

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

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28 **Key words:** SARS-CoV-2, COVID-19, antibody neutralization, Omicron variant, B.1.1.529, Delta variant,

29 B.1.617.2, breakthrough infection, boosted breakthrough infection, variant severity, virus-like particle (VLP),

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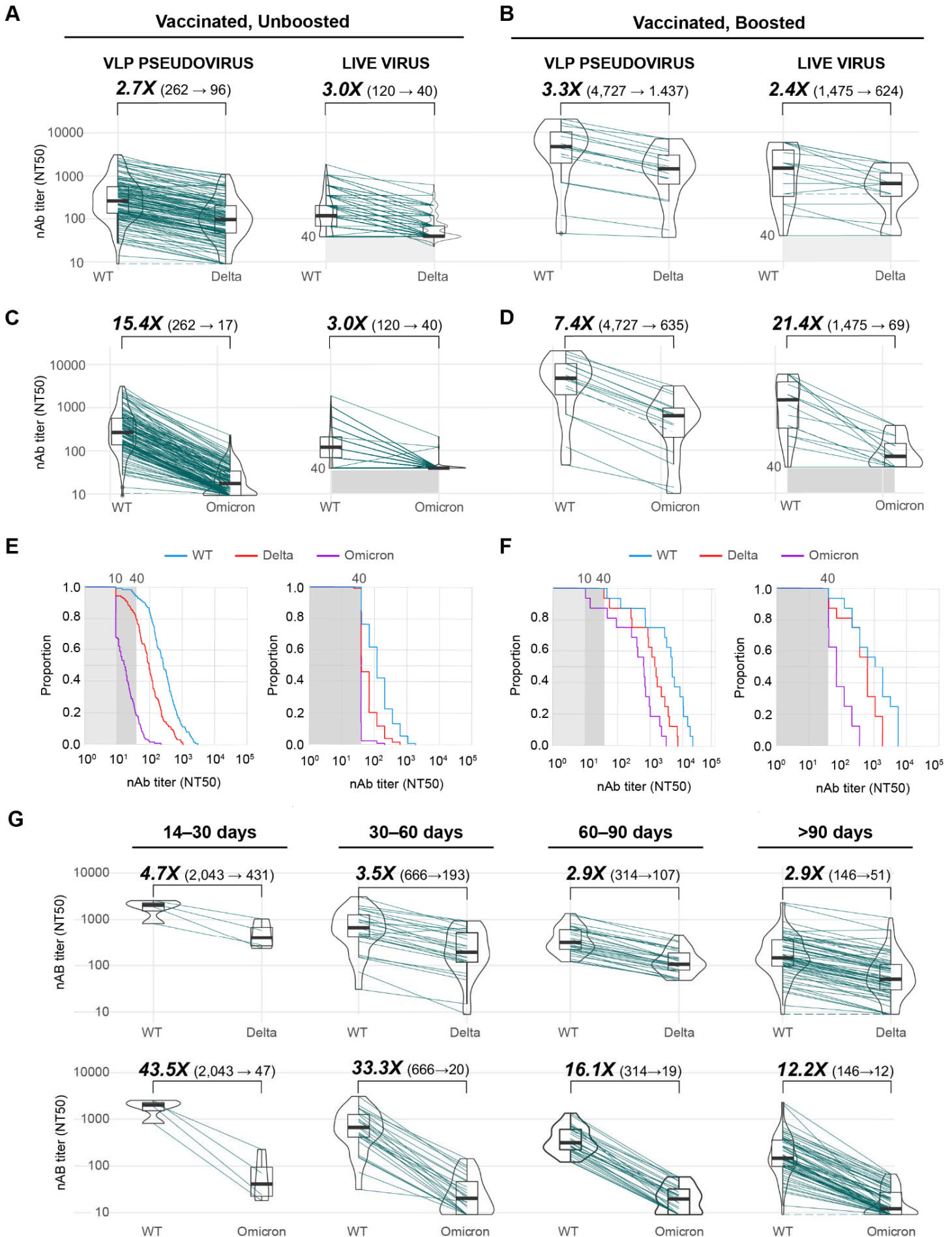
31 **Summary**

32 Virus-like particle (VLP) and live virus assays were used to investigate neutralizing immunity to Delta
33 and Omicron SARS-CoV-2 variants in 239 samples from 125 fully vaccinated individuals. In uninfected, non-
34 boosted individuals, VLP neutralization titers to Delta and Omicron were reduced 2.7-fold and 15.4-fold,
35 respectively, compared to wild-type (WT), while boosted individuals (n=23) had 18-fold increased titers. Delta
36 breakthrough infections (n=39) had 57-fold and 3.1-fold titers whereas Omicron breakthrough infections (n=14)
37 had 5.8-fold and 0.32-fold titers compared to uninfected non-boosted and boosted individuals, respectively. The
38 difference in titers (p=0.049) was related to a higher proportion of moderate to severe infections in the Delta
39 cohort (p=0.014). Correlation of neutralizing and spike quantitative antibody titers was decreased with Delta or
40 Omicron compared to WT. Neutralizing antibodies in Delta and Omicron breakthrough infections increase
41 overall, but the relative magnitude of increase is greater in more clinically severe infection and against the
42 specific infecting variant.

43 **Introduction**

45 Variants of concern have emerged throughout the COVID-19 pandemic, causing multiple waves of
46 infection (Dyson *et al.* 2021). The Omicron (B.1.1.529) variant has been shown to be highly transmissible with
47 decreased susceptibility to therapeutic monoclonal antibodies and neutralizing antibodies conferred by
48 vaccination or prior infection (Flemming 2022; VanBlargan *et al.* 2022). These characteristics are likely due to
49 a large number of mutations in the spike protein (n=30) (Cao *et al.* 2021). Omicron has spread to become the
50 predominant circulating lineage worldwide as of early January 2022 amidst high background levels of Delta
51 (B.1.617.2) variant infection (Gangavarapu *et al.*, 2020). The surge in Omicron has led to a reinstatement of
52 public health interventions to decrease transmission and a renewed focus on vaccination efforts, although
53 evidence to date suggests that Omicron causes less severe disease than other SARS-CoV-2 variants (Wolter *et*
54 *al.* 2022; Davies *et al.* 2022).

