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1 **Neutralizing immunity induced against the Omicron BA.1 and BA.2 variants in vaccine**
2 **breakthrough infections**

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1 All authors listed have contributed significantly to the study, have seen, and have approved the
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3 publication by a journal other than *Journal of Infectious Diseases*.
4

5 **Running Title:** Neutralizing immunity against Omicron
6

7 **Key Points:** Neutralizing immunity was stronger against BA.2 than BA.1, regardless of infecting
8 variant. Cross-variant neutralization was minimal in unvaccinated infections but moderate to
9 strong in vaccine breakthrough infections. Observed differences in neutralizing immunity
10 between unboosted and boosted breakthrough infections were comparable.

ACCEPTED MANUSCRIPT

1 **Abstract**

2 **Background**

3 As of early 2022, the Omicron variants are the predominant circulating lineages globally.
4 Understanding neutralizing antibody responses against Omicron BA.1 and BA.2 following
5 vaccine breakthrough infections will provide insights into BA.2 infectivity and susceptibility to
6 subsequent re-infection.

7 **Methods**

8 Live virus neutralization assays were used to study immunity against Delta and Omicron BA.1
9 and BA.2 variants in samples from 86 individuals, 24 unvaccinated (27.9%) and 63 vaccinated
10 (72.1%), who were infected with Delta (n=42, 48.8%) or BA.1 (n=44, 51.2%). Among the 63
11 vaccinated individuals, 39 were unboosted (45.3%), while 23 were boosted (26.7%).

12 **Results**

13 In unvaccinated infections, neutralizing antibodies (nAbs) against the three variants were weak
14 or undetectable, except against Delta for Delta-infected individuals. Both Delta and BA.1
15 breakthrough infections resulted in strong nAb responses against ancestral wild-type and Delta
16 lineages, but moderate nAb responses against BA.1 and BA.2, with similar titers between
17 unboosted and boosted individuals. Antibody titers against BA.2 were generally higher than
18 those against BA.1 in breakthrough infections.

19 **Conclusions**

20 These results underscore the decreased immunogenicity of BA.1 as compared to BA.2,
21 insufficient neutralizing immunity against BA.2 in unvaccinated individuals, and moderate to
22 strong neutralizing immunity induced against BA.2 in Delta and BA.1 breakthrough infections.

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1 Word count: 197

2

3 **Key Words:** Omicron BA.2, Omicron BA.2, and Delta SARS-CoV-2 variants; COVID-19;
4 breakthrough infection; neutralizing antibodies; vaccine boosting

5

6 **Introduction**

7 Following its emergence in November 2021, the Severe Acute Respiratory Syndrome
8 Coronavirus 2 (SARS-CoV-2) Omicron (B.1.1.529) variant spread rapidly to become the
9 predominant variant globally by early 2022 [1-3]. Akin to the evolution of most SARS-CoV-2
10 variants of concern/variants of interest (VOC/VOI), continual mutations gave rise to multiple
11 Omicron sub-lineages, including BA.1 and BA.2 [4]. At the end of February 2022, the World
12 Health Organization (WHO) issued a statement of concern regarding the increasing prevalence
13 of the BA.2 sub-lineage worldwide. BA.2 is more transmissible than BA.1 without causing more
14 severe clinical outcomes [5-7]. Despite being classified as the same variant, the BA.1 and BA.2
15 sub-lineages differ by 40 mutations, and the two sub-lineages are antigenically distinct [8].

16 Unvaccinated individuals infected with BA.1 generate weak cross-neutralizing antibody
17 responses against other VOCs [9, 10], while vaccinated individuals with BA.1 or Delta
18 breakthrough infections generate moderate to strong cross-neutralizing responses [11-13].

19 Whether this induced broad-based immunity extends to the BA.2 sub-lineage remains largely
20 unexplored. Either vaccination alone without prior or subsequent infection or infection alone
21 without vaccination fails to generate a robust neutralizing antibody response against BA.2 in
22 convalescent samples [14]. However, little is known regarding the “hybrid” immunity induced
23 against BA.2 in vaccinated individuals who are subsequently infected with BA.1 or Delta. This

1 study used live virus neutralization assays to evaluate differential neutralizing antibody
2 responses against BA.1, BA.2, Delta, and ancestral WA-1 wildtype (WT) lineages in
3 unvaccinated and vaccinated individuals infected with either Delta or BA.1.

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7 **Methods**

8 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

9 **Human Subjects**

10 Human subjects in this study included patients hospitalized with COVID-19 at University
11 of California, San Francisco (UCSF) and consenting SARS-CoV-2 infected individuals enrolled
12 in one of two longitudinal prospective studies at UCSF. The first UMPIRE (UCSF EMPLOYEE
13 and community member Immune REsponse) study focused on collection of prospective whole
14 blood and plasma samples to evaluate the immune response to COVID-19 vaccination [11],
15 while the second study of SARS-CoV-2 household transmission at UCSF has been federally
16 designated as a public health surveillance project [15] (**Table 1**). For hospitalized UCSF patients,
17 remnant samples were biobanked and retrospective medical chart reviews for demographic and
18 clinical metadata were performed under a waiver of consent and according to protocols approved
19 by the UCSF Institutional Review Board (protocol numbers 10-01116 and 11-05519). Informed
20 consent for participation in the UMPIRE study and protocols for data and sample collection were
21 approved by the UCSF Institutional Review Board (protocol number 20-33083). For the
22 household transmission study, written informed consent was obtained from all subjects and
23 protocols for sample and data collection were approved by the UCSF Institutional Review Board.

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1 **Cell Lines**

2 For SARS-CoV-2 isolation in cell cultures and live virus assay, Vero E6-TMPRSS2-
3 T2A-ACE2 and Vero-81 derived from African green monkey kidney, were cultured at 37°C in
4 Modified Eagle Medium (MEM) supplemented with 1x penicillin-streptomycin (Gibco),
5 glutamine (Gibco), and 10% fetal calf serum (Hyclone). The Vero E6-TMPRSS2-T2A-ACE2
6 were supplemented with 10ug/mL puromycin. The Vero-81 cell line was authenticated by and
7 ordered from the American Tissue Culture Collection (ATCC). Re-authentication of this cell line
8 was not conducted prior to use.

