as SARS-CoV-2 becomes an endemic virus and broad swaths of the population acquire vaccine-mediated and/or natural immunity. However, in the current study, we found that Omicron breakthrough infections generate a slower rise in and lower levels of neutralizing antibodies than Delta. A muted neutralizing antibody response with Omicron breakthrough infections relative to Delta may be due to an increased proportion of asymptomatic or mild infections in the Omicron cohort (55.0% versus 28.6% for Delta, p=0.083), or decreased replication and virulence along with attenuated disease associated with Omicron infection (Halfmann, et al., 2022; Hui, et al., 2022). Thus, immunity from Omicron breakthrough infection may be less durable than breakthrough infection from other variants such as Delta in preventing infection from another, more pathogenic variant, should it emerge in the future. However, it is reassuring that breakthrough infections in vaccine recipients are associated with both shorter overall duration of infection

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(Kissler, et al., 2021) and decreased risk of hospitalization and death (Tenforde, et al., 2022) compared to infection in unvaccinated individuals.

In the current study, live virus neutralization studies showed 2.5-fold lower titers than those using VLPs, which are similar to spike-pseudotyped viruses. Most SARS-CoV-2 neutralization studies reported to date have used pseudoviruses because the protocols for running these assays have been reliable, safe, and convenient. Of note, the VLPs used in this study incorporate all the Omicron-specific mutations found in the structural spike, nucleocapsid, matrix, and fusion proteins (Syed et al. 2022), and not only in the spike protein, as is the case for most pseudovirus assays. One possibility for the discrepant neutralization results may be the use of different cell lines for the VLP (293T) and live virus (Vero) assays, although both cell lines are highly susceptible and permissive to SARS-CoV-2 given stable expression of TMPRSS2 (transmembrane serine protease 2) and the ACE2 (angiotensin converting enzyme 2) receptor (Hoffmann et al. 2020; Case et al. 2020). A more likely explanation is that pseudoviruses and VLPs typically only measure the capacity of the virus to enter cells during a single round of infection, whereas live virus assays measure virus infection over several rounds of infection since the reporting endpoints rely on the appearance of cytopathic effect, during which the viruses have already spread from cell-to-cell. Therefore, the reported extent of immune evasion associated with Omicron infection may be underestimated with the use of pseudovirus assays alone.

The utility of the FDA authorized serologic assay results as correlates of immune protection with respect to infection from different variants is still under investigation (Gilbert et al. 2021). Here we found that spike IgG quantitative and neutralizing antibody results are less correlated with Delta and Omicron infections and thus less predictive of neutralizing immunity. The degree of correlation was inversely related to the extent of neutralizing antibody evasion associated with the variant, which is to be expected since the IgG quantitative assay targets the spike protein from an ancestral WA-1 lineage. Despite the presence of multiple spike mutations, measured antibody levels of 10^3–10^4 for Delta and $>10^5$ for Omicron still reliably predicted neutralization. Nevertheless, serologic assays tailored to individual variants or assays directly measuring neutralization will likely be needed for more accurate assessments of neutralizing immunity.

Limitations of study

There are several limitations to the current study. One limitation is the use of remnant biobanked samples from patients with Delta or Omicron breakthrough infections. As a result, acute and convalescent samples collected longitudinally were only available for a subset of patients. In addition, the times of collection for Delta and Omicron breakthrough infections were not matched, and Omicron breakthrough samples available for analysis had been collected a median 4 days earlier than Delta breakthrough samples, although this difference was not statistically significant. Another limitation is the low total sample numbers, especially since further stratification of samples by immunocompromised and/or boosted status was necessary given the potential confounding effect on neutralizing antibody titers. The low sample numbers also precluded analysis of other comorbidities, such as obesity, pre-existing lung disease, and diabetes, that may account for the differences in neutralizing antibody titers. Collection and analysis of additional samples from patients with breakthrough infections at both acute and convalescent time points will be needed to reproduce our findings and explore how other comorbidities potentially affect neutralizing immunity. Finally, the data collected on breakthrough infections was reliant on retrospective chart review and not collected as part of a prospective study, and inconsistencies and/or incomplete entries in the medical records may have decreased the accuracy of the abstracted clinical metadata.

STAR Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Charles Chiu (charles.chiu@ucsf.edu).

Materials availability

Passaged aliquots of the cultured SARS-CoV-2 Omicron virus, synthetic VLPs (virus-like particles), and available remaining clinical nasal swab and plasma samples are available upon request**.**

Data and code availability

Assembled SARS-CoV-2 genomes in this study were uploaded to GISAID (Shu and McCauley, 2017) (accession numbers included in Table S1). Scripting code used for the data analysis and visualization, SARS-CoV-2 genome FASTA files, and Table S1 are available in a Zenodo data repository (doi: 10.5281/zenodo.5899518).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human Subjects

The human subjects in this study include patients hospitalized with COVID-19 at UCSF and individuals enrolled through the UMPIRE (UCSF EMPloyee and community member Immune REsponse) study **(Table 1).** For hospitalized UCSF patients, remnant samples were biobanked and retrospective medical chart reviews for relevant demographic and clinical metadata were performed under a waiver of consent and according to protocols approved by the UCSF Institutional Review Board (protocol numbers 10-01116 and 11-05519). Informed consent for participation in the UMPIRE study and collection of data and samples were obtained according to a protocol approved by the UCSF Institutional Review Board (protocol number 20-33083). The UMPIRE study cohort included fully vaccinated individuals with either 2 doses of Emergency Use Authorization (EUA) authorized mRNA vaccine (Pfizer or Moderna) or 1 dose of the EUA authorized Johnson and Johnson vaccine and boosted individuals who received an additional dose of vaccine after completing the primary series.

