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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),

30 quantitative antibody assay, variant of concern, pseudovirus infectivity studies, humoral immunity

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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

44 **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).

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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.
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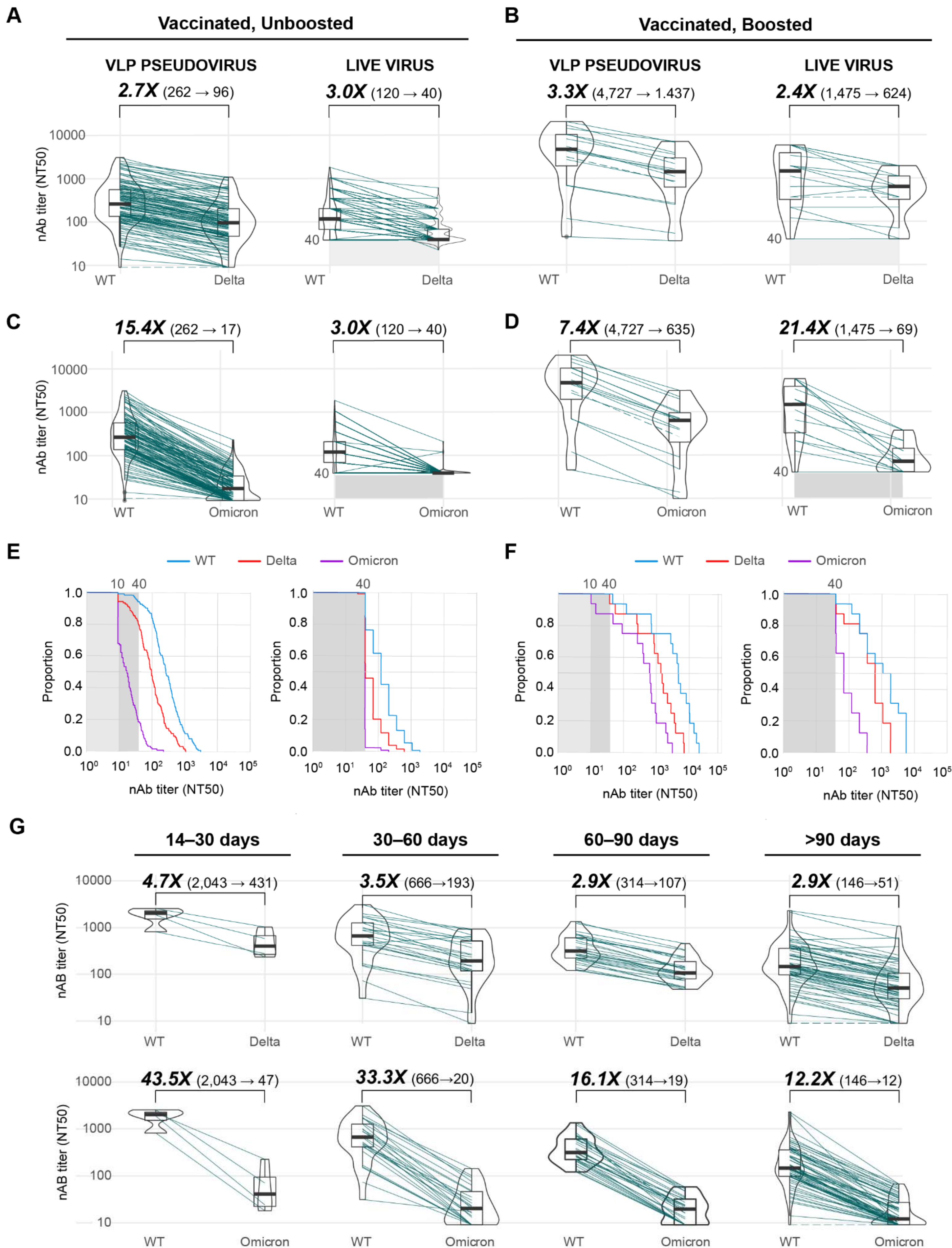

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67 **Figure 1. Neutralizing antibody levels in fully vaccinated, uninfected individuals.** **(A, B)** Box-violin plots
68 showing median neutralizing antibody titers using VLP (left) and live virus (right) assays against the SARS-
69 CoV-2 WA-1 ancestral lineage (wild-type, or "WT") and Delta variant in vaccinated, unboosted **(A)** and
70 vaccinated, boosted **(B)** individuals **(C, D)** Box-violin plots of titers against the WT and Omicron variant in
71 vaccinated, unboosted **(C)** and vaccinated, boosted **(D)** individuals. **(E, F)** Cumulative distribution function
72 plots of titers to WT, Delta, and Omicron using VLP (left) and live virus (right) assays in vaccinated, unboosted
73 **(E)** and vaccinated, boosted **(F)** individuals, showing the proportion of samples at or above a given titer. **(G)**
74 Longitudinal box-violin plots of VLP titers to Delta (top) and Omicron (bottom) stratified by time ranges
75 following completion of a primary vaccine series.
76