10 **METHOD DETAILS**

11 **Human Sample Collection**

12 Blood samples were collected through three different protocols. First, remnant plasma
13 samples from patients of any age and gender hospitalized with COVID-19 at UCSF were
14 retrieved from UCSF Clinical Laboratories . Clinical data from hospitalized UCSF patients were
15 extracted through retrospective chart review. Second, plasma samples were collected through the
16 UMPIRE study from unboosted and boosted subjects. Consenting participants came to a UCSF
17 Clinical Research Service Laboratory for blood collection at approximately 1, 2, and 6-month
18 intervals following breakthrough infection. Demographic and clinical metadata from UMPIRE
19 participants were obtained through Qualtric surveys performed at enrollment and each visit.
20 Finally, blood samples and patient clinical metadata were obtained through the household
21 transmission study: a field team collected blood samples from non-hospitalized participants at
22 their residences and interviewers administered questionnaires by phone to collect
23 sociodemographic and clinical data. Subjects were enrolled within 5 days of symptom onset of

1 the first SARS-CoV-2-positive case in the household and samples and metadata were collected
2 during weekly visits for 28 days (25).

3

4 **Clinical Chart Review**

5 Moderately severe infections included hospitalization for COVID-19 pneumonia with an
6 oxygen requirement of >2 L by nasal cannula or another infectious complication of the disease.
7 Severe infections included COVID-19 pneumonia with severe hypoxemia with an oxygen
8 requirement of >6 L, the need for CPAP, BIPAP, intubation with mechanical ventilation,
9 COVID-19 associated end-organ failure, and/or death. Outpatients and hospitalized patients not
10 meeting criteria for moderate or severe infection were classified as having a mild or
11 asymptomatic infection.

12 Immunocompromised patients included patients on immunosuppressive therapy due to
13 active malignancies, patients on immunosuppressive medication following solid organ or bone
14 marrow transplantation, and patients with any disease resulting in a severe immunodeficiency.

15

16 **SARS-CoV-2 Whole-Genome Sequencing**

17 Viral whole-genome sequencing of SARS-CoV-2 was performed as previously described
18 [11]. Remnant clinical nasopharyngeal/oropharyngeal (NP/OP) swab samples collected in
19 universal transport media or viral transport media (UTM/VTM) were diluted with DNA/RNA
20 shield (Zymo Research, # R1100-250) in a 1:1 ratio (100 µl primary sample + 100 µl shield)
21 prior to viral RNA extraction. The Omega BioTek MagBind Viral DNA/RNA Kit (Omega
22 Biotek, # M6246-03) and the KingFisher™ Flex Purification System with a 96 deep-well head
23 (ThermoFisher, 5400630) were used for viral RNA extraction. Extracted RNA was reverse
24 transcribed to complementary DNA and tiling multiplexed amplicon PCR was performed using

1 Artic version 3 and/or VarSkip SARS-CoV-2 primers (New England Biolabs). Adapter ligation
2 was performed using the NEBNext® ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina®)(New
3 England Biolabs, # E7658L). Libraries were barcoded using NEBNext Multiplex Oligos for
4 Illumina (96 unique dual-index primer pairs) (New England Biolabs, # E6440L) and purified
5 with AMPure XP (Beckman-Coulter, #63880). Amplicon libraries were sequenced using
6 Illumina MiSeq or NextSeq 550 as 2x150 base pair paired-end reads.

7

8 **Genome Assembly, Variant Identification and Mutation Analysis**

9 Raw sequencing data were demultiplexed, converted to FASTQ files, and screened for
10 SARS-CoV-2 sequences using BLASTn (BLAST+ package 2.9.0). Reads containing adapters,
11 ARTIC and/or VarSkip primer sequences and/or of low-quality were filtered using BBDuk
12 (version 38.87) and mapped to the Wuhan-Hu-1 SARS-CoV-2 reference genome (National
13 Center for Biotechnology Information (NCBI) GenBank accession number NC_045512.2) using
14 BMap (version 38.87) [16]. Consensus sequences were generated using iVar (version 1.3.1)
15 [17] and lineages were assigned using Pangolin (version 3.1.17) [18] and NextClade (version
16 1.11.0) [19] softwares.

17 For PCA plot generation, 756 available full-length SARS-CoV-2 genomic sequences
18 from California were downloaded from the Global Initiative on Sharing All Influenza Data
19 (GISAID) database. The datasets included 459 Delta genomes from samples collected in San
20 Francisco County from September, 2021 – November 2021, 214 BA.1. genomes from samples
21 collected in California from November, 2021 – February, 2022, and 83 BA.2 genomes from
22 samples collected in California in April, 2022. Individual genomes corresponding to the ancestral
23 wild-type lineage (GenBank accession number NC_045512.2) and representative Beta, Alpha,
24 Gamma, Epsilon, Lambda, and Mu variants were included. Coding mutations in the full-length

1 genome and spike gene were called using Nextclade (version 1.11.0) [19]. PCA was performed
2 using the FactoMineR (version 2.4) package in R (version 4.0.2). Coordinates of the centroids
3 and selected genomes were extracted, and Euclidean distances were calculated using custom R
4 scripts.

5 6 **SARS-CoV-2 Isolation in Cell Cultures**

7 BA.1, BA.2, and Delta lineages were isolated from de-identified patient NP swabs sent to
8 the California Department of Public Health. To isolate Delta, 200 µl of a NP sample previously
9 identified as Delta was diluted 1:3 in PBS, supplemented with 0.75% bovine serum albumin
10 (BSA-PBS) and added to confluent Vero-81 cells. Following a 1-hour absorption period,
11 additional media was added, and the flask was incubated at 37°C with 5% CO₂ with daily
12 monitoring for cytopathic effect (CPE). When 50% CPE was detected, the contents were
13 collected, clarified by centrifugation, and stored at -80°C as passage 0 stock. Passaged stock of
14 Delta was made by inoculation of Vero-81 confluent T150 flasks with 1:10 diluted p0 stock and
15 harvested at approximately 50% CPE. Omicron viral stock for the two lineages of interest was
16 similarly produced from a sequence confirmed NP sample using Vero E6-TMPRSS2-T2A-
17 ACE2. All viral stocks were sequenced to confirm lineage and TCID₅₀ (tissue culture infective
18 dose 50, or the dose at which 50% of inoculated cells in culture are infected) was determined by
19 titration.