Cell Lines

For the VLP assay, 293T cells derived from human embryonic kidney 293 cells, were used to generate the VLPs, while 293T-ACE2-TMPRSS2 cells were used to receive the VLPs mixed with the heat inactivated plasma. Both cell lines were cultured at 37°C on either 10cm or 15cm plates containing Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1x penicillin/streptomycin added. Cells were passaged at 50%-80% confluence, and the number of passages was not recorded. The 293T cell line was obtained from ATCC and authenticated by the University of California, Berkeley sequencing facility using short tandem repeat (STR) profiling. The 293T-ACE2-TMPRSS2 cells were generated using lentiviral transfection of the authenticated 293T cells followed by antibiotic selection. ACE2/TMPRSS2 expression was confirmed using Western blotting.

For SARS-CoV-2 isolation in cell cultures and the live virus assay, Vero E6-TMPRSS2-T2A-ACE2 and Vero CCL-81 cells derived from African green monkey kidney were cultured at 37°C in Modified Eagle Medium (MEM) supplemented with 1x penicillin-streptomycin (Gibco), glutamine (Gibco), and 10% fetal calf serum (Hyclone). The Vero E6-TMPRSS2-T2A-ACE2 were also supplemented with 10ug/mL puromycin. Cells were passaged at 50%-80% confluence, and the number of passages was not recorded. The Vero CCL-81 and Vero E6-TMPRSS2-T2A-ACE2 cell lines was obtained from ATCC and BEI Resources, respectively. The Vero CCL-81 cell line tested negative for Mycoplasma contamination by PCR. The Vero E6-TMPRSS2-T2A-ACE2 cell line was authenticated by the manufacturer with confirmation of ACE2 and TMPRSS2 expression by indirect fluorescent antibody assay, confirmation of African green monkey origin by multiplex PCR amplification of the cytochrome C oxidase I gene, and exclusion of Mycoplasma contamination by PCR.

METHOD DETAILS

Human Sample Collection

Blood samples were collected through two methods. First, remnant whole blood and plasma samples from patients hospitalized with COVID-19 at UCSF were retrieved from UCSF Clinical Laboratories daily based on availability. Clinical data from hospitalized UCSF patients in the study was retrieved through

retrospective chart review. Samples were obtained from pediatric and adult patients of all genders. No analyses based on sex or age were conducted. Second, plasma samples were also collected through the UMPIRE study, a longitudinal COVID-19 research study focused on collection of prospective whole blood and plasma samples from enrolled subjects to evaluate the immune response to vaccination, with and without boosting, and/or vaccine breakthrough infection. Consented participants came to a UCSF CTSI Clinical Research Service (CRS) Laboratory where their blood was drawn by nurses and phlebotomists. At each visit, two to four 3mL EDTA (ethylenediaminetetraacetic acid) tubes of whole blood were drawn, and one or two EDTA tubes were processed to plasma from each timepoint. Relevant demographic and clinical metadata from UMPIRE participants were obtained through participant Qualtric surveys performed at enrollment and at each blood draw. Plasma samples were heat inactivated at 56°C for 30 mins prior to use in VLP and live virus assays.

Clinical Chart Review

The criteria for an infection of moderate severity included hospitalization for COVID-19 pneumonia with an oxygen requirement of >2L of oxygen by nasal cannula or another infectious complication of the disease (e.g. acute renal injury, diarrhea with electrolyte disturbances, necrosis of the extremities, encephalopathy, etc.). The criteria for a severe infection included COVID-19 pneumonia with severe hypoxemia with an oxygen requirement of >6L, including the need for CPAP (continuous positive airway pressure), BIPAP (bilevel positive airway pressure), or intubation with mechanical ventilation, COVID-19 associated end-organ failure, and/or death. Outpatients and hospitalized patients not meeting criteria for moderate-severe infection were classified as having an asymptomatic or mild infection.

Viral Whole-Genome Sequencing

Remnant clinical nasopharyngeal/oropharyngeal (NP/OP) swab samples collected in universal transport media or viral transport media (UTM/VTM) were diluted with DNA/RNA shield (Zymo Research, # R1100-250) in a 1:1 ratio (100 µl primary sample + 100 µl shield) prior to viral RNA extraction. The Omega BioTek

MagBind Viral DNA/RNA Kit (Omega Biotek, # M6246-03) and the KingFisherTM Flex Purification System with a 96 deep-well head (ThermoFisher, 5400630) were then used for viral RNA extraction. Extracted RNA was reverse transcribed to complementary DNA and tiling multiplexed amplicon PCR was performed using SARS-CoV-2 primers version 3 according to a published protocol (Quick et al. 2017). Adapter ligation was performed using the NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®)(New England Biolabs, # E7658L). Libraries were barcoded using NEBNext Multiplex Oligos for Illumina (96 unique dual-index primer pairs) (New England Biolabs, # E6440L) and purified with AMPure XP (Beckman-Coulter, #63880). Amplicon libraries were then sequenced on either Illumina Miseq or NextSeq 550 as 2x150 paired-end reads (300 cycles).