77 **Results**

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

80 VLP and live virus neutralization assays were performed in parallel on 144 plasma samples collected
81 from 81 subjects enrolled in a prospectively enrolled longitudinal cohort (the UMPIRE, or "UCSF eMPloyee
82 and community Immune REsponse study"), 18 (22.2%) of whom had received a booster and none of whom
83 were previously infected. We chose available samples from the earliest and most recent time points
84 collected from each subject ≥14 days after the last vaccine dose for neutralization testing. Sample collection
85 dates for fully vaccinated, non-boosted individuals (n=63) ranged from 14 to 305 days (median = 91 days)
86 following completion of the primary series of 2 doses for an mRNA vaccine (BNT162b2 from Pfizer or mRNA-
87 1273 from Moderna) or 1 dose of the adenovirus vector vaccine (Ad26.COV2.S from Johnson and Johnson); for
88 boosted individuals (n=18), collection dates ranged from 2 to 74 days (median = 23 days) following the booster
89 dose. Overall, median neutralizing antibody titers were 2.5-fold lower using live viruses compared to VLPs
90 **(Supplementary Figure 1).**

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In non-boosted vaccinated individuals, median VLP neutralizing antibody titers to Delta and Omicron relative to the ancestral wild-type (WT) virus WA-1 lineage were reduced 2.7-fold (262 → 17,expressed as NT50 titers, or titers needed to neutralize 50% of VLP activity) and 15.4-fold (262 → 96), respectively **(Figure 1A and B).** In comparison, live virus neutralization titers to Delta and Omicron were both reduced 3.0-fold (120 → 40), with the lower reduction for Omicron accounted for by the higher limit of detection (LOD) for the live virus (NT50 = 40) compared to the VLP neutralization assay (NT50 = 10). Using VLPs, the proportion of individuals with neutralizing antibodies to 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, baseline VLP titers to WT were 18-fold higher (4,727 versus 262) than in non-boosted individuals **(Figure 1A, B, D, and E, left),** and decreases in titers to 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 to all 3 lineages above an NT50 cutoff of 40 **(Figure 1F, left).** In contrast, live virus neutralization titers in boosted individuals showed a 21.4-fold (1,475→69) decrease in titers to Omicron relative to WT **(Figure 1E, right)**, with only ~62% of boosted individuals having neutralizing antibodies to Omicron **(Figure 1F, right).** Median VLP neutralization titers to WT decreased by 93% (14-fold, 2,043→146) over >3 months following vaccination, with decreases in titers to Delta and Omicron of 2.9-–4.7-fold and 12.2-43.5-fold, respectively, relative to WT **(Figure 1G).**

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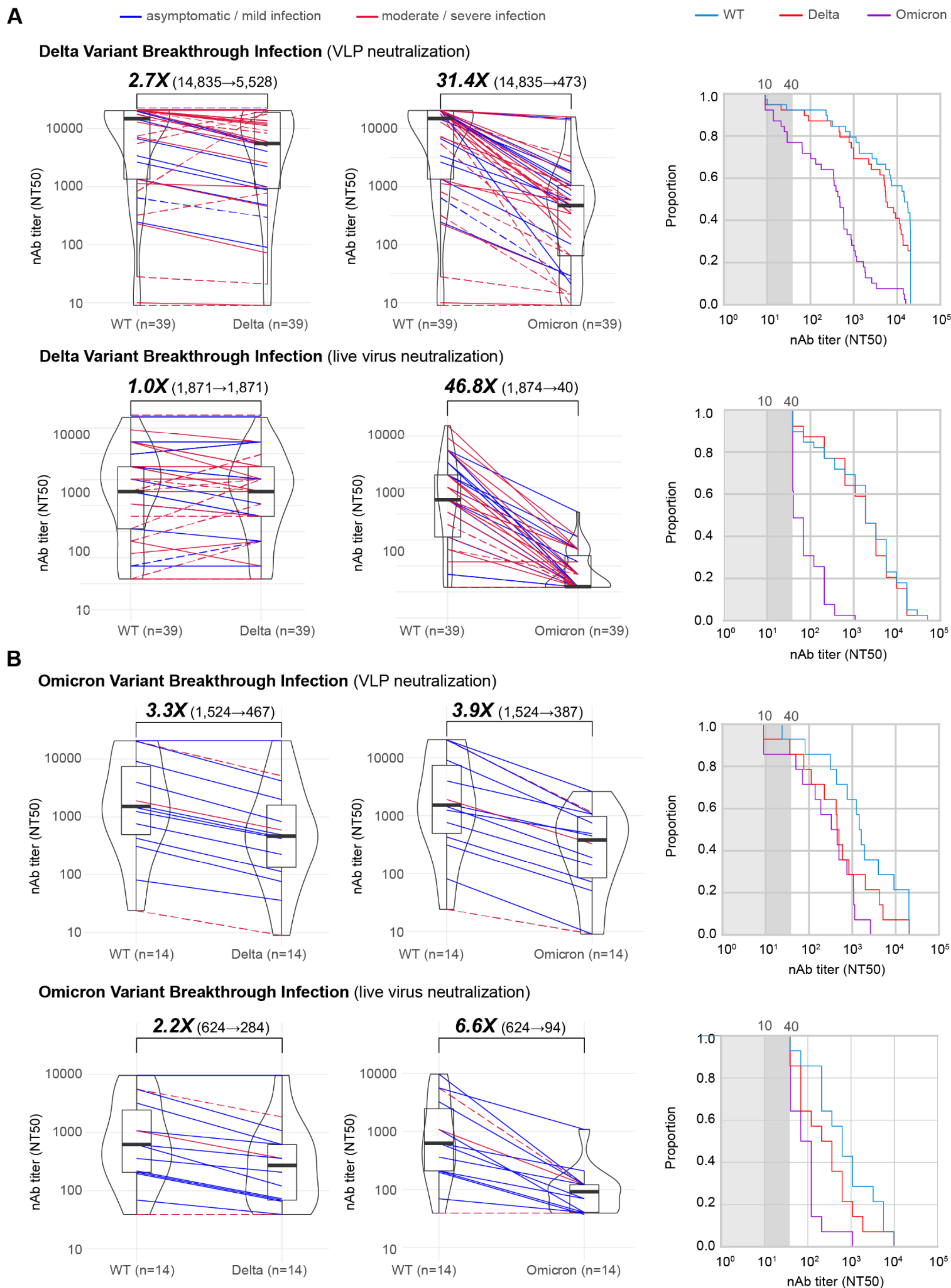