20 21 **Live Virus Neutralization Assay**

22 CPE endpoint neutralization assays were done following the limiting dilution model
23 using p1 stocks of BA.1, BA.2 and Delta in Vero E6-TMPRSS2-T2A-ACE2. Patient plasma was
24 diluted 1:10 in 0.75% BSA-PBS and heat inactivated at 56C for 30 minutes. Serial 3-fold

1 dilution of plasma were made in BSA-PBS. Plasma dilutions were mixed with 100 TCID₅₀ of
2 each virus diluted in BSA-PBS at a 1:1 ratio and incubated for 1 hour at 37°C. Final plasma
3 dilutions in plasma-virus mixture ranged from 1:40 to 1:84480. 100ul of the plasma-virus
4 mixtures were added in duplicate to 96-well plates pre-seeded with Vero E6-TMPRSS2-T2A-
5 ACE2 at a density of 2.5x10⁴/well and incubated in a 37°C incubator with 5% CO₂ until
6 consistent CPE was seen in the virus control wells. Positive and negative controls were included
7 with cell control wells and a viral back titration to verify TCID₅₀ viral input. Individual wells
8 were scored for CPE as having a binary outcome of “infection” or “no infection” and the ID₅₀
9 (inhibitory dose 50, the concentration of plasma needed to inhibit virus-induced CPE by 50%),
10 was calculated using the Spearman-Kärber method.

11 12 **Quantification and Statistical Analysis**

13 Statistical analyses and data visualization were performed using R (version 4.0.2) with
14 the ggplot2 package (version 3.3.5) [20]. Fisher’s exact test was used to evaluate associations of
15 demographic and clinical variables with variant-specific breakthrough infections. Wilcoxon
16 signed-rank and Wilcoxon-Mann-Whitney U tests were used to determine significance for paired
17 and unpaired samples, respectively. All tests were conducted as two-sided at the 0.05
18 significance level.

19 20 **Data Repository Submission**

21 The SARS-CoV-2 genomes used in this study were deposited into the GISAID database.
22 P-values relevant to the study, coordinates for the PCA plots, and custom scripts used for plot

1 generation were deposited into a publicly accessible Zenodo database repository (doi:
2 10.5281/zenodo.6485708).

3 4 **Results**

5 **Study cohort**

6 This study included individual plasma samples from 86 patients, including 24 (27.9%)
7 unvaccinated, 39 (45.3%) vaccinated with a primary series of either two doses of an mRNA
8 vaccine or one dose of an adenovirus vector vaccine (“unboosted”), and 23 (26.7%) vaccinated
9 with an additional booster dose (“boosted”). All patients with breakthrough infections contracted
10 COVID-19 \geq 14 days after their last vaccine dose.

11 Among the 42 Delta infections (**Table 1**), 37 (88.1%) were sequenced and classified as
12 Delta, while the remaining 5 (11.9%) were presumed Delta because they were collected from
13 patients infected with SARS-CoV-2 when Delta comprised 98.0-99.1% of the circulating
14 variants in California (CDPH, 2022). Of the 42 Delta infected patients, 22 (52.4%) were
15 immunocompromised, and 29 (69.0%) had moderate to severe COVID-19. 14 (33.3%) were
16 unvaccinated, 25 (59.5%) were unboosted, and 3 (7.1%) were boosted. Among the 28 vaccinated
17 patients, sample collection dates ranged from 14 to 49 days (median = 26 days) following
18 symptom onset date or PCR positivity, whichever came earlier.

19 Among the 44 Omicron BA.1 infections (**Table 1**), 32 (72.7%) were sequenced and
20 classified as BA.1, while the remaining 12 (27.3%) were presumed BA.1 because they were
21 collected from patients infected with SARS-CoV-2 when BA.1 comprised 97.4-99.8% of the
22 circulating variants in California (CDPH, 2022). Of the 44 BA.1 infected patients, 19 (43.2%)
23 were immunocompromised, and 20 (45.5%) had moderate to severe COVID-19. 10 (22.7%)

1 were unvaccinated, 14 (31.8%) were unboosted, and 14 (45.5%) were boosted. Among the 28
2 vaccinated patients, sample collection dates ranged from 15 to 43 days (median = 24 days)
3 following symptom onset date or PCR positivity, whichever came earlier.

5 **Neutralizing antibody responses in patients infected with Delta**

6 Among the 14 unvaccinated patients out of 42 infected with Delta, median neutralizing
7 antibody responses against Delta were strong (median $NT_{50}=1,871$, where NT_{50} refers to the
8 neutralization test titers associated with a $\geq 50\%$ inhibition of the plasma sample), with titers
9 significantly higher than that against BA.1 (48X, $p=0.0011$) and BA.2 (27X, $p=0.0017$) (**Figure**
10 **1A**). In comparison, neutralizing antibody responses against BA.2 and BA.1 were weak (median
11 $NT_{50}<100$), with titers against BA.2 $>1.8X$ higher than against BA.1 ($p=0.12$) (**Figure 1A**).