Genome Assembly and Variant Identification

Raw sequencing data were simultaneously demultiplexed and converted to FASTQ files and screened for SARS-CoV-2 sequences using BLASTn (BLAST+ package 2.9.0). Reads containing adapters, the ARTIC and/or VarSkip primer sequences, and low-quality reads were filtered using BBDuk (version 38.87) and then mapped to the Wuhan-Hu-1 SARS-CoV-2 reference genome (National Center for Biotechnology Information (NCBI) GenBank accession number NC_045512.2) using BBMap (version 38.87). Consensus sequences were generated using iVar (version 1.3.1) (Grubaugh et al. 2019) and lineages were assigned using Pangolin (Rambaut et al. 2020) (version 3.1.17).

Serologic testing

SARS-CoV-2 quantitative IgG levels were determined using the Abbott AdviseDx SARS-CoV-2 IgG II (spike RBD-based) test according to the manufacturer's specifications.

VLP neutralization assay

For transfection in a 15 cm dish, plasmids CoV2-N (0.67), CoV2-M-IRES-E (0.33), CoV-2-Spike (0.0016) and LucT20 (1.0) at indicated mass ratios for a total of 40 µg of DNA were diluted in 1000 µL Opti-

MEM (Modified Eagle Medium). 120 µg PEI (polyethyleneimine) was diluted in 1000 µL Opti-MEM and added to the plasmid dilution quickly to complex the DNA. The transfection mixture was incubated for 20 minutes at room temperature and then added dropwise to 293T cells in a 15cm dish containing 20 mL of DMEM (Dulbecco's Modified Eagle Medium), 10% fetal bovine serum and 1x penicillin/streptomycin. Media was changed after 24 hours of transfection. At 48 hours post-transfection, the VLP containing supernatant was collected and filtered using a 0.45 µm syringe filter.

Each heat inactivated plasma sample was serially diluted from a 1:20 to a 1:20480 dilution in complete DMEM media prior to incubation (1hr at 37°C) with 40µL VLPs at total volume of 50µL, prior to plating onto receiver cells (50,000 293T ACE2-TMPRSS2 cells). The following day, the supernatant was removed, and the cells were lysed in 20 µL passive lysis buffer (Promega) for 15 minutes at room temperature with gentle rocking. The lysates were transferred to an opaque white 96-well plate and 30 µL of reconstituted luciferase assay buffer was added and mixed with each lysate. Luminescence was measured immediately after mixing using a TECAN plate reader. Neutralization titer (NT50) was estimated by fitting the points and interpolating the dilution at which 50% infectivity was observed.

SARS-CoV-2 Isolation in Cell Culture

SARS-CoV-2 Delta and Omicron variants were isolated from de-identified patient nasopharyngeal (NP) swabs sent to the California Department of Public Health from hospitals in California for surveillance purposes. To isolate the Delta variant, 200ul of a patient sample that was previously identified as Delta by virus whole-genome sequencing was diluted 1:3 in PBS supplemented with 0.75% bovine serum albumin (BSA-PBS) and added to confluent Vero CCL-81 cells in a T25 flask. Following a 1-hour absorption period, additional media was added, and the flask was incubated at 37˚C with 5% CO2 with daily monitoring for cytopathic effect (CPE). When 50% CPE was detected, the contents were collected, clarified by centrifugation, and stored at -80C as passage 0 stock. Passaged stock of Delta was made by inoculation Vero CCL-81 confluent T150 flasks with 1:10 diluted p0 stock and harvesting at approximately 50% CPE. Omicron viral stock was similarly

produced from a sequence confirmed NP sample using Vero E6-TMPRSS2-T2A-ACE2 in a T25 flask and harvested at 90% CPE with no subsequent passaging. Both viral stocks were sequenced to confirm lineage and TCID50 was determined by titration.

Live Virus Neutralization Assay

CPE endpoint neutralization assays were done following the limiting dilution model using p0 stock of Omicron and p1 stock of Delta in Vero E6-TMPRSS2-T2A-ACE2. Patient plasma was diluted 1:10 in bovine serum albumin-phosphate buffered saline (BSA-PBS) and heat inactivated at 56C for 30 minutes. Serial 3-fold dilution of plasma were made in BSA-PBS. Plasma dilutions were mixed with 100 TCID50 (tissue culture infective dose 50, or the dose at which 50% of inoculated cells in culture are infected) of each virus diluted in BSA-PBS at a 1:1 ratio and incubated for 1 hour at 37C. Final plasma dilutions in plasma-virus mixture ranged from 1:40 to 1:84480. 100ul of the plasma-virus mixtures was added in duplicate to flat bottom 96-well plates pre-seeded with Vero E6-TMPRSS2-T2A-ACE2 at a density of 2.5 x 104/well and incubated in a 37˚C incubator with 5% CO2 until consistent CPE was seen in the virus control (no neutralizing plasma added) wells. Positive and negative controls were included as well as cell control wells and a viral back titration to verify TCID50 viral input. Individual wells were scored for CPE as having a binary outcome of ‘infection” or ‘no infection’ and the ID50 (inhibitory dose 50, the concentration of plasma needed to inhibit virus-induced CPE by 50%), was calculated using the Spearman-Karber method. All steps were done in a Biosafety Level 3 lab using approved protocols.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses and data visualization were performed using R (version 4.0.3) and Python (version 3.7.10). Fisher’s exact test was used to evaluate associations of demographic and clinical variables with variant-specific breakthrough infections (**Table 1**). Fold decreases in neutralizing activity were measured by comparing median neutralizing antibody titers. Statistical details of each comparison can be found in the main text of the

study as well as in the figures themselves. Significance testing was performed using the Wilcoxon signed-rank test and Mann-Whitney U test for paired and unpaired samples, respectively. Correlation coefficients were calculated using Spearman's rank analysis. Plots were generated using ggplot2 package (version 3.3.5) in R and seaborn package (version 0.11.0) in Python. All statistical tests were conducted as two-sided at the 0.05 significance level. Exact values of n are listed in the main text of the paper for each portion of the study, where n represents the number of COVID infected individuals. Subjects were excluded if they were identified to be infected with a variant that was neither Delta nor Omicron.