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Figure 2. Neutralizing antibody levels in Delta and Omicron breakthrough infections. (A) Box-violin plots of median neutralizing antibody titers against Delta (left) and Omicron (middle) variants compared to WT, along with cumulative distribution function 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,**

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

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

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

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

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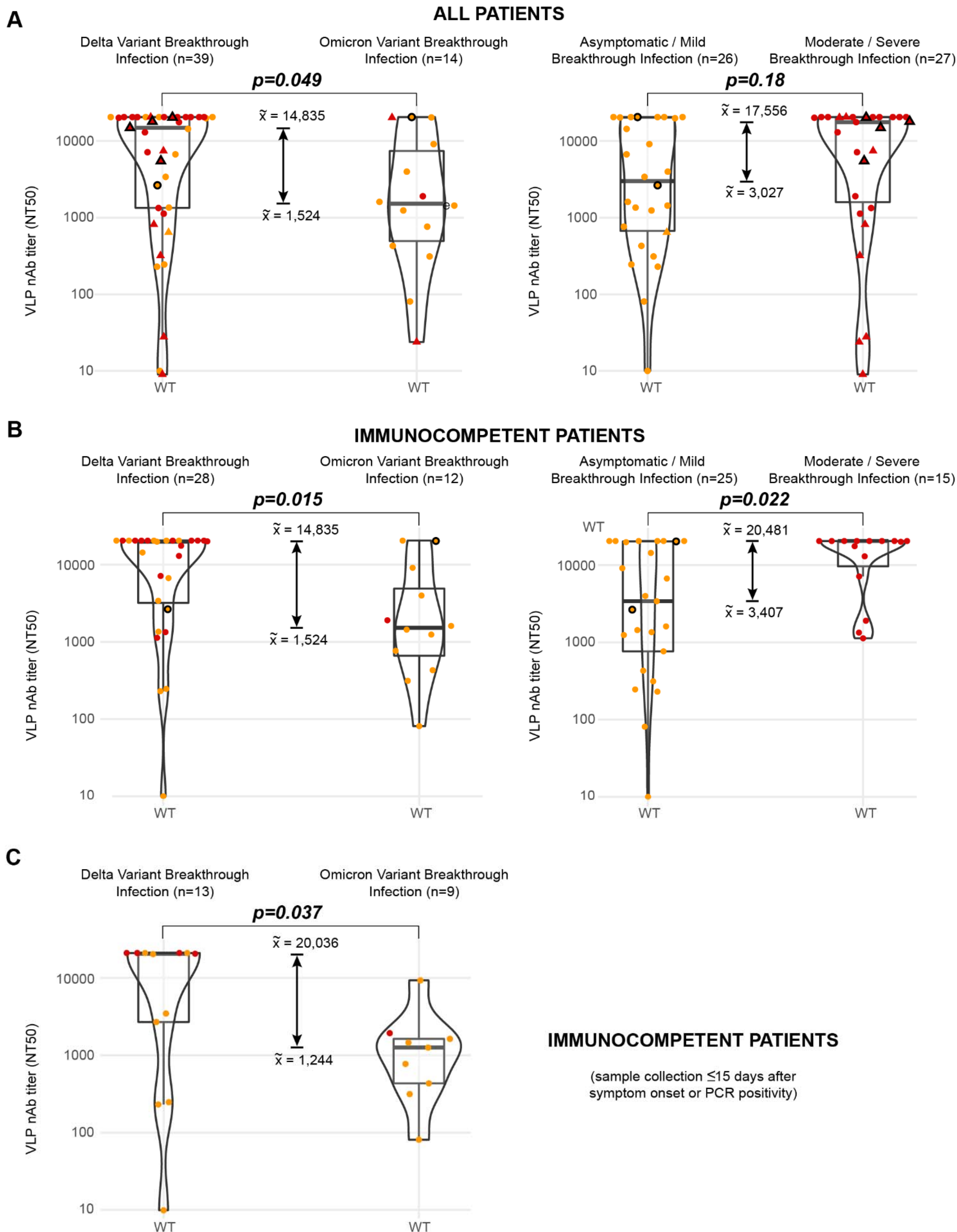