55 The development of neutralizing antibody responses in Delta and Omicron breakthrough infections
56 remain largely unexplored. Here we evaluated neutralizing antibody responses in fully vaccinated individuals,
57 some of whom were boosted and/or subsequently developed a COVID-19 breakthrough infection.
58 Neutralization was assessed using two independent methods that incorporated either SARS-CoV-2 virus-like
59 particles (VLP) incorporating all of the Omicron mutations in the spike, nucleocapsid, matrix, and fusion
60 structural proteins (Syed *et al.* 2021; Syed *et al.* 2022) or live viruses in the assay. We also correlated the
61 neutralization results with quantitative spike antibody levels and investigated associations between neutralizing
62 antibody titers and infecting variant or clinical severity of the breakthrough infection.



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

46

47 **Results**

48 **Neutralizing antibody levels in vaccinated individuals wane over time and are reduced against the Delta** 49 **and Omicron variants**

50 VLP and live virus neutralization assays were performed in parallel on 144 plasma samples collected
51 from 81 subjects enrolled in a prospectively enrolled longitudinal cohort (the UMPIRE, or “UCSF eMPLOYEE
52 and community Immune REsponse study”), 18 (22.2%) of whom had received a booster and none of whom
53 were previously infected. We chose available samples from the earliest and most recent time points
54 collected from each subject ≥ 14 days after the last vaccine dose for neutralization testing. Sample collection
55 dates for fully vaccinated, non-boosted individuals (n=63) ranged from 14 to 305 days (median = 91 days)
56 following completion of the primary series of 2 doses for an mRNA vaccine (BNT162b2 from Pfizer or mRNA-
57 1273 from Moderna) or 1 dose of the adenovirus vector vaccine (Ad26.COV2.S from Johnson and Johnson); for
58 boosted individuals (n=18), collection dates ranged from 2 to 74 days (median = 23 days) following the booster
59 dose. Overall, median neutralizing antibody titers were 2.5-fold lower using live viruses compared to VLPs
60 (Supplementary Figure 1).

31 In non-boosted vaccinated individuals, median VLP neutralizing antibody titers to Delta and Omicron
32 relative to the ancestral wild-type (WT) virus WA-1 lineage were reduced 2.7-fold (262 → 17, expressed as
33 NT50 titers, or titers needed to neutralize 50% of VLP activity) and 15.4-fold (262 → 96), respectively (**Figure**
34 **1A and B**). In comparison, live virus neutralization titers to Delta and Omicron were both reduced 3.0-fold (120
35 → 40), with the lower reduction for Omicron accounted for by the higher limit of detection (LOD) for the live
36 virus (NT50 = 40) compared to the VLP neutralization assay (NT50 = 10). Using VLPs, the proportion of
37 individuals with neutralizing antibodies to Omicron above an NT50 cutoff of 40 was ~20%, as compared to
38 ~80% and ~95% for Delta and WT, respectively (**Figure 1C, left**). The corresponding proportions using live
39 viruses were ~5%, ~45%, and ~75% for Omicron, Delta, and WT, respectively (**Figure 1C, right**). In boosted
40 individuals, baseline VLP titers to WT were 18-fold higher (4,727 versus 262) than in non-boosted individuals
41 (**Figure 1A, B, D, and E, left**), and decreases in titers to Delta and Omicron relative to WT were more modest
42 at 3.3-fold and 7.4-fold, respectively (**Figure 1D and E, left**). The increase in VLP neutralization titers
43 corresponded to >93% of boosted individuals having neutralizing antibodies to all 3 lineages above an NT50
44 cutoff of 40 (**Figure 1F, left**). In contrast, live virus neutralization titers in boosted individuals showed a 21.4-
45 fold (1,475→69) decrease in titers to Omicron relative to WT (**Figure 1E, right**), with only ~62% of boosted
46 individuals having neutralizing antibodies to Omicron (**Figure 1F, right**). Median VLP neutralization titers to
47 WT decreased by 93% (14-fold, 2,043→146) over >3 months following vaccination, with decreases in titers to
48 Delta and Omicron of 2.9–4.7-fold and 12.2–43.5-fold, respectively, relative to WT (**Figure 1G**).