12 Like the unvaccinated cases, neutralizing antibody responses against Delta in unboosted
13 Delta breakthrough infections ($n=25$) were strong, with titers (median $NT_{50}=3,240$) significantly
14 higher than against BA.1 (16X, $p<0.001$) and BA.2 (5.2X, $p<0.001$) (**Figure 1A**). Although
15 moderate (median NT_{50} 100-1,000), neutralizing antibody responses against BA.1 and BA.2 in
16 unboosted Delta-infected individuals were 5.3X ($p=0.0037$) and 9.0X ($p=0.024$) higher,
17 respectively, compared to those in unvaccinated individuals (**Figure 1A, 2A, and 2B**). Median
18 neutralizing titers against BA.2 were 3.0X higher than against BA.1 ($p=0.15$). The pattern of
19 neutralizing antibody responses in the 3 boosted patients with Delta breakthrough infections, all
20 of whom were immunocompromised, was comparable to that in unboosted patients (**Figure 1A**).

21

22

1 **Neutralizing antibody responses in patients infected with Omicron BA.1**

2 Among the 10 unvaccinated patients out of 44 infected with BA.1, neutralizing antibody
3 responses against BA.1 were low to moderate (median $NT_{50}=164$), but titers were still $>4.2X$
4 those of Delta ($p=0.034$) and $3.0X$ those of BA.2 ($p=0.080$) (**Figure 1A**). Titers against both
5 Delta and WA-1 were below the limit of detection (**Figure 1A**), consistent with the previously
6 observed lack of cross-variant neutralization responses in unvaccinated individuals infected with
7 BA.1 [9, 10].

8 In contrast, BA.1 vaccine breakthrough infections ($n=14$) induced moderate to strong
9 antibody responses against BA.1 ($NT_{50}=852$) and BA.2 ($NT_{50}=1,080$), with $1.3X$ higher median
10 neutralizing antibody titers against BA.2 than BA.1 ($p=0.34$) (**Figure 1A**). Compared to
11 unvaccinated infections, these titers were $5.2X$ higher against BA.1 ($p=0.087$) and $19.8X$ higher
12 against BA.2 ($p=0.0081$) (**Figure 2A and 2B**). In boosted individuals, the increases in titers
13 compared to unvaccinated BA.1 infections were similar ($3.8X$ and $15.6X$ higher titers against
14 BA.1 and BA.2, respectively), with $1.4X$ higher titers against BA.2 than BA.1 ($p=0.22$).
15 Interestingly, median neutralizing antibody titers were slightly lower for boosted compared to
16 unboosted individuals against BA.1 and BA.2, although these differences were not significant
17 ($p=0.55$ and $p=0.63$, respectively). Thus, BA.1 breakthrough infections were found to generate
18 higher neutralizing antibody titers against BA.2 than against BA.1.

19 When patients in each vaccination/breakthrough infection category were further stratified
20 based on immunocompromised status, disease severity, and type of vaccine received, only a few
21 significant differences were observed (**Table S2; Figure 2E and 2F**). Titers against BA.1 and
22 BA.2 were significantly higher in unvaccinated patients with moderate to severe Delta infections
23 compared to those with mild or asymptomatic infections. Because no significant differences

1 were observed in median neutralizing antibody titers between BA.1 unboosted and boosted
2 breakthrough infections (**Figure 2A-D; Table S2**), we combined all BA.1 breakthrough
3 infections and further stratified the data based on immunocompromised status, disease severity,
4 and type of vaccine received (**Figure 1B and C**). Titers against BA.2 were significantly higher
5 than BA.1 for (1) immunocompetent, (2) mild or asymptomatic, and (3) immunocompetent, mild
6 or asymptomatic subgroups ($p=0.039$, $p=0.0053$, and $p=0.0038$, respectively). All other pairwise
7 comparisons were not significant. Titers against Delta in immunocompetent patients were
8 significantly higher than those in immunocompromised patients ($p=0.0039$) while titers against
9 Delta in patients with mild or asymptomatic infections were significantly higher than those with
10 moderate to severe infections ($p=0.021$). Similar outcomes were observed with the titers against
11 WT with immunocompetent versus immunocompromised patients ($p=0.018$) and mild or
12 asymptomatic versus moderate to severe infections ($p=0.018$).

13 We sought to investigate why observed neutralizing antibody titers against BA.2 were
14 comparable to, albeit slightly higher than BA.1 for BA.1 breakthrough infections. Principal
15 component analysis (PCA) plots revealed that BA.1 was more closely related to WT and Delta
16 based on antigenic distance as compared to BA.2 (**Figure 3A**). In addition, the antigenic distance
17 between BA.2 and BA.1 was large, similar to that between BA.2 and the ancestral WT lineage
18 targeted by the vaccine (**Figure 3A**, 6.56 versus 6.53 and **Figure 3B**, 4.93 versus vs. 4.99).
19 However, as expected, the centroid of the BA.1 cluster, representing an approximation of the
20 infecting BA.1 variant, was positioned much closer to the cultured BA.1 virus used in the
21 neutralization experiments than the BA.2 virus (**Figure 3A**, 0.92 versus 6.53 and **Figure 3B**,
22 0.20 versus 4.93). Thus, antigenic similarity alone did not explain why titers of BA.2 were found
23 to be higher than BA.1 in BA.1 breakthrough infections.

1

2 **Discussion**

3 This study employed live virus assays to quantify neutralizing antibody titers in 86 Delta
4 or BA.1 infected subjects who were either unvaccinated, vaccinated but unboosted, or boosted.
5 Notably, neutralizing antibody responses against BA.2 were mostly higher, albeit slightly, than
6 those against BA.1, regardless of vaccination status or infecting variant (BA.1 or Delta). In
7 unvaccinated BA.1 and Delta infections, cross-variant neutralizing responses were weak or non-
8 existent, while in vaccinated breakthrough infections, neutralization responses were either strong
9 (against Delta) or moderate (against BA.1 and BA.2). We also did not detect any significant
10 differences in neutralizing antibody titers between unboosted and boosted breakthrough
11 infections. Taken together, these findings indicate that breakthrough infections in vaccinated but
12 not unvaccinated individuals elicit moderate to strong neutralizing responses against BA.2, and
13 that prior boosting does not significantly enhance this response.