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364 **Figure 3. Comparison of neutralizing antibody titers against the WT lineage in Delta and Omicron**
365 **breakthrough infections.** **(A)** Box-violin plots comparing neutralizing antibody titers against the WT lineage
366 between Delta and Omicron breakthrough infections (left) and between asymptomatic or mild and moderate or
367 severe breakthrough infections (right) for all patients in the study. **(B)** Corresponding box-violin plots for
368 immunocompetent patients only. **(C)** Box-violin plots between Delta and Omicron breakthrough infections for
369 samples that were collected ≤15 days after symptom onset or PCR positivity. P-values for significance were
370 determined using the Mann-Whitney U test.
371

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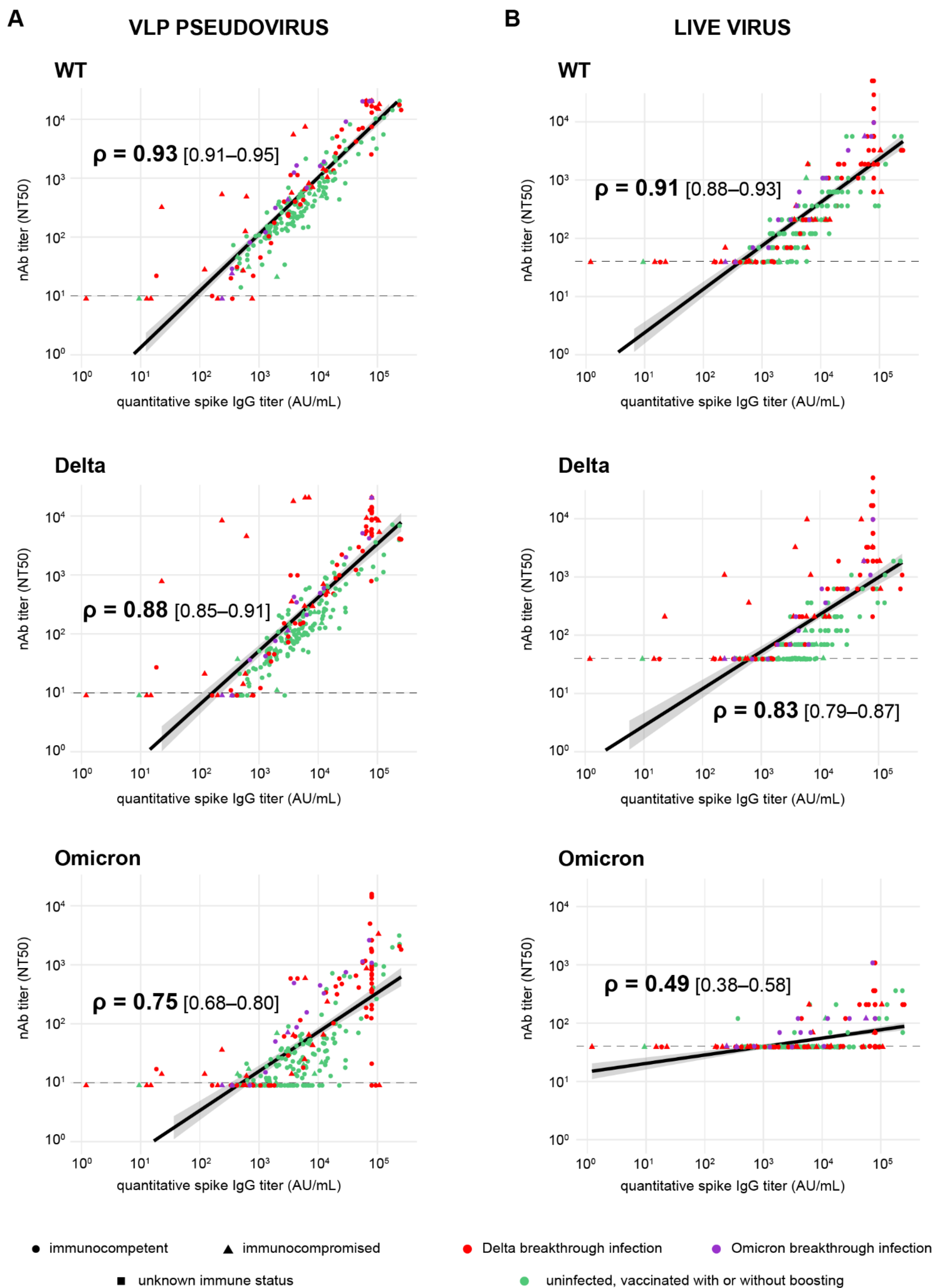