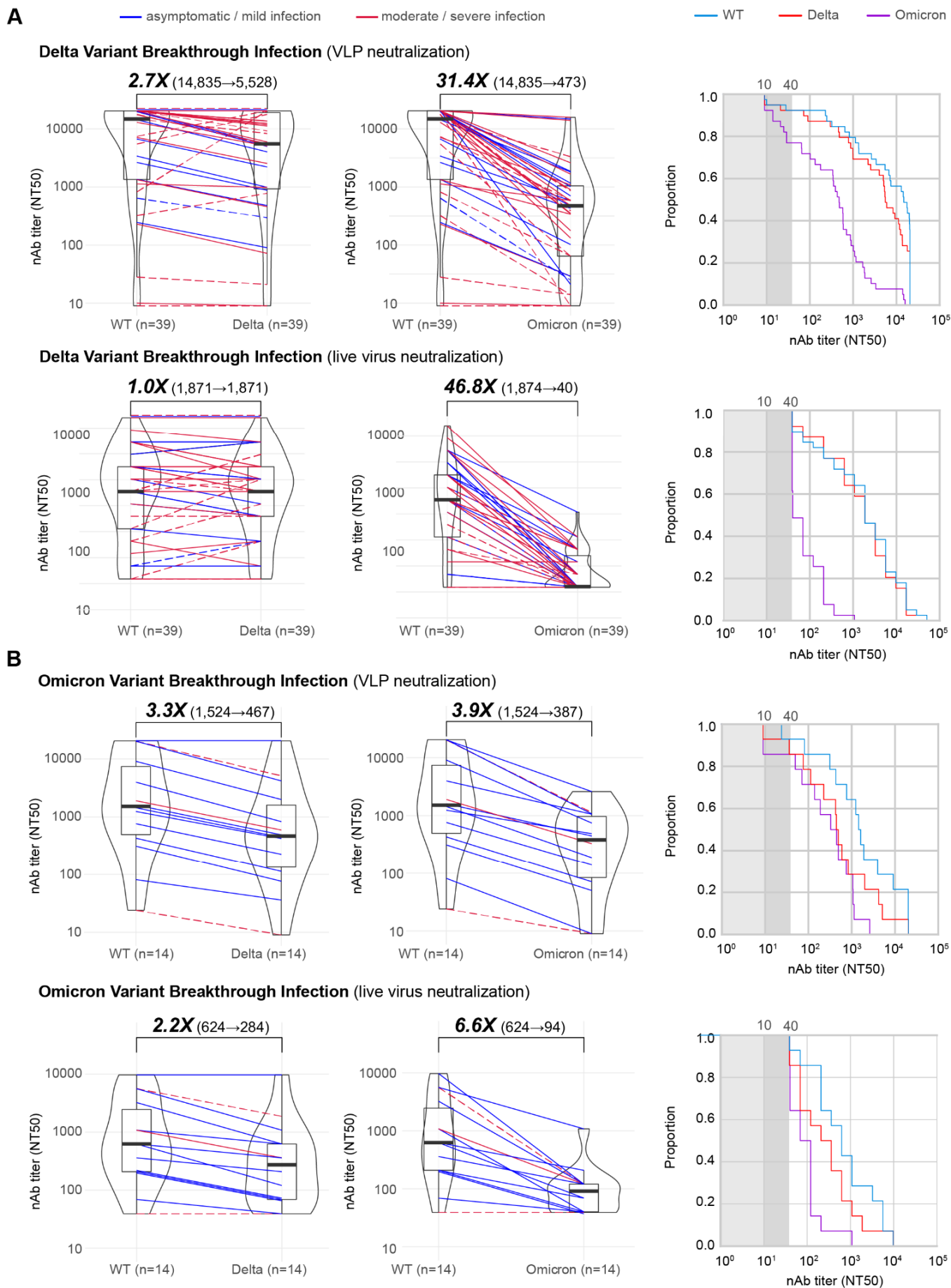


Figure 2. Neutralizing antibody levels in Delta and Omicron breakthrough infections. (A) Box-violin plots of median neutralizing antibody titers against Delta (left) and Omicron (middle) variants compared to WT, along with cumulative distribution function plots of titers to WT, Delta, and Omicron (right), showing the proportion of samples at or above a given titer, in patients with Delta breakthrough infections using VLP (top row) and live virus (bottom row) assays. (B) Corresponding box-violin and cumulative distribution plots in patients with Omicron breakthrough infections. 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 or severe infection). The solid lines denote immunocompetent and the dashed lines immunocompromised patients.

Breakthrough infection increases baseline neutralizing antibody levels and variant-specific immunity

To investigate neutralizing antibody responses and the extent of cross-variant immunity, we analyzed plasma samples from 53 patients with confirmed SARS-CoV-2 breakthrough infections. Of the 53 cases, 28 and 14 were identified as Delta and Omicron variants, respectively, by viral whole-genome sequencing. For the remaining 11, we were unable to identify the lineage because of lack of a respiratory swab sample or insufficient genome coverage for definitive identification. These 11 were presumptively identified as Delta because they were collected from July 30 to December 1, 2021, during a period of when Delta accounted for 97.1 – 99.6% of the circulating lineages in California (CDPH 2022). The number of days between sample collection and symptom onset or PCR positivity, whichever was earlier, ranged from 1 to 55 days (median = 15 days). Of the 53 breakthrough cases, 28 (52.8%) were hospitalized with moderate to severe COVID-19 disease and 13 (24.5%) were immunocompromised.

Using VLP assays, we found that Delta breakthrough infections (n=39), 5 of which were boosted, had increased median baseline WT neutralization titers of 57-fold (14,835 versus 262) and 3.1-fold (14,835 versus 4,727) compared to those from non-boosted and boosted individuals, respectively (**Figure 1A, top and 2A, top**). In addition, neutralization titers to Delta rose to the same level as WT in the live virus assay (**Figure 2A,**

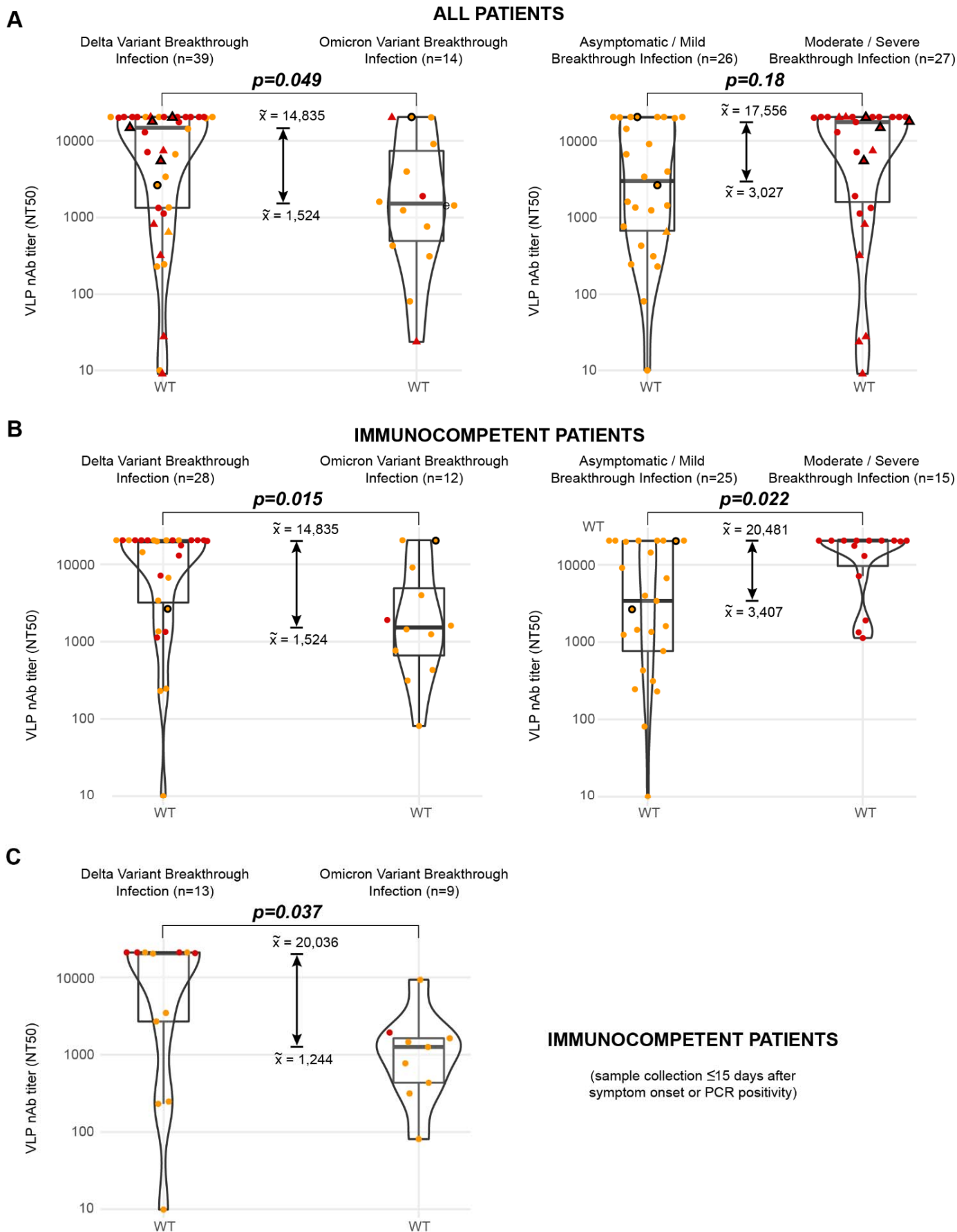
37 **bottom**). Partial neutralization to Omicron was observed mainly due to the increase in baseline titers, as there
38 appeared to be limited cross-variant immunity with 31.1-fold and 46.8-fold decreases in Omicron neutralization
39 relative to WT for the VLP and live assays, respectively (**Figure 2A**). The proportion of individuals with
40 neutralizing antibodies to Omicron above an NT50 cutoff of 40 was calculated at ~75% (**Figure 2A, top right**)
41 and ~43% (**Figure 2A, bottom right**) for the VLP and live virus assays, respectively.