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Tables

Characteristic		Delta variant	Delta variant (%)	Omicron variant	Omicron variant (%)	p-value
Reported sex	Female	14	40.0%	11	55.0%	0.40
	Male	21	60.0%	9	45.0%	
Age	>65	19	54.3%	10	50.0%	0.79
	18-65	16	45.7%	10	50.0%	
Received COVID-19 vaccine booster dose	Yes	5	14.3%	8	40.0%	0.048
	No	30	85.7%	12	60.0%	
Disease severity	Moderate-severe	25	71.4%	9	45.0%	0.083
	Asymptomatic or mild	10	28.6%	11	55.0%	
Immune status	Immunocompromised	12	34.3%	2	10.0%	0.059
	Immunocompetent	23	65.7%	18	90.0%	
Median difference in days between sample collection and symptom onset or PCR test positivity		16		12		0.34
	Total	**35**		**20**		

Table 1. Clinical and demographic characteristics in Delta and Omicron variant breakthrough infections

P-values for significance were determined using two-tailed Fisher's Exact Test for the categorical variables and the Mann-Whitney U test for the median difference in days between sample collection and symptom onset or PCR test positivity. The table includes all breakthrough infections (n=55) for which a sample was collected from 4 – 32 days after symptom onset or PCR test positivity.

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Figure Legends

Figure 1. Neutralizing antibody levels in fully vaccinated, uninfected individuals. (A, D) Box-violin plots showing median neutralizing antibody titers using VLP (left) and live virus (right) assays against the SARS-CoV-2 WA-1 ancestral lineage (wild-type, or "WT") and Delta variant in vaccinated, unboosted **(A)** and vaccinated, boosted **(D)** individuals **(B, E)** Box-violin plots of titers against the WT and Omicron variant in vaccinated, unboosted **(B**) and vaccinated, boosted **(E)** individuals. **(C, F)** Cumulative distribution function plots of titers to WT, Delta, and Omicron using VLP (left) and live virus (right) assays in vaccinated, unboosted **(C)** and vaccinated, boosted **(F)** individuals, showing the proportion of samples at or above a given titer. **(G)** Longitudinal box-violin plots of VLP titers to Delta (top) and Omicron (bottom) stratified by time ranges following completion of a primary vaccine series.

Figure 2. Neutralizing antibody levels in Delta and Omicron breakthrough infections. (A) Box-violin plots of median neutralizing antibody titers against Delta (left) and Omicron (middle) variants compared to WT, along with cumulative distribution function plots of titers against WT, Delta, and Omicron (right), showing the proportion of samples at or above a given titer, in patients with Delta breakthrough infection using a VLP neutralization assay. **(B)** Corresponding plots in patients with Delta breakthrough infection using a live virus neutralization assay. **(C)** Corresponding plots in patients with Omicron breakthrough infection using a VLP neutralization assay. **(D)** Corresponding plots in patients with Omicron breakthrough infection using a live virus assay. For the box-violin plots, the median is represented by the thick black line inside the box. The lines connecting the paired points are color-coded based on severity of infection (blue = asymptomatic or mild infection, red = moderate-severe infection). The solid lines denote immunocompetent and the dashed lines immunocompromised patients. Boosted samples are denoted with knobs at the ends of the lines.

Figure 3. Comparison of neutralizing antibody titers against the WT lineage in Delta and Omicron breakthrough infections. (A) All patients in the study with breakthrough infection and available samples collected from 4 – 32 days after symptom onset or SARS-CoV-2 PCR test positivity. **(left)** Kernel density plot showing distribution of collection days for samples from Delta and Omicron breakthrough infections. **(middle)** Box-violin plot comparing VLP neutralizing antibody titers against the WT lineage between Delta and Omicron breakthrough infections. **(right)** Box-violin plot comparing VLP neutralizing antibody titers against the WT lineage between asymptomatic or mild and moderate-severe breakthrough infections. **(B)** Corresponding kernel density plot (left) and box-violin plots (middle and right) for immunocompetent, unboosted patients. **(C)** Longitudinal plots of VLP neutralizing antibody titers against the WT lineage versus days after symptom onset or SARS-CoV-2 PCR test positivity for Delta (left) and Omicron (right) breakthrough infections. Serial samples from the same patient are plotted as lines, shown color-coded based on clinical severity of the breakthrough infection. Circular knobs at the ends of the lines denote boosted status, whereas dotted lines denote immunocompromised status. Singleton time points for individual patients are shown as diamonds. The insets show longitudinal plots corresponding to immunocompetent, unboosted patients, along with a regression line. For the kernel density and box-violin plots, p-values for significance were determined using the Mann-Whitney U test. For the regression analysis, p-values for significance were determined using a t distribution with $n - 2$ degrees of freedom (df).

Figure 4. Correlation between quantitative spike IgG and neutralizing antibody titers. (A) Plots showing correlation between spike IgG titers and neutralizing antibodies directed against WT (top), Delta (middle) and Omicron (bottom) lineages using a VLP-based assay. **(B)** Plots showing correlation between spike IgG titers and neutralizing antibodies directed against WT (top), Delta (middle) and Omicron (bottom) lineages using a live virus-based assay. The Spearman's rank coefficient (ρ) was used to assess the strength of correlation and to determine the p-value for significance.