14 Our finding of consistently higher neutralizing antibody responses against BA.2 in the
15 setting of BA.1 breakthrough infection is unexpected. The failure to mount an enhanced response
16 to BA.1 in BA.1 breakthrough infections relative to BA.2, which is antigenically distinct (**Figure**
17 **3**) [8], suggests at least three non-mutually exclusive possibilities. First, BA.1 infection, whether
18 in unvaccinated or vaccinated individuals, may be inherently less immunogenic, including
19 against itself (BA.1), than infection from other variants [9-11]. Interestingly, neutralizing
20 antibody levels were not substantially different in a primate study comparing the original
21 approved WT vaccine to an updated vaccine specifically targeting the BA.1 variant [21].
22 Another study looking at boosted but uninfected individuals found higher neutralizing antibody
23 titers against BA.2 as compared to BA.1 [22]. The apparent decreased immunogenicity of BA.1
24 is possibly related to different conformational changes in the S (spike) protein of BA.1 that may

1 affect fusion of the virus to the cell membrane and thus influence antigenicity and the humoral
2 immune response [23, 24]. Second, hybrid neutralizing immunity may be primarily driven
3 through vaccination and not the infecting variant, consistent with our results and those from
4 another study [25]. Third, here we used live virus to evaluate neutralization, in contrast to other
5 reports typically using pseudoviruses [26]. Differences in capsid proteins other than the S
6 (spike), including the E (fusion), M (matrix), and N (nucleoprotein) and their potential
7 involvement during infection may explain the differences in antibody response to BA.1
8 compared to other variants. Importantly, the observed boost in immunity against BA.1 and BA.2
9 induced by BA.1 breakthrough infection will not necessarily protect against future infection as
10 the Omicron subvariants BA.2.12.1 and BA.4/BA.5 have continued to evolve with increasing
11 escape from neutralizing antibodies [32].

12 Vaccinated patients with mild or asymptomatic BA.1 breakthrough infections had
13 significantly higher neutralizing antibody titers against BA.2 than BA.1 (Figure 1B), regardless
14 of their immune status. Additionally, BA.1 breakthrough infections in immunocompetent, but
15 not immunocompromised patients, had higher neutralizing antibody titers against BA.2 than
16 BA.1. Taken together, these results suggest that immunocompromised patients hospitalized with
17 BA.1 are less likely to mount effective antibody responses against BA.2 after recovery. As
18 expected, neutralizing antibody titers against Delta and wild-type lineages in immunocompetent
19 patients were significantly higher than those in immunocompromised patients. However, there
20 was no significant difference in titers against BA.1 or BA.2 between immunocompetent and
21 immunocompromised patients. These findings are consistent with the effectiveness of the
22 vaccine in boosting immune protection against more less divergent lineages such as Delta and
23 WT but decreasing effectiveness against the more divergent Omicron lineages.

1 In this study, we did not detect significant differences in antibody titers against BA.1 or
2 BA.2 between unboosted and boosted individuals following BA.1 breakthrough infection,
3 consistent with findings from another report [27]. This may be due in part to the timing of the
4 breakthrough infection relative to when vaccinated individuals received their last dose. Antibody
5 titers have been shown to wane over time, with boosted individuals generally starting at a higher
6 baseline [11]. Alternatively, boosting with a vaccine targeted against wild-type ancestral virus,
7 while further enhancing protection against hospitalizations and death from severe COVID-19
8 [28, 29] as well as immunity against closely related strains such as Delta [11, 30], may contribute
9 much less towards neutralizing immunity against highly divergent variants such as Omicron
10 BA.1 and BA.2.

11
12

13 **Limitations of the Study**

14 There are several limitations of the study. We analyzed remnant Delta or BA.1 positive
15 biobanked samples, and thus the number of convalescent samples was limited by availability.
16 The analyses stratified by clinical severity were likely underpowered to detect small effects. We
17 were unable to definitively confirm the variant identification for a small percentage of samples.
18 Vaccination status and other clinical metadata were gathered using retrospective chart review
19 rather than prospectively; thus, any inconsistency or error in the electronic medical records
20 would lead to inaccuracies in the extracted clinical metadata.

21

22

1 **Author Contributions**

2 N.B., M.K.M., C.H., and C.Y.C. conceived and designed the study. N.B., V.S., K.A., P.S., A.S.-
3 G., J.N., J.P.-R., R.D.S., and D.A.W. identified and collected patient samples and clinical
4 metadata. B.W., K.F., A.S.G., and Y.Z. sequenced SARS-CoV-2 genomes for variant
5 identification. M.K.M. and S.B. ran the live virus neutralization assays. N.B., M.K.M., V.S.,
6 C.H., and C.Y.C analyzed the neutralization, sequencing, and clinical data. M.O., D.A.W., R.A.,
7 J.D.K., C.H., and C.Y.C. provided administrative, technical, or material support. C.H. and
8 C.Y.C. supervised the study. N.B., M.K.M., and C.Y.C. wrote the manuscript and prepared the
9 figures. All authors read and edited the manuscript and agree to its contents.

10

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12 Technologies, Ltd. The other authors declare no conflicts of interest.

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17

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1 **Tables**

Characteristic		Unvaccinated Delta	Unvaccinated Delta (%)	Unvaccinated BA.1	Unvaccinated BA.1 (%)	p-value
Reported sex	Female	4	28.6%	6	60.0%	0.21
	Male	10	71.4%	4	40.0%	
Age	>65	2	14.3%	4	40.0%	0.19
	18-65	12	85.7%	6	60.0%	
Disease Severity	asymptomatic	1	7.1%	3	30.0%	-
	mild	4	28.6%	2	20.0%	
	moderate	5	35.7%	2	20.0%	
	severe	4	28.6%	3	30.0%	
Status	immunocompetent	9	64.3%	6	60.0%	1
	immunocompromised	5	35.7%	4	40.0%	
Total		14		10		