42 In contrast to Delta, Omicron breakthrough infections (n=14), of which only 1 was boosted, exhibited
43 much smaller increases in baseline WT titers, 5.8-fold those from non-boosted individuals and only to about
44 one-third of the titers from boosted individuals (**Figure 1A and 2B, top left**). However, a relative increase in
45 neutralization titers to Omicron was observed, with Omicron-specific titers in breakthrough infections only 3.9-
46 fold lower than WT (**Figure 2B, top left**). In contrast, no corresponding increase in Delta-specific titers was
47 observed, with a 3.3-fold reduction in titer that was comparable to that observed previously in uninfected
48 vaccinated individuals (**Figure 1A and 2B, top left**). Thus, Omicron breakthrough infection resulted in ~90%
49 of individuals having neutralizing antibodies to Omicron above an NT50 cutoff of 40, comparable in proportion
50 to those having neutralizing antibodies to Delta (**Figure 2B, bottom left**).

51 A head-to-head comparison of Omicron and Delta breakthrough infections was performed after
52 inclusion of samples collected from 0 to 30 days after symptom onset (**Figure 2B, left and right**). The
53 comparison showed that Delta breakthrough infections resulted in more pronounced increases in baseline WT
54 titers relative to non-boosted vaccinated individuals, 61.8-fold (16,195 versus 262) versus 5.8-fold (1,524 versus
55 262) (**Figure 1A and 2B**). The difference in median neutralization titers between Delta and Omicron variants
56 was significant when including all breakthrough patients (9.7-fold, 14,835→1,524, p=0.049) and when
57 considering only immunocompetent patients (13.1-fold, 19,921→1,524, p=0.015) (**Figure 3A and 3B, left**).
58 While the breakthrough infection conferred additional neutralizing immunity against the infecting variant
59 (**Figure 1A, bottom and Figure 1B, left**), the extent of cross-variant immunity beyond increases in baseline
60 WT titers was limited, with a 3.3-fold reduction in Delta titers in Omicron breakthroughs and a 36.1-fold

31 reduction in Omicron titers in Delta breakthroughs (**Figure 2A and B**), comparable to the reductions observed
32 in uninfected vaccinated individuals (**Figure 1A**).

- asymptomatic / mild infection, immunocompetent
- asymptomatic / mild infection, immunocompromised
- moderate / severe infection, immunocompetent
- moderate / severe infection, immunocompromised
- asymptomatic / mild infection, immunocompetent, boosted
- asymptomatic / mild infection, immunocompromised, boosted
- moderate / severe infection, immunocompetent, boosted
- moderate / severe infection, immunocompromised, boosted



34 **Figure 3. Comparison of neutralizing antibody titers against the WT lineage in Delta and Omicron**
35 **breakthrough infections. (A)** Box-violin plots comparing neutralizing antibody titers against the WT lineage
36 between Delta and Omicron breakthrough infections (left) and between asymptomatic or mild and moderate or
37 severe breakthrough infections (right) for all patients in the study. **(B)** Corresponding box-violin plots for
38 immunocompetent patients only. **(C)** Box-violin plots between Delta and Omicron breakthrough infections for
39 samples that were collected ≤ 15 days after symptom onset or PCR positivity. P-values for significance were
70 determined using the Mann-Whitney U test.