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Supplemental Figures

Figure S1. VLP and live virus neutralization assay median neutralizing antibody titers, related to Figures 1-4. Plot showing the difference in median neutralizing antibody titers to WT lineage between VLP-based and live virus-based assay.

Supplemental Tables

Excel spreadsheet "serology_v6.xlsx"

Table S1. **Metadata for the 259 plasma samples included in this study, related to Figures 1-4, Table 1, and Figure S1**.

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Acknowledgments

We thank Baolin Wang for her assistance in preparing SARS-CoV-2 genomic sequencing libraries. We thank the staff at the UCSF Clinical Laboratories (Katrina De La Cruz, Cynthia Chu and Ray Chan) for help in collecting plasma samples. This work was funded by US CDC Epidemiology and Laboratory Capacity (ELC) for Infectious Diseases Grant 6NU50CK000539 to the California Department of Public Health (COVIDnet) (M-K.M., C.H., D.A.W., C.Y.C.), the Innovative Genomics Institute (IGI) at UC Berkeley and UC San Francisco (J.D., M.O., C.Y.C.), US Centers for Disease Control and Prevention contract 75D30121C10991 (C.Y.C.), the Roddenberry Foundation (M.O.), National Institutes of Health (NIH) grants R37AI083139 (M.O.), R21AI59666 (J.A.D.), and U54HL147127 (M.M.K.), the Howard Hughes Medical Institute (J.A.D.), the Gladstone Institutes (J.A.D. and M.O.), Abbott Laboratories (C.Y.C.), and the Sandler Program for Breakthrough Biomedical Research (C.Y.C.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings and conclusions in this article are those of the author(s) and do not necessarily represent the views or opinions of the California Department of Public Health or the California Health and Human Services Agency.

Author contributions

C.Y.C., M.O., J. D., and C.H. conceived and designed the study. C.Y.C, V.S., N.B., and P.S. coordinated the sequencing efforts and laboratory studies. A.S., M.K.M., A.S-G., N.B., V.S., M.G.K., B.W., B.S., M.M.K, A.C., P.Y.C, Y.Z., M.R., and J.P. performed experiments. C.Y.C., V.S., N.B., P.S, A.S., M.K.M, A.S-G., J.N., A.G., M.R., J.P., J.H.Jr., C.H. analyzed data. C.Y.C. and V.S. performed genome assembly. V.S., N.B., P.S., J.N., and A.G. collected samples. C.Y.C., V.S., N.B., and P.S. wrote the manuscript. C.Y.C. and V.S. prepared the figures. C.Y.C., V.S., A.S., M.K.M., N.B., P.S., M.G-K., Y.Z., J.N., A.G., J.H.Jr., C.H., and D.A.W. edited the manuscript. C.Y.C. and V.S. revised the manuscript. All authors read the manuscript and agree to its contents.

Declaration of Interests

C.Y.C. is the director of the UCSF-Abbott Viral Diagnostics and Discovery and receives research support for SARS-CoV-2 studies from Abbott Laboratories. The other authors declare no competing interests.

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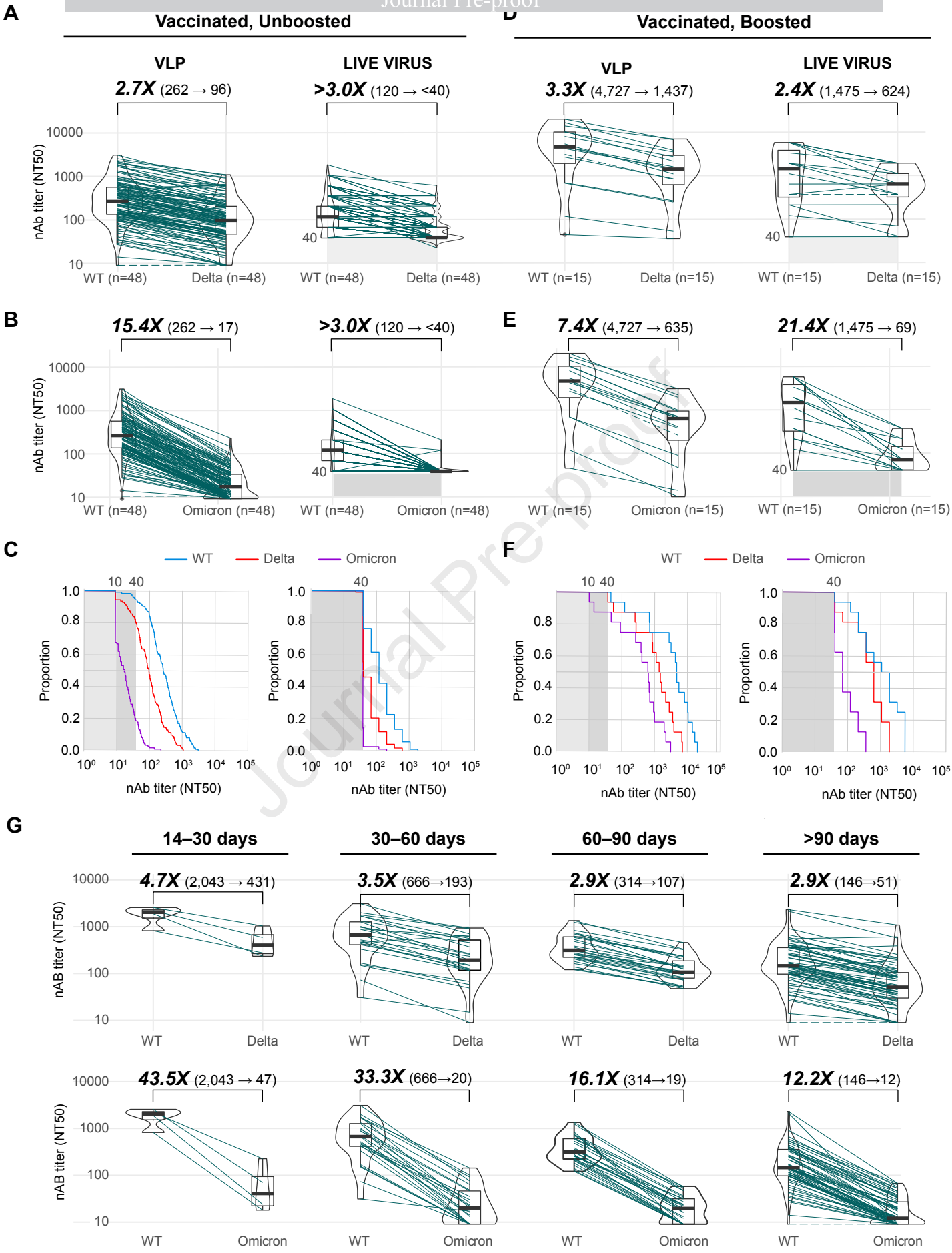