Characteristic		Vaccine Breakthrough Delta	Vaccine Breakthrough Delta (%)	Vaccine Breakthrough BA.1	Vaccine Breakthrough BA.1 (%)	p-value
Reported sex	Female	6	24.0%	7	50.0%	0.16
	Male	19	76.0%	7	50.0%	
Age	>65	12	48.0%	5	35.7%	0.52
	18-65	13	52.0%	9	64.3%	
Disease Severity	asymptomatic	1	4.0%	2	14.3%	-
	mild	7	28.0%	6	42.9%	
	moderate	4	16.0%	1	7.1%	
	severe	13	52.0%	5	35.7%	
Vaccine type	Moderna	9	36.0%	4	28.6%	-
	Pfizer	11	44.0%	7	50.0%	
	J&J	4	16.0%	2	14.3%	
	unk	1	4.0%	1	7.1%	

Status	immunocompetent	17	68.0%	9	64.3%	1
	immunocompromised	8	32.0%	5	35.7%	
Total		25		14		
Characteristic		Booster Breakthrough Delta	Booster Breakthrough Delta (%)	Booster Breakthrough BA.1	Booster Breakthrough BA.1 (%)	P-value
Reported sex	Female	2	66.7%	9	45.0%	0.59
	Male	1	33.3%	11	55.0%	
Age	>65	2	66.7%	5	25.0%	0.21
	18-65	1	33.3%	15	75.0%	
Disease Severity	asymptomatic	0	0.0%	1	5.0%	-
	mild	0	0.0%	10	50.0%	
	moderate	1	33.3%	0	0.0%	
	severe	2	66.7%	9	45.0%	
Booster type	Moderna	3	100.0%	11	55.0%	0.25
	Pfizer	0	0.0%	9	45.0%	
Status	immunocompetent	0	0.0%	13	65.0%	0.068
	immunocompromised	3	100.0%	7	35.0%	
Total		3		20		

Table 1. Clinical and demographic metadata in unvaccinated, vaccinated, and boosted patients infected with Delta or BA.1. P-values were calculated using Fisher's exact test for two-sided analysis of categorical contingency tables. The table includes all individuals in the study (n=86).

1 **FIGURE LEGENDS**

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4 **Figure 1. Comparison of neutralizing antibody titers within subgroups of unvaccinated,**

5 **vaccinated but unboosted, or boosted patients infected with BA.1 or Delta.** The bar plots

6 show median neutralizing antibody titers against BA.1 (lavender), BA.2 (dark purple), Delta

7 (red), and ancestral wild-type (WT) (green) lineages. **(A)** Median neutralizing antibody titers

8 against BA.1, BA.2, Delta, and WT. **(B)** Median neutralizing antibody titers against BA.1, BA.2,

9 Delta., and WT for combined unboosted and boosted patients with BA.1 breakthrough infection,

10 stratified by immunocompromised status, disease severity, and type of vaccine received. **(C)**

11 Neutralizing antibody titers for individual patients are plotted. The neutralizing antibody

12 response is defined as weak ($NT_{50} < 100$), moderate ($NT_{50} \geq 100$ and $< 1,000$), or strong

13 ($NT_{50} \geq 1000$). P-values are calculated using the Wilcoxon signed-rank nonparametric test for

14 paired samples or using the Wilcoxon-Mann-Whitney nonparametric test for unpaired samples.

15 Abbreviations NS, non-significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

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Figure 2. Comparison of neutralizing antibody titers between subgroups of unvaccinated, vaccinated but unboosted, or boosted patients infected with BA.1 or Delta. Bar plots show median neutralizing antibody titers against BA.1 (lavender), BA.2 (dark purple), Delta (red), and ancestral WT (green) lineages. Median neutralizing antibody titers against BA.1 (**A**), BA.2 (**B**), Delta (**C**), and ancestral wild-type (WT) (**D**) lineages are shown. (**E,F**) Median neutralizing antibody titers against BA.1 (**E**) or BA.2 (**F**) in patients infected with BA.1 or Delta, stratified by immunocompromised status, disease severity, and type of vaccine received. P-values are calculated using the Wilcoxon-Mann-Whitney nonparametric test for unpaired samples. Abbreviations NS, non-significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

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Figure 3. Principal component analysis plots showing the antigenic relatedness of the SARS-CoV-2 variants based on coding mutations. (A) Antigenic relatedness between SARS-CoV-2 variants based on all coding mutations in the viral genome. (B) Antigen relatedness based on coding mutations in the spike gene. The 95% confidence ellipses associated with datasets of viral genomes representing the Delta (red), BA.1 (lavender), and BA.2 (purple) variants are shown, along with the centroid of each cluster (white outlined circle). Individual genomes associated with other variants (ancestral wild-type (WT), Beta, Alpha, Gamma, Epsilon, Lambda, and Mu) are denoted by colored circles, while genomes associated with the Delta, BA.1, and BA.2 cultures used in the neutralization assay are denoted by colored circles highlighted with a black outline. Lines connecting the individual genomes and/or centroids related to the neutralizing antibody response against BA.1 (solid) and BA.2 (dotted) are shown.