71

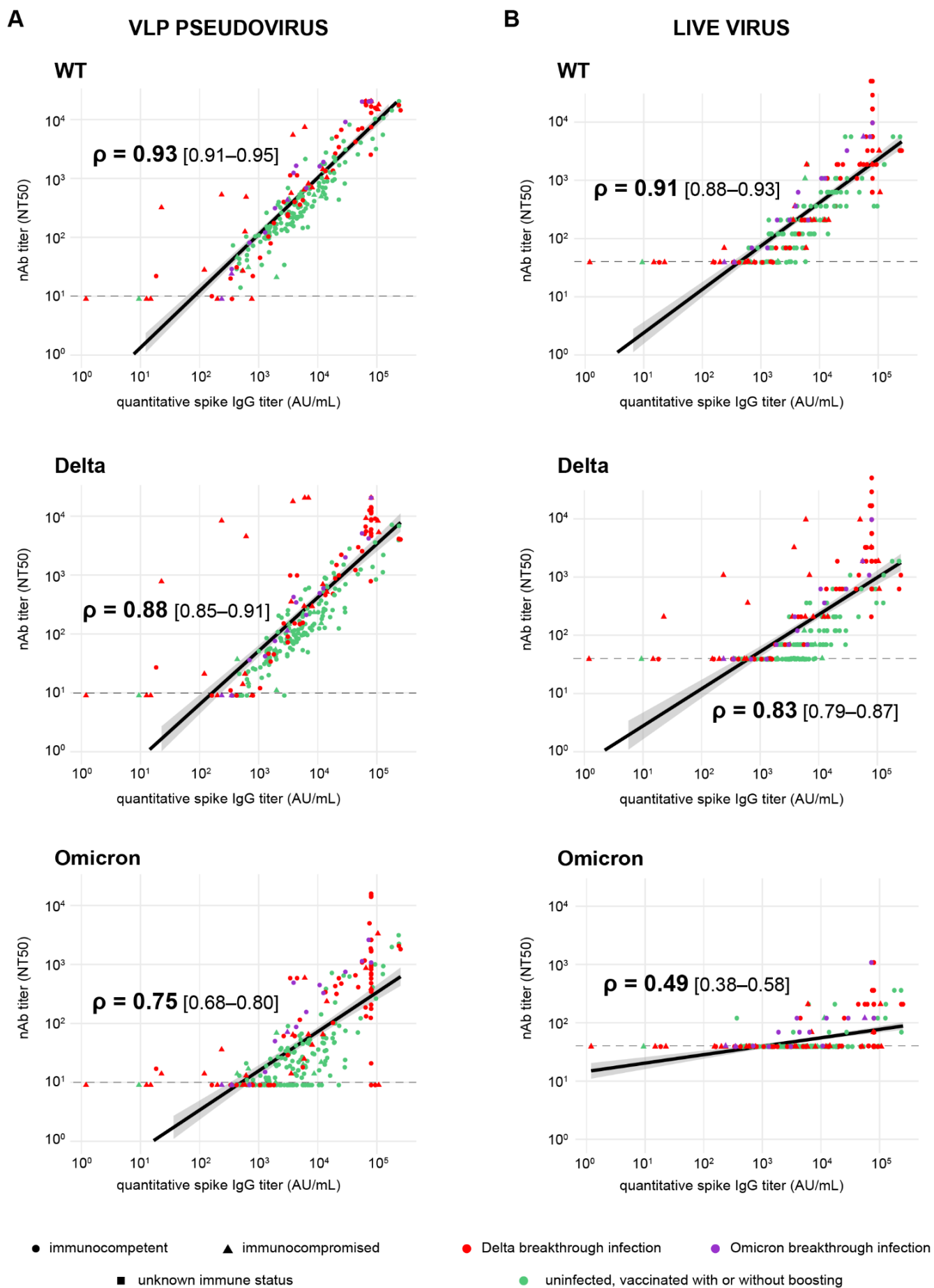


Figure 4. Correlation between quantitative spike IgG and neutralizing antibody titers. (A) Plots showing correlation between spike IgG titers and neutralizing antibodies directed against WT (top), Delta (middle) and Omicron (bottom) lineages using a VLP-based assay. (B) Plots showing correlation between 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.

Characteristic		Delta variant	Delta variant(%)	Omicron variant	Omicron variant(%)	p-value	lower 95%CI	upper 95%CI	odds ratio
gender	female	17	43.59%	8	57.14%	0.5343	0.14	2.35	0.59
	male	22	56.41%	6	42.86%				
age	>65	19	48.72%	6	42.86%	0.7632	0.31	5.32	1.26
	18-65	20	51.28%	8	57.14%				
received booster	yes	5	12.82%	1	7.14%	1	0.18	97.23	1.89
	no	34	87.18%	13	92.86%				
severity	moderate to severe	24	61.54%	3	21.43%	0.01351	1.23	36.82	5.67
	asymptomatic to mild	15	38.46%	11	78.57%				
hospitalized for covid	yes	25	64.10%	3	21.43%	0.01116	1.36	41.17	6.31
	no	14	35.90%	11	78.57%				
immune status	immunocompromised	11	28.21%	2	14.29%	0.4729	0.41	24.74	2.32
	immunocompetent	28	71.79%	12	85.71%				
median collection interval from symptom onset									
all samples		17 days		8.5 days		0.008			
≤ 15 days		9 days		6 days		0.1231			

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 days between sample collection and symptom onset or PCR positivity.

Clinical severity is a potential determinant of neutralizing immunity

We sought to identify clinical factors that may explain the difference between neutralizing antibody levels between Delta and Omicron breakthrough infections. Univariate analysis revealed that among the factors examined, only clinical severity, hospitalization for COVID-19, and median numbers of days between symptom onset or PCR positivity and sample collection, were significant (**Table 1**). Notably, there was a higher proportion of moderate to severe infections in Delta compared to Omicron breakthrough cases (**Table 1, p=0.014**). In immunocompetent patients, moderate to severe breakthrough infections from Delta and Omicron were found to elicit higher median levels of neutralizing antibodies as compared to mild or asymptomatic infections (**Figure 3B, right, 20,481 versus 3,407, p=0.022**). The corresponding comparison was not significant for all patients (**Figure 3A, right, 17,556 versus 3,027, p=0.18**), as there were several vaccinated patients in the current study who were hospitalized for moderate to severe COVID-19 breakthrough infection yet failed to generate a robust antibody response due to their immunocompromised state (**Figure 3A, red triangles**). Since the median number of days between symptom onset or PCR positivity and sample collection for Delta and Omicron infections was also significant (**Table 1, 17 versus 8.5 days, p=0.008**), we performed a sub-analysis of neutralization titers between Delta and Omicron breakthrough infections for samples collected ≤ 15 days from symptom onset or PCR positivity. The difference in levels of neutralizing antibodies from Delta or Omicron breakthrough infection was still significant (**Figure 3C, right, p=0.037**), even at comparable time intervals between symptom onset or PCR positivity and sample collection (**Table 1, 9 versus 6 days, p=0.12**).