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773 **Figure 4. Correlation between quantitative spike IgG and neutralizing antibody titers. (A)** Plots showing
774 correlation between spike IgG titers and neutralizing antibodies directed against WT (top), Delta (middle) and
775 Omicron (bottom)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

778

779

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			

780

781 **Table 1. Clinical and demographic characteristics in Delta and Omicron variant breakthrough infections.**
782 P-values for significance were determined using two-tailed Fisher's Exact Test for the categorical variables and
783 the Mann-Whitney U test for the days between sample collection and symptom onset or PCR positivity.

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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 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

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311 moderate levels of spike IgG antibody failed to neutralize Omicron in the live virus assay **(Figure 4B, bottom).**
312 Quantitative NT50 titers of 10^3–10^4 and >10^5 reliably predicted Delta and Omicron neutralization, respectively.
313

314 **Discussion**

315 Here we used VLP and live virus neutralization assays to investigate neutralizing antibody responses in
316 125 vaccinated individuals, both non-boosted and boosted, and after Delta and Omicron vaccine breakthrough
317 infections. Our results suggest that vaccine boosting and breakthrough infections can restore broad neutralizing
318 hybrid immunity by increasing baseline titers, with higher relative titers against the infecting variant. Notably,
319 Delta-specific titers in Delta breakthroughs rose to become comparable to levels against WT, while Omicron-
320 specific titers in Omicron breakthroughs rose to become comparable to levels against Delta. However, we also
321 found that the magnitude of increase in baseline titers is dependent on the clinical severity of the breakthrough
322 infection. Increased neutralizing antibody levels in Delta related to Omicron breakthrough cases (p=0.049) was
323 likely due to an increased proportion of moderate to severe infections in the Delta compared to the Omicron
324 cohort (p=0.014, 61.5% versus 21.4%). Indeed, moderate to severe clinical infections in both Delta and
325 Omicron breakthrough cohorts were associated with significantly higher neutralization titers than mild or
326 asymptomatic infections among immunocompetent patients (p=0.022).

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

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

Our findings have implications with regard to the likelihood of Omicron infections providing mass immunization on the population level against SARS-CoV-2. Widespread infections from Omicron globally both in vaccinated and unvaccinated persons, have been reported, and Omicron has 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 suggests that Omicron may be rapidly outcompeting more pathogenic variants such as Delta (Outbreak.info, 2022). These observations raise the prospect of Omicron being 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, we found a significantly smaller rise of neutralization titers associated with milder Omicron breakthrough infection in vaccinated individuals, to only approximately one-third of the rise associated with boosting. We also identified limited cross-variant immunity to Delta. Thus, breakthrough infection from Omicron may enhance cross-protection against Delta, and vice-versa, inasmuch as there is a sufficiently large increase in baseline neutralizing immunity, which appears to be related to the clinical severity of the infection. Our findings parallel those from another study from our group that demonstrated limited cross-variant immunity after milder Omicron variant infection in unvaccinated individuals in a mouse model and in human patients (Suryawanshi *et al*., 2022). Taken together, our results suggest that Omicron-induced immunity may not be sufficient to prevent infection from another, more pathogenic variant, should it emerge in the future. They also highlight the continued importance of vaccine boosters in enhancing immunity, as breakthrough infection alone may not be reliable in eliciting protective titers against re-infection or future infection from different variants. Furthermore, the relative increase in immunity against the infecting variant in breakthrough infections indicates that the use of variant-specific immunogens in vaccine development remains a viable strategy for addressing VOCs that continue to circulate in the population.

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361 Results from the live virus neutralization studies consistently showed lower titers than those using
362 VLPs, which are similar to spike-pseudotyped viruses. The majority of SARS-CoV-2 neutralization studies
363 reported to date have used pseudoviruses because the protocols for running these assays have been reliable,
364 safe, and convenient. Of note, the VLPs used in this study incorporate all of the Omicron-specific mutations
365 found in the structural spike, nucleocapsid, matrix, and fusion proteins (Syed *et al.* 2022), and not only in the
366 spike protein, as is the case for most pseudovirus assays. One possibility for the discrepant neutralization results
367 may be the use of different cell lines for the VLP (293T) and live virus (Vero) assays, although both cell lines
368 are highly susceptible and permissive to SARS-CoV-2 given stable expression of the ACE2 and TMPRSS2
369 receptors (Hoffmann *et al.* 2020; Case *et al*. 2020). A more likely explanation is that pseudoviruses and VLPs
370 typically only measure the capacity of the virus to enter cells during a single round of infection, whereas live
371 virus assays measure virus infection over several rounds of infection since the reporting endpoints rely on the
372 appearance of cytopathic effect, during which the viruses have already spread from cell-to-cell. Therefore, the
373 reported extent of immune evasion associated with Omicron infection may be underestimated with the use of
374 pseudovirus assays alone. In this study, for example, we found 7.4X decreases in median neutralization titers
375 (4,727→635) against Omicron in boosted individuals using the VLP assay but a 21.4X decrease (1,475→69) in
376 titers using the live virus assay, corresponding to 93% and 62% of boosted individuals, respectively, having
377 neutralizing antibody levels above an NT50 cutoff of 40.