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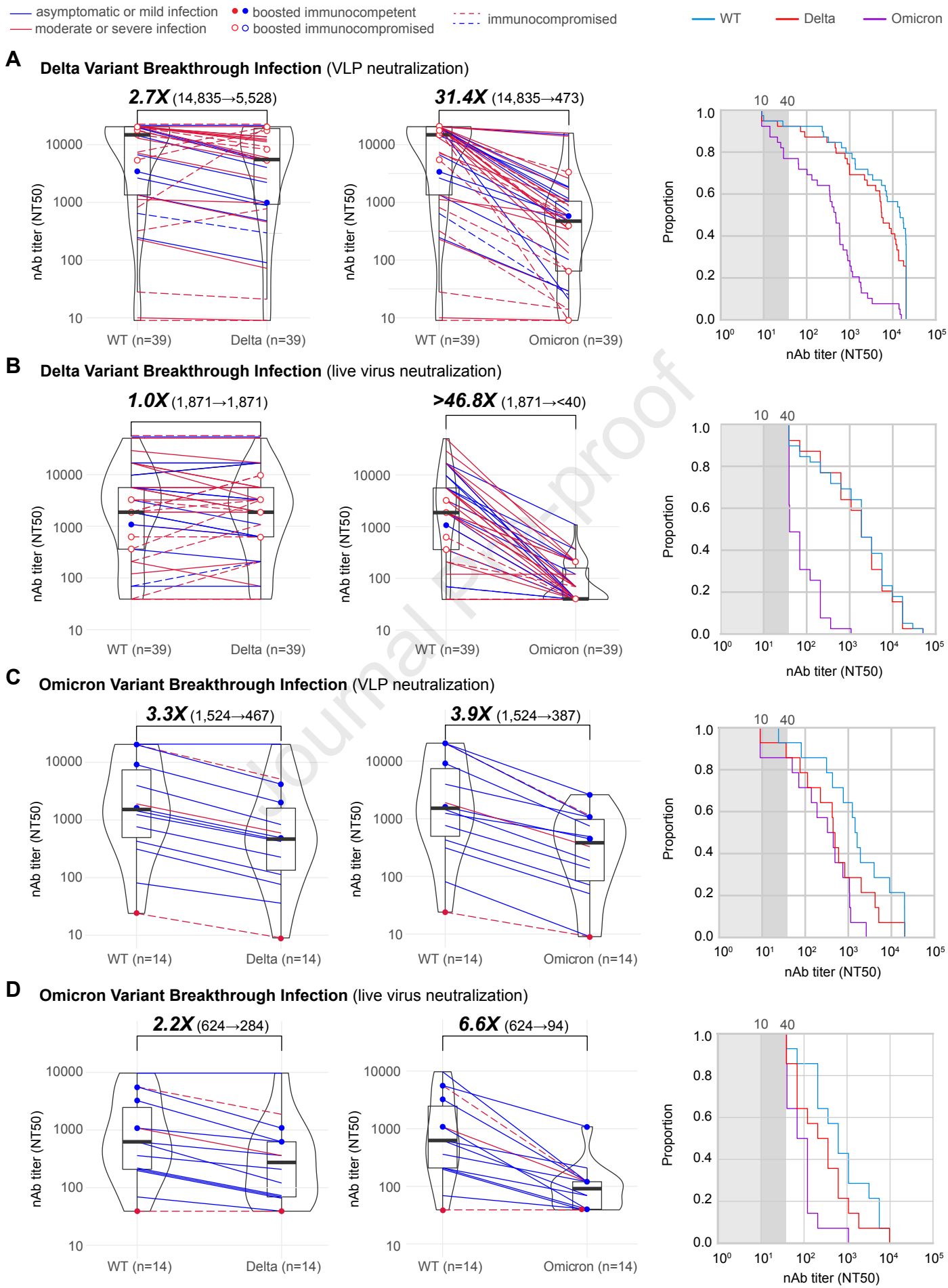