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 quantitative spike antibody results from an FDA Emergency Use Authorization (EUA) authorized commercial assay (**Figure 4**). The results showed decreased correlation of neutralization and quantitative antibody titers with Omicron (Spearman's $\rho=0.49-0.75$) and Delta ($\rho=0.83-0.88$) relative to WT ($\rho=0.91-0.93$). Of note, many cases of Delta breakthrough infection with low to

11 moderate levels of spike IgG antibody failed to neutralize Omicron in the live virus assay (**Figure 4B, bottom**).
12 Quantitative NT50 titers of 10^3 – 10^4 and $>10^5$ reliably predicted Delta and Omicron neutralization, respectively.
13

14 **Discussion**

15 Here we used VLP and live virus neutralization assays to investigate neutralizing antibody responses in
16 125 vaccinated individuals, both non-boosted and boosted, and after Delta and Omicron vaccine breakthrough
17 infections. Our results suggest that vaccine boosting and breakthrough infections can restore broad neutralizing
18 hybrid immunity by increasing baseline titers, with higher relative titers against the infecting variant. Notably,
19 Delta-specific titers in Delta breakthroughs rose to become comparable to levels against WT, while Omicron-
20 specific titers in Omicron breakthroughs rose to become comparable to levels against Delta. However, we also
21 found that the magnitude of increase in baseline titers is dependent on the clinical severity of the breakthrough
22 infection. Increased neutralizing antibody levels in Delta related to Omicron breakthrough cases ($p=0.049$) was
23 likely due to an increased proportion of moderate to severe infections in the Delta compared to the Omicron
24 cohort ($p=0.014$, 61.5% versus 21.4%). Indeed, moderate to severe clinical infections in both Delta and
25 Omicron breakthrough cohorts were associated with significantly higher neutralization titers than mild or
26 asymptomatic infections among immunocompetent patients ($p=0.022$).

27 A limitation of the current study is the lack of both acute and convalescent samples from patients with
28 Delta or Omicron breakthrough infections. Indeed, we found a significant difference in the median days
29 between symptom onset or PCR positivity and sample collection (**Table 1, 17 versus 8.5 days, $p=0.008$**).
30 However, when we analyzed a more comparable subset of samples collected ≤ 15 days following PCR positivity
31 and sample collection, the difference in neutralization titers between Omicron and Delta was still significant
32 ($p=0.037$). To confirm these findings, collection and analysis of samples from patients with Omicron
33 breakthrough infections at later time points is ongoing. Other studies have looked at the effect of boosting on
34 neutralization of Omicron and the role cross-variant immunity plays in Omicron breakthrough infections. A
35 study from Laurie, et al. (2022) reported a 4 to 8-fold reduction in neutralization titer in sera from boosted

36 individuals using a pseudovirus assay, comparable to the 7.4-fold reduction that we observed using a VLP
37 assay. Similar to our findings, the study by Khan, et al. (2021) found that sera from patients with Omicron
38 breakthrough infections can enhance Delta virus neutralization to a limited extent (4.4-fold), but that immunity
39 elicited against the specific infecting variant (Omicron) is higher (17.4-fold).

40 Our findings have implications with regard to the likelihood of Omicron infections providing mass
41 immunization on the population level against SARS-CoV-2. Widespread infections from Omicron globally both
42 in vaccinated and unvaccinated persons, have been reported, and Omicron has shown to cause milder disease
43 with reduced risk of hospitalization and death relative to prior lineages (Wolter *et al.*, 2022). In addition,
44 epidemiologic data to date suggests that Omicron may be rapidly outcompeting more pathogenic variants such
45 as Delta (Outbreak.info, 2022). These observations raise the prospect of Omicron being a harbinger of the end
46 of the pandemic as SARS-CoV-2 becomes an endemic virus and broad swaths of the population acquire
47 vaccine-mediated and/or natural immunity. However, we found a significantly smaller rise of neutralization
48 titers associated with milder Omicron breakthrough infection in vaccinated individuals, to only approximately
49 one-third of the rise associated with boosting. We also identified limited cross-variant immunity to Delta. Thus,
50 breakthrough infection from Omicron may enhance cross-protection against Delta, and vice-versa, inasmuch as
51 there is a sufficiently large increase in baseline neutralizing immunity, which appears to be related to the
52 clinical severity of the infection. Our findings parallel those from another study from our group that
53 demonstrated limited cross-variant immunity after milder Omicron variant infection in unvaccinated individuals
54 in a mouse model and in human patients (Suryawanshi *et al.*, 2022). Taken together, our results suggest that
55 Omicron-induced immunity may not be sufficient to prevent infection from another, more pathogenic variant,
56 should it emerge in the future. They also highlight the continued importance of vaccine boosters in enhancing
57 immunity, as breakthrough infection alone may not be reliable in eliciting protective titers against re-infection
58 or future infection from different variants. Furthermore, the relative increase in immunity against the infecting
59 variant in breakthrough infections indicates that the use of variant-specific immunogens in vaccine development
60 remains a viable strategy for addressing VOCs that continue to circulate in the population.

31 Results from the live virus neutralization studies consistently showed lower titers than those using
32 VLPs, which are similar to spike-pseudotyped viruses. The majority of SARS-CoV-2 neutralization studies
33 reported to date have used pseudoviruses because the protocols for running these assays have been reliable,
34 safe, and convenient. Of note, the VLPs used in this study incorporate all of the Omicron-specific mutations
35 found in the structural spike, nucleocapsid, matrix, and fusion proteins (Syed *et al.* 2022), and not only in the
36 spike protein, as is the case for most pseudovirus assays. One possibility for the discrepant neutralization results
37 may be the use of different cell lines for the VLP (293T) and live virus (Vero) assays, although both cell lines
38 are highly susceptible and permissive to SARS-CoV-2 given stable expression of the ACE2 and TMPRSS2
39 receptors (Hoffmann *et al.* 2020; Case *et al.* 2020). A more likely explanation is that pseudoviruses and VLPs
40 typically only measure the capacity of the virus to enter cells during a single round of infection, whereas live
41 virus assays measure virus infection over several rounds of infection since the reporting endpoints rely on the
42 appearance of cytopathic effect, during which the viruses have already spread from cell-to-cell. Therefore, the
43 reported extent of immune evasion associated with Omicron infection may be underestimated with the use of
44 pseudovirus assays alone. In this study, for example, we found 7.4X decreases in median neutralization titers
45 (4,727→635) against Omicron in boosted individuals using the VLP assay but a 21.4X decrease (1,475→69) in
46 titers using the live virus assay, corresponding to 93% and 62% of boosted individuals, respectively, having
47 neutralizing antibody levels above an NT50 cutoff of 40.

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

36

37

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51 **Author contributions**

52 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
53 sequencing efforts and laboratory studies. A.S., M.K.M., A.S-G., N.B., V.S., M.G.K., B.S., M.M.K, A.C.,
54 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.,
55 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
56 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
57 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
58 manuscript. C.Y.C. and V.S. revised the manuscript. All authors read the manuscript and agree to its contents.