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

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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.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

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12 C.Y.C. is the director of the UCSF-Abbott Viral Diagnostics and Discovery and receives research support for
13 SARS-CoV-2 studies from Abbott Laboratories. The other authors declare no competing interests.
14
15 **STAR Methods**
16
17 **RESOURCE AVAILABILITY**
18 **Lead contact**
19 Further information and requests for resources and reagents should be directed to and will be fulfilled by the
20 Lead Contact, Charles Chiu (charles.chiu@ucsf.edu).
21
22 **Materials availability**
23 Passaged aliquots of the cultured SARS-CoV-2 Omicron virus, synthetic VLPs (virus-like particles), and
24 available remaining clinical nasal swab and serum samples are available upon request**.**
25
26 **Data and code availability**
27 Assembled SARS-CoV-2 genomes in this study were uploaded to GISAID (Shu and McCauley, 2017)
28 (accession numbers pending). Scripting code used for the data analysis and visualization, a table showing
29 deidentified clinical and demographic metadata, and consensus genome FASTA files are available in a Zenodo
30 data repository pending)
31
32 **Human Sample Collection and Ethics Statement**
33 Blood samples were collected through two methods. First, remnant whole blood and plasma samples
34 from patients hospitalized with COVID-19 at UCSF were retrieved from UCSF Clinical Laboratories daily
35 based on availability. Remnant samples were biobanked and retrospective medical chart reviews for relevant
36 demographic and clinical metadata were performed under a waiver of consent and according to protocols

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approved by the UCSF Institutional Review Board (protocol numbers 10-01116 and 11-05519). 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 (UCSF EMPloyee and community member Immune REsponse) 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. 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 cohorts included (1) 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. 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 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. Serum 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 to severe infection were classified as having a mild or asymptomatic infection.

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562

563 **Viral Whole-Genome Sequencing**

564 Remnant clinical nasopharyngeal/oropharyngeal (NP/OP) swab samples collected in universal transport
565 media or viral transport media (UTM/VTM) were diluted with DNA/RNA shield (Zymo Research, # R1100-
566 250) in a 1:1 ratio (100 µl primary sample + 100 µl shield). The Omega BioTek MagBind Viral DNA/RNA Kit
567 (Omega Biotek, # M6246-03) and the KingFisherTM Flex Purification System with a 96 deep-well head
568 (ThermoFisher, 5400630) were then used for viral RNA extraction. Extracted RNA was reverse transcribed to
569 complementary DNA and tiling multiplexed amplicon PCR was performed using SARS-CoV-2 primers version
570 3 according to a published protocol (Quick *et al*. 2017). Adapter ligation was performed using the NEBNext®
571 ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®)(New England Biolabs, # E7658L). Libraries were
572 barcoded using NEBNext Multiplex Oligos for Illumina (96 unique dual-index primer pairs) (New England
573 Biolabs, # E6440L) and purified with AMPure XP (Beckman-Coulter, #. Amplicon libraries were then
574 sequenced on either Illumina Miseq or NextSeq 550 as 2x150 paired-end reads (300 cycles).

575 **Genome Assembly and Variant Identification**

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

583 **Serologic testing**

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

VLP neutralization assay

For a 6-well, 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 4 μg of DNA were diluted in 200 μL optimem. 12 μg PEI was diluted in 200 μL Opti-MEM and added to plasmid dilution quickly to complex the DNA. Transfection mixture was incubated for 20 minutes at room temperature and then added dropwise to 293T cells in 2 mL of DMEM containing fetal bovine serum and penicillin/streptomycin. Media was changed after 24 hours of transfection and At 48 hours posttransfection, VLP containing supernatant was collected and filtered using a 0.45 μm 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 10- cm plate and 60 μg for 15-cm plate. Optimem volumes were 100 μL, 400 μL, 1 mL and 3 mL respectively and PEI was always used at 3:1 mass ratio.

Each heat inactivated serum sample was serially diluted from 1:20 to 1:20480 dilution in complete DMEM media prior to incubation (1hr at 37°C) with 40μL VLPs with total volume of 50μL, then plated onto receiver cells (50000 293T ACE2-TMPRSS2 cells). Next day, supernatant was removed and cells were lysed in 20 μL passive lysis buffer (Promega) for 15 minutes at room temperature with gentle rocking. 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 interpolating the dilution of serum at which 50% infectivity was observed.