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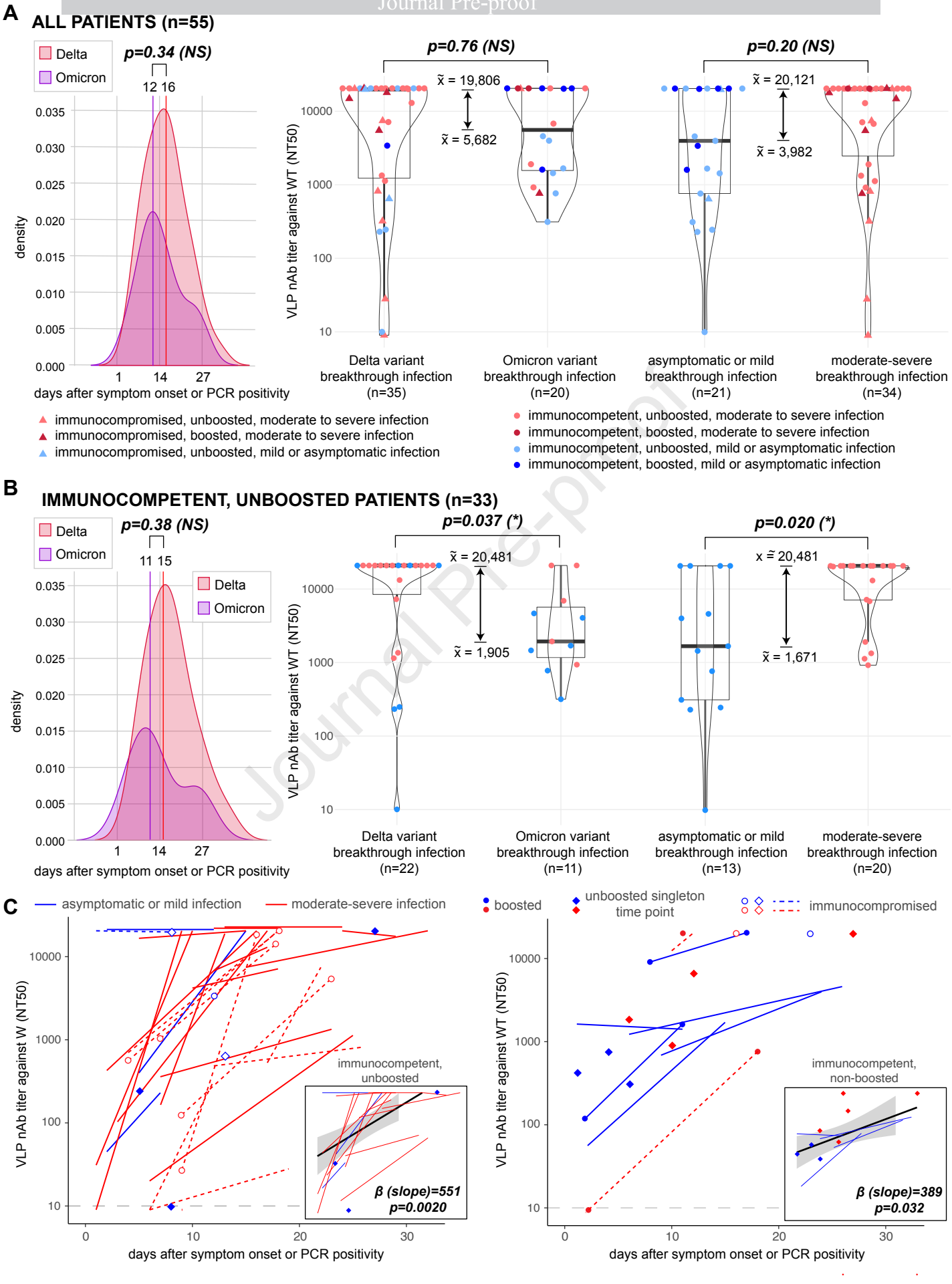