59 **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.

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 serum 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 pending). Scripting code used for the data analysis and visualization, a table showing deidentified clinical and demographic metadata, and consensus genome FASTA files are available in a Zenodo data repository pending)

Human Sample Collection and Ethics Statement

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. 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

37 approved by the UCSF Institutional Review Board (protocol numbers 10-01116 and 11-05519). Samples were
38 obtained from pediatric and adult patients of all genders. No analyses based on sex or age were conducted.
39 Second, plasma samples were also collected through the UMPIRE (UCSF EMPLOYEE and community member
40 Immune REsponse) study, a longitudinal COVID-19 research study focused on collection of prospective whole
41 blood and plasma samples from enrolled subjects to evaluate the immune response to vaccination, with and
42 without boosting, and/or vaccine breakthrough infection. Informed consent for participation in the UMPIRE
43 study and collection of data and samples were obtained according to a protocol approved by the UCSF
44 Institutional Review Board (protocol number 20-33083). The UMPIRE study cohorts included (1) fully
45 vaccinated individuals with either 2 doses of Emergency Use Authorization (EUA) authorized mRNA vaccine
46 (Pfizer or Moderna) or 1 dose of the EUA authorized Johnson and Johnson vaccine. Consented participants
47 came to a UCSF CTSI Clinical Research Service (CRS) Laboratory where their blood was drawn by nurses and
48 phlebotomists. At each visit, two to four 3mL EDTA tubes of whole blood were drawn, and one or two EDTA
49 tubes were processed to plasma from each timepoint. Relevant demographic and clinical metadata from
50 UMPIRE participants were obtained through participant Qualtrics surveys performed at enrollment and at each
51 blood draw. Serum samples were heat inactivated at 56°C for 30 mins prior to use in VLP and live virus assays.

53 **Clinical Chart Review**

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

32

33 **Viral Whole-Genome Sequencing**

34 Remnant clinical nasopharyngeal/oropharyngeal (NP/OP) swab samples collected in universal transport
35 media or viral transport media (UTM/VTM) were diluted with DNA/RNA shield (Zymo Research, # R1100-
36 250) in a 1:1 ratio (100 µl primary sample + 100 µl shield). The Omega BioTek MagBind Viral DNA/RNA Kit
37 (Omega Biotek, # M6246-03) and the KingFisher™ Flex Purification System with a 96 deep-well head
38 (ThermoFisher, 5400630) were then used for viral RNA extraction. Extracted RNA was reverse transcribed to
39 complementary DNA and tiling multiplexed amplicon PCR was performed using SARS-CoV-2 primers version
70 3 according to a published protocol (Quick *et al.* 2017). Adapter ligation was performed using the NEBNext®
71 ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®)(New England Biolabs, # E7658L). Libraries were
72 barcoded using NEBNext Multiplex Oligos for Illumina (96 unique dual-index primer pairs) (New England
73 Biolabs, # E6440L) and purified with AMPure XP (Beckman-Coulter, #. Amplicon libraries were then
74 sequenced on either Illumina Miseq or NextSeq 550 as 2x150 paired-end reads (300 cycles).

75 **Genome Assembly and Variant Identification**

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

33 **Serologic testing**

34 SARS-CoV-2-specific antibodies were determined using the Abbott ARCHITECT SARS-CoV-2 IgG
35 (N-based), AdviseDx SARS-CoV-2 IgM (spike receptor-binding domain (RBD)-based), and AdviseDx SARS-
36 CoV-2 IgG II (spike RBD-based) tests according to the manufacturer's specifications.

37 **VLP neutralization assay**

38 For a 6-well, plasmids CoV2-N (0.67), CoV2-M-IRES-E (0.33), CoV-2-Spike (0.0016) and LucT20
39 (1.0) at indicated mass ratios for a total of 4 µg of DNA were diluted in 200 µL optimem. 12 µg PEI was diluted
40 in 200 µL Opti-MEM and added to plasmid dilution quickly to complex the DNA. Transfection mixture was
41 incubated for 20 minutes at room temperature and then added dropwise to 293T cells in 2 mL of DMEM
42 containing fetal bovine serum and penicillin/streptomycin. Media was changed after 24 hours of transfection
43 and At 48 hours posttransfection, VLP containing supernatant was collected and filtered using a 0.45 µm
44 syringe filter. For other culture sizes, the mass of DNA used was 1 µg for 24-well, 4 µg for 6-well, 20 µg for
45 10- cm plate and 60 µg for 15-cm plate. Optimem volumes were 100 µL, 400 µL, 1 mL and 3 mL respectively
46 and PEI was always used at 3:1 mass ratio.

47 Each heat inactivated serum sample was serially diluted from 1:20 to 1:20480 dilution in complete
48 DMEM media prior to incubation (1hr at 37°C) with 40µL VLPs with total volume of 50µL, then plated onto
49 receiver cells (50000 293T ACE2-TMPRSS2 cells). Next day, supernatant was removed and cells were lysed in
50 20 µL passive lysis buffer (Promega) for 15 minutes at room temperature with gentle rocking. Lysates were
51 transferred to an opaque white 96-well plate and 30 µL of reconstituted luciferase assay buffer was added and
52 mixed with each lysate. Luminescence was measured immediately after mixing using a TECAN plate reader.
53 Neutralization titer (NT50) was estimated by fitting the interpolating the dilution of serum at which 50%
54 infectivity was observed.

55 **SARS CoV-2 isolation in cell cultures**

06 Vero E6-TMPRSS2-T2A-ACE2 and Vero-81 were cultured with MEM supplemented with 1x
07 penicillin-streptomycin (Gibco), glutamine (Gibco) and 10% Fetal calf serum (Hyclone). The Vero E6-
08 TMPRSS2-T2A-ACE2 were also supplemented with 10ug/mL puromycin.