SARS CoV-2 isolation in cell cultures

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Vero E6-TMPRSS2-T2A-ACE2 and Vero-81 were cultured with 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.

The Omicron and Delta variants were isolated from a patient NP swab. To isolate Delta, 200ul of a nasopharyngeal (NP) sample that was previously sequence identified as Delta, was diluted 1:3 in PBS supplemented with 0.75% bovine serum albumin (BSA-PBS) and added to confluent Vero-81 cells in a T25 flask, allowed to adsorb for 1 hour, then additional media was added and the flask was incubated at 37˚C with 5% CO2 with daily monitoring for 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 of Vero-81 confluent T150 flasks with 1:10 diluted p0 stock, monitored and harvested 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 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 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 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 were 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 IC50

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

532 **Statistical Analyses and Data Visualization**

533 Statistical analyses and data visualization were performed using R (version 4.0.3) and Python (version
534 3.7.10). Fisher's exact test was used to evaluate associations of demographic and clinical variables with variant-
535 specific breakthrough infections. Fold decreases in neutralizing activity were measured by comparing median
536 neutralizing antibody titers. Significance testing was performed using the Wilcoxon signed-rank test and Mann-
537 Whitney U test for paired and unpaired samples, respectively. Correlation coefficients were calculated using
538 Spearman's rank analysis. Plots were generated using ggplot2 (version 3.3.5) in R and seaborn package (version
539 0.11.0) in Python. All statistical tests were conducted as two-sided at the 0.05 significance level.

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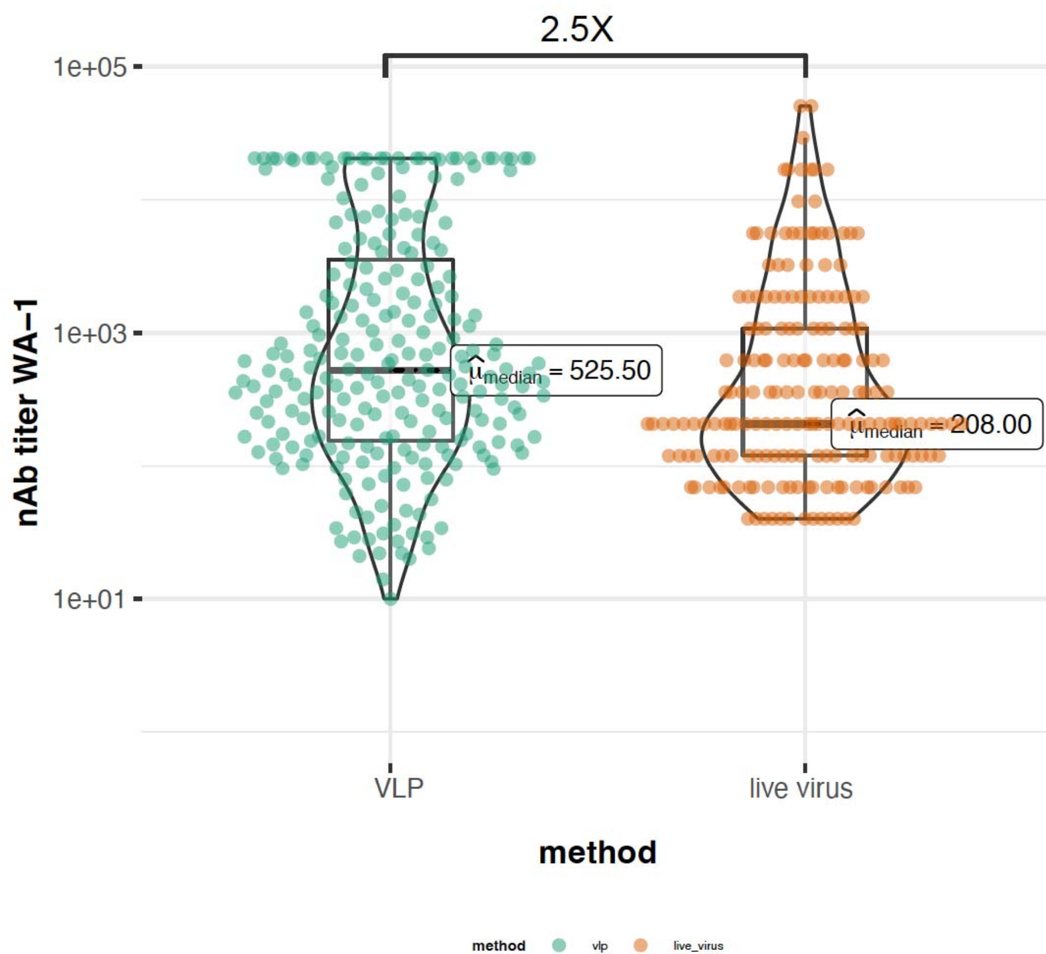
442 **Supplemental Figures**



443

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

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References

Cao, Y., Wang, J., Jian, F. *et al*. (2021). Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* (2021). doi: https://doi.org/10.1038/s41586-021-04385-3.