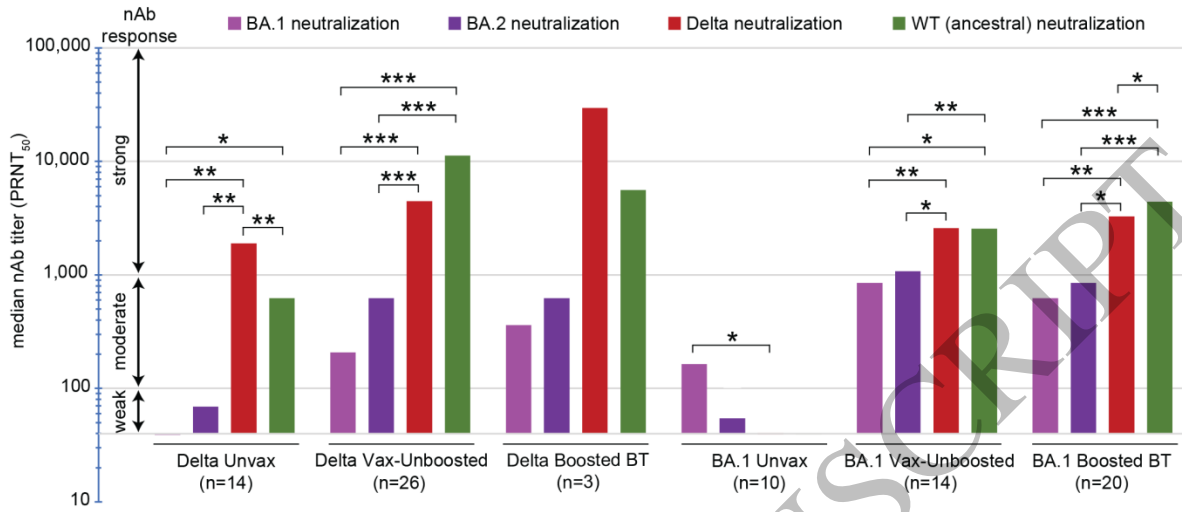
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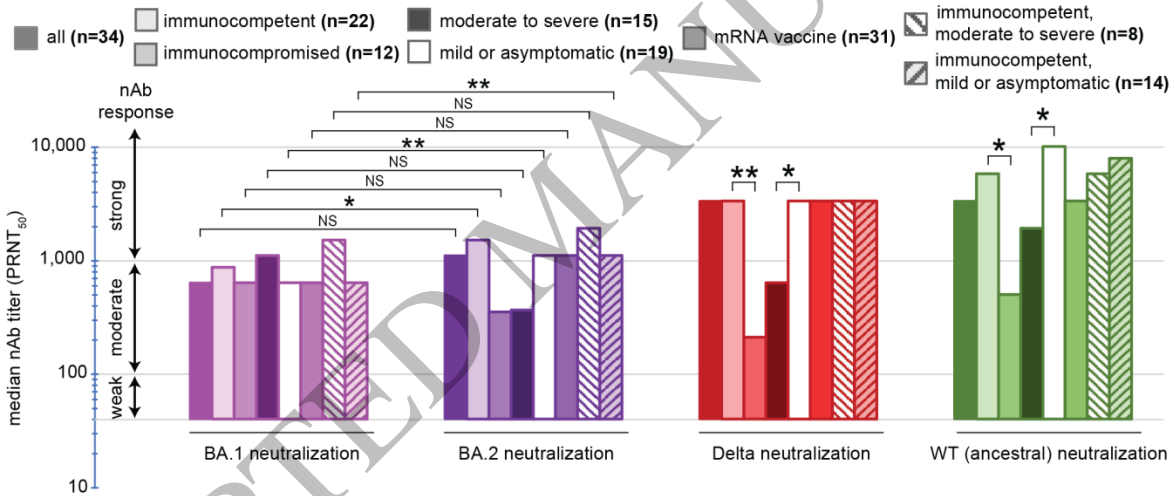
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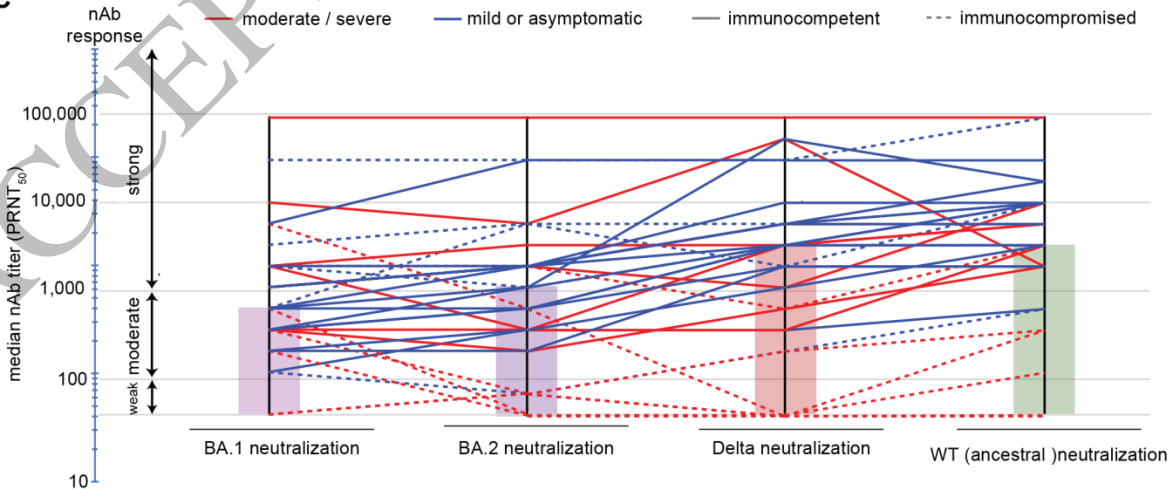
A Delta and BA.1 infections in unvaccinated, vaccinated-unboosted, and boosted individuals (n=86)



B BA.1 breakthrough infections in combined vaccinated-unboosted and boosted individuals



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Figure 1
193x251 mm (x DPI)