09 The Omicron and Delta variants were isolated from a patient NP swab. To isolate Delta, 200ul of a
10 nasopharyngeal (NP) sample that was previously sequence identified as Delta, was diluted 1:3 in PBS
11 supplemented with 0.75% bovine serum albumin (BSA-PBS) and added to confluent Vero-81 cells in a T25
12 flask, allowed to adsorb for 1 hour, then additional media was added and the flask was incubated at 37°C with
13 5% CO₂ with daily monitoring for CPE. When 50% CPE was detected, the contents were collected, clarified by
14 centrifugation and stored at -80C as passage 0 stock. Passaged stock of Delta was made by inoculation of Vero-
15 81 confluent T150 flasks with 1:10 diluted p0 stock, monitored and harvested at approximately 50% CPE.
16 Omicron viral stock was similarly produced from a sequence confirmed NP sample using Vero E6-TMPRSS2-
17 T2A-ACE2 in a T25 and harvested at 90% CPE with no subsequent passaging. Both viral stocks were
18 sequenced to confirm lineage and TCID₅₀ was determined by titration.

19 **Live virus neutralization assay**

20 CPE endpoint neutralization assays were done following the limiting dilution model using p0 stock of
21 Omicron and p1 stock of Delta in Vero E6-TMPRSS2-T2A-ACE2. Patient plasma was diluted 1:10 in BSA-
22 PBS and heat inactivated at 56C for 30 minutes. Serial 3-fold dilution of plasma were made in BSA-PBS.
23 Plasma dilutions were mixed with 100 TCID₅₀ of each virus diluted in BSA-PBS at a 1:1 ratio and incubated
24 for 1 hour at 37C. Final plasma dilutions in plasma-virus mixture ranged from 1:40 to 1:84480. 100ul of the
25 plasma-virus mixtures were added in duplicate to flat bottom 96-well plates pre-seeded with Vero E6-
26 TMPRSS2-T2A-ACE2 at a density of 2.5 x 10⁴/well and incubated in a 37°C incubator with 5% CO₂ until
27 consistent CPE was seen in the virus control (no neutralizing plasma added) wells. Positive and negative
28 controls were included as well as cell control wells and a viral back titration to verify TCID₅₀ viral input.
29 Individual wells were scored for CPE as having a binary outcome of ‘infection’ or ‘no infection’ and the IC₅₀

30 was calculated using the Spearman-Karber method. All steps were done in a Biosafety Level 3 lab using
31 approved protocols.

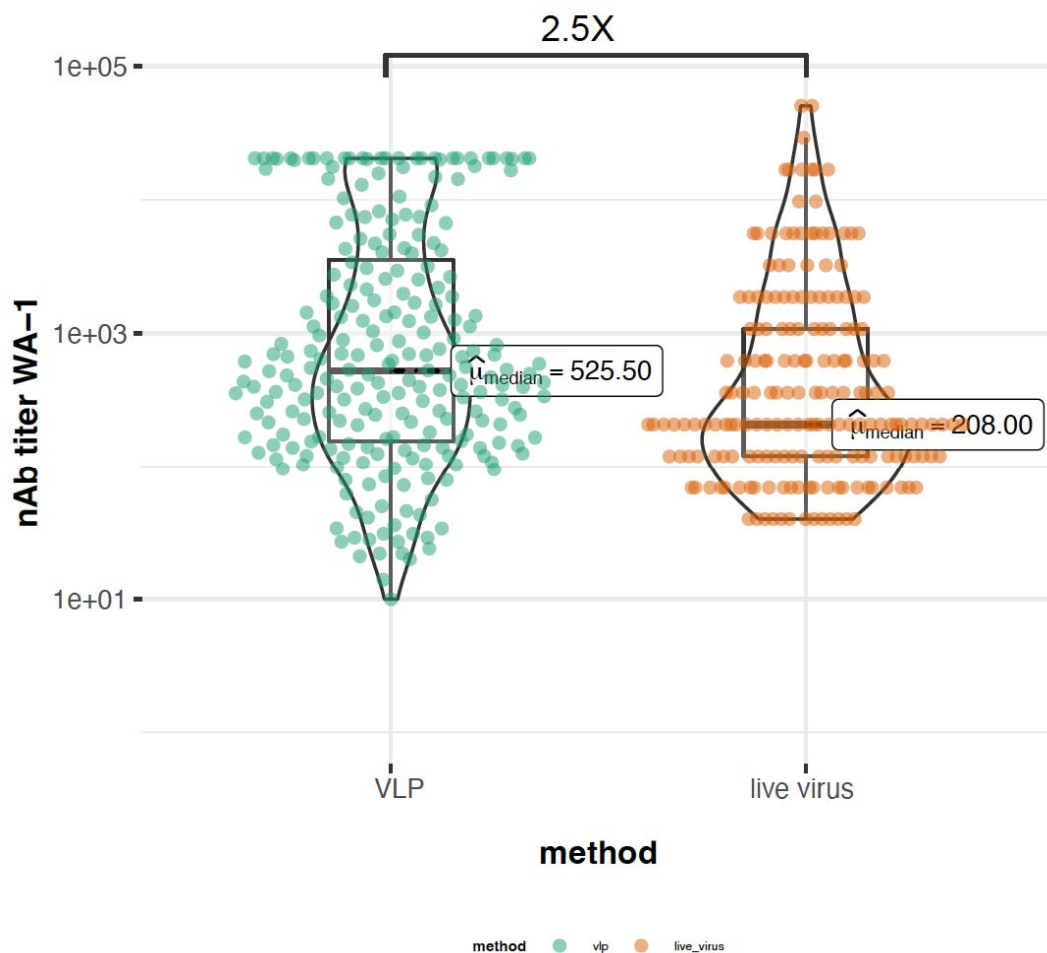
32 **Statistical Analyses and Data Visualization**

33 Statistical analyses and data visualization were performed using R (version 4.0.3) and Python (version
34 3.7.10). Fisher's exact test was used to evaluate associations of demographic and clinical variables with variant-
35 specific breakthrough infections. Fold decreases in neutralizing activity were measured by comparing median
36 neutralizing antibody titers. Significance testing was performed using the Wilcoxon signed-rank test and Mann-
37 Whitney U test for paired and unpaired samples, respectively. Correlation coefficients were calculated using
38 Spearman's rank analysis. Plots were generated using ggplot2 (version 3.3.5) in R and seaborn package (version
39 0.11.0) in Python. All statistical tests were conducted as two-sided at the 0.05 significance level.

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12 Supplemental Figures



13

14 **Figure S1. VLP and live virus neutralization assay median neutralizing antibody titers.** Plot showing the
15 difference in median neutralizing antibody titers to WT lineage between VLP-based and live virus-based assay.

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