Case, J. B., Rothlauf, P. W., Chen, R. E. *et al*. (2020). Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2. *Cell Host Microbe*. 28(3): 475-485. doi: 10.1016/j.chom.2020.06.021.

CDC COVID-19 Response Team. (2021). SARS-CoV-2 B.1.1.529 (Omicron) Variant — United States, December 1–8, 2021. *MMWR Morb Mortal Wkly Rep*. 70(50): 1731–1734. doi: 10.15585/mmwr.mm7050e1.

CDPH. (2022). Variants (California Department of Public Health). Retrieved from: https://covid19.ca.gov/variants/. Accessed 1/24/22.

Davies, M., Kassanjee, R., Rousseau, P. *et al*. (2022). Outcomes of laboratory-confirmed SARS-CoV-2 infection in the Omicron-driven fourth wave compared with previous waves in the Western Cape Province, South Africa. *medRxiv*. doi: 10.1101/2022.01.12.22269148 (preprint).

Dyson, L., Hill, E.M., Moore, S. *et al*. (2021) Possible future waves of SARS-CoV-2 infection generated by variants of concern with a range of characteristics. *Nat Commun*. 12: 5730. doi: https://doi.org/10.1038/s41467-021-25915-7.

Flemming, A. (2022). Omicron, the great escape artist. *Nat Rev Immunol*. doi: https://doi.org/10.1038/s41577-022-00676-6.

medRxiv preprint doi: https://doi.org/10.1101/2022.01.25.22269794; this version posted January 26, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
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Gangavarapu, K., Alkuzweny, M., Cano, M. *et al*. (2020). outbreak.info), https://outbreak.info/

Gilbert, P. B., Montefiori, D. C., McDermott, A. B. *et al*. (2021). Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*. 375(6576): 43-50. doi: 10.1126/science.abm3425.

Grubaugh, N.D., Gangavarapu, K., Quick, J. *et al*. (2019). An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol*. 20: 8. doi: https://doi.org/10.1186/s13059-018-1618-7.

Hoffman, M., Kleine-Weber, H., Schroeder, S. *et al*. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 181(2): 271–280. doi: 10.1016/j.cell.2020.02.052.

Khan, K., Karim, F., Cele, S. *et al*. (2021). Omicron infection enhances neutralizing immunity against the Delta variant. *medRxiv*, doi: 10.1101/2021.12.27.21268439 (preprint).

Laurie, M. T., Liu, J., Sunshine, S. *et al*. (2022). SARS-CoV-2 variant exposures elicit antibody responses with differential cross-neutralization of established and emerging strains including Delta and Omicron. *The Journal of Infectious Diseases*. doi: https://doi.org/10.1093/infdis/jiab635.

Outbreak.info. (2022). Retrieved from: https://outbreak.info/. Accessed 1/24/21.

medRxiv preprint doi: https://doi.org/10.1101/2022.01.25.22269794; this version posted January 26, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted medRxiv a license to display the preprint in perpetuity.
It is made available under a CC-BY-NC-ND 4.0 International license .

Quick, J., Grubaugh, N., Pullan, S. *et al*. (2017). Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. *Nat Protoc*. 12: 1261–1276. doi: https://doi.org/10.1038/nprot.2017.066.

Rambaut, A., Holmes, E.C., O'Toole, Á. *et al*. (2020). A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol*. 5: 1403–1407. doi: https://doi.org/10.1038/s41564-020-0770-5.

Shu Y., McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22:30494.

Suryawanshi, R.K., Chen, I.P., Ma, T. *et al.* (2022). Limited cross-variant immunity after infection with the SARS-CoV-2 Omicron variant without vaccination. *medRxiv*, doi: 10.1101/2022.01.13.22269243 (preprint).

Syed, A. M., Taha, T. Y., Tabata, T. *et al*. (2021). Rapid assessment of SARS-CoV-2–evolved variants using virus-like particles. *Science*. 374(6575): 1626-1632. doi: 10.1126/science.abl6184.

Syed, A.M., Ceiling, A., Khalid, M.M. *et al.* (2022). Omicron mutations enhance infectivity and reduce antibody neutralization of SARS-CoV-2 virus-like particles. *medRxiv*. doi: 10.101/2021.12.20.21268048 (preprint).

VanBlargan, L.A., Errico, J.M., Halfmann, P.J. *et al*. (2022). An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nat Med*. doi: https://doi.org/10.1038/s41591-021-01678-y.

medRxiv preprint doi: https://doi.org/10.1101/2022.01.25.22269794; this version posted January 26, 2022. The copyright holder for this preprint
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21 Wolter, N., Jassat, W., Walaza, S. *et al*. (2022). Early assessment of the clinical severity of the SARS-CoV-2
22 omicron variant in South Africa: a data linkage study. *Lancet*. S0140-6736(22): 00017-4. doi: 10.1016/S0140-
23 6736(22)00017-4.