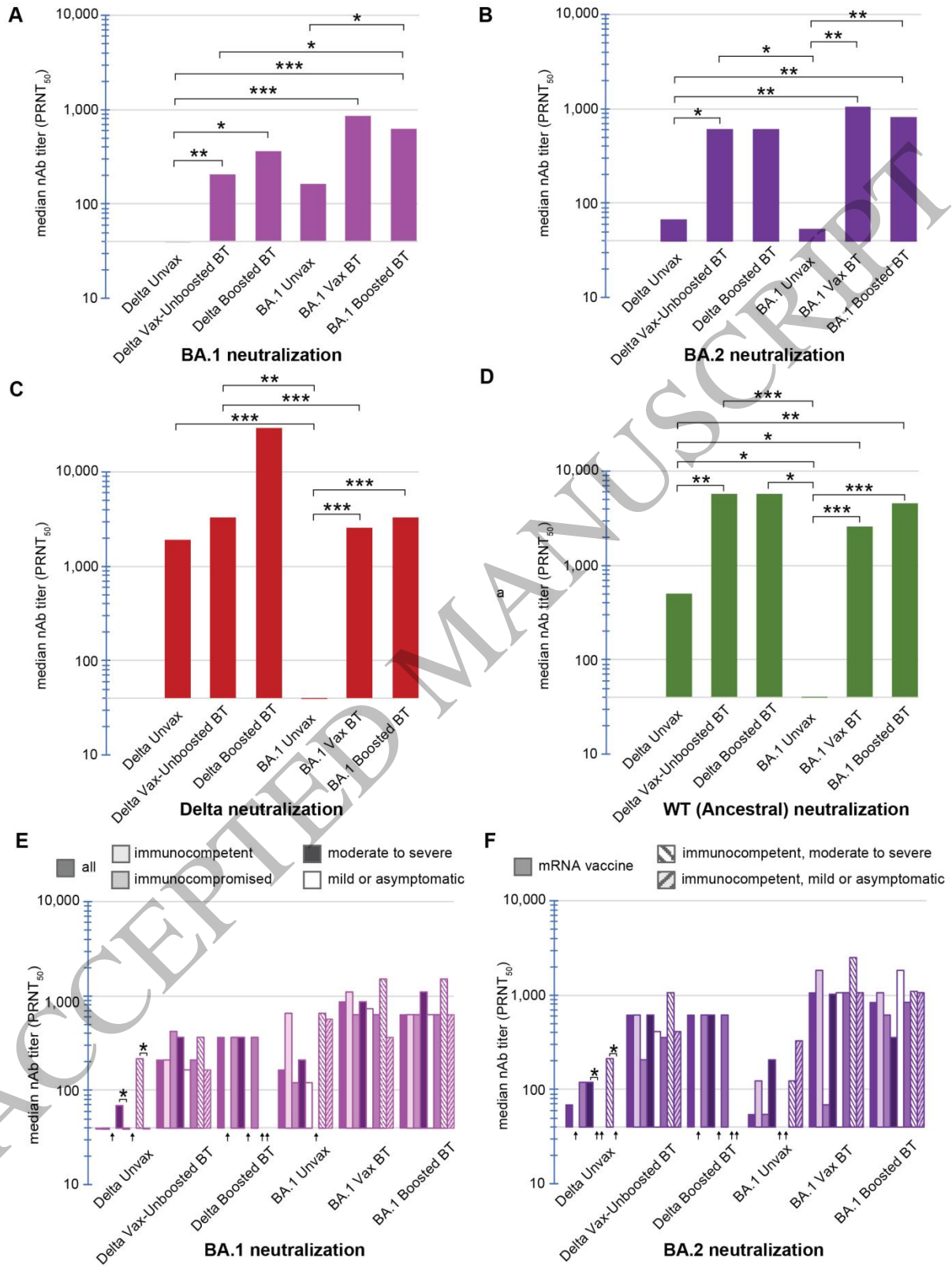
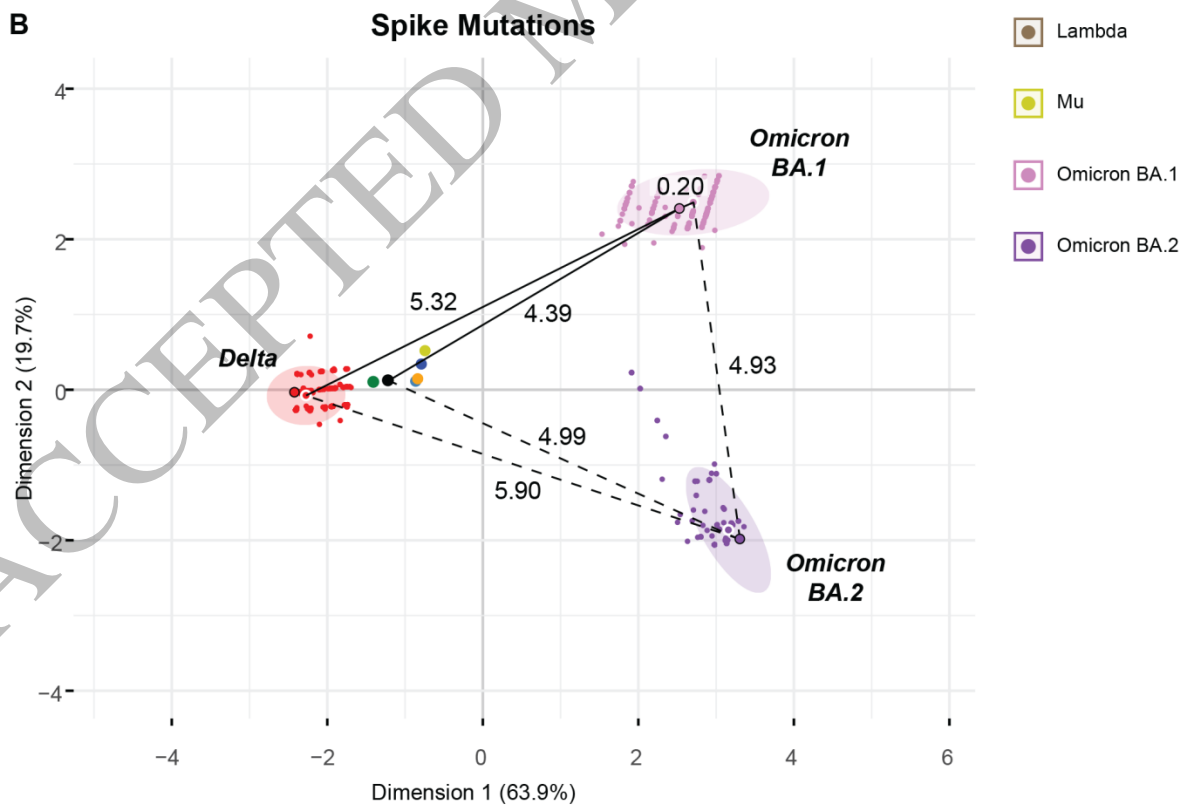