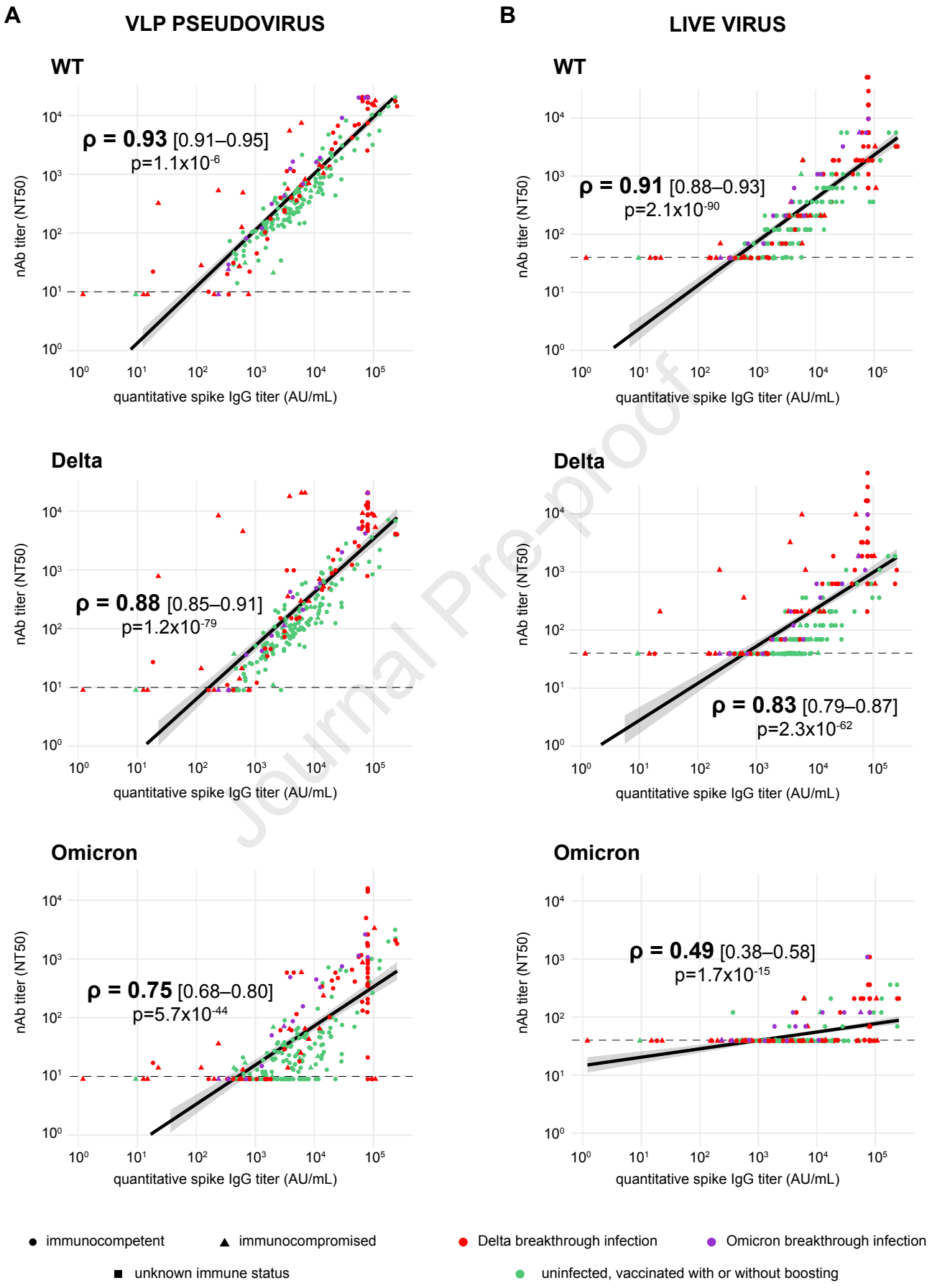

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HIGHLIGHTS

1. In breakthrough infections, variant-specific cross-neutralizing immunity is limited.
2. Higher antibody titers are observed in severe versus mild breakthrough infections.
3. Delta breakthroughs exhibited 10.8X higher antibody titers compared to Omicron.
4. The rise in antibody titers from Omicron breakthroughs was 1/3 of that from boosting.

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In comparing breakthrough infections from the SARS-CoV-2 Delta and Omicron variants, the latter, though milder than Delta infections, were associated with lower antibody titers and limited cross-neutralizing immunity, suggesting reduced protection against re-infection or infection from a future variant.

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KEY RESOURCES TABLE

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and virus strains		
SARS-CoV-2 Delta strain	P2 culture	EPI_ISL_4279956
SARS-CoV-2 Omicron strain	P2 culture	EPI_ISL_9275812
Biological samples		
Remnant nasal/nasopharyngeal swab samples in universal transport media	Obtained from patients under IRB-approved biobanking protocol	N/A
Peripheral blood plasma	Obtained from patients and vaccinated recipients under IRB-approved biobanking and prospective study protocols	N/A
Chemicals, peptides, and recombinant proteins		
DNA/RNA shield	Zymo Research	Cat# R1100-250
Critical commercial assays		
Omega BioTek MagBind Viral DNA/RNA Kit	Omega Biotek	Cat# M6246-03
KingFisherTM Flex Purification System	ThermoFisher	Cat# 5400630
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit	New England Biolabs	Cat# E7658L
NEBNext Multiplex Oligos	New England Biolabs	Cat# E6440L
Luciferase Assay System	Promega	Cat# E1501
Deposited data		
SARS-CoV-2 genomes in GISAID	Chiu Laboratory	Accession numbers included in Table S1
Scripting code used for the data analysis and visualization, FASTA files	Chiu and Servellita, 2022	doi: 10.5281/zenodo.5899518

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Experimental models: cell lines		
Vero CCL-81	ATCC	
Vero E6-TMPRSS2-T2A-ACE2	BEI Resources	Cat # NR-54970
293T ACE2/TMPRSS2	Deposition into biorepository pending. Part of this study: https://www.medrxiv.org/content/10.1101/2021.12.20.21268048v3	
Experimental models: organisms/strains	This study	N/A
Oligonucleotides		
ARTIC v3 primers for SARS-CoV-2 virus whole-genome sequencing	Quick et al., 2017	https://artic.network/ncov-2019
Varskip primers for SARS-CoV-2 virus whole-genome sequencing	New England Biolabs	Cat# E7658L
Recombinant DNA		
VLP plasmids (M,E,N)	Syed et al., 2021. https://www.addgene.org/browse/article/28220280/	
VLP plasmids Spike	Deposit pending. Part of this study: https://www.medrxiv.org/content/10.1101/2021.12.20.21268048v3	
Software and algorithms		
BBTools suite, v38.87	Bushnell, 2021, https://jgi.doe.gov/data-and-tools/bbtools/	
iVar v1.3.1	Grubaugh, 2019, https://andersen-lab.github.io/ivar/html/manualpage.html	
PANGOLIN v.3.1.17	https://github.com/cov-lineages/pangolin	

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R v4.0.3	https://www.R-project.org/	
Python v3.7.10	Python Software Foundation, https://www.python.org/	
Adobe Illustrator v23.1.1	Adobe, https://www.adobe.com/	
MS Excel v16.57	Microsoft, https://www.microsoft.com/en-us/microsoft-365/excel	
Other		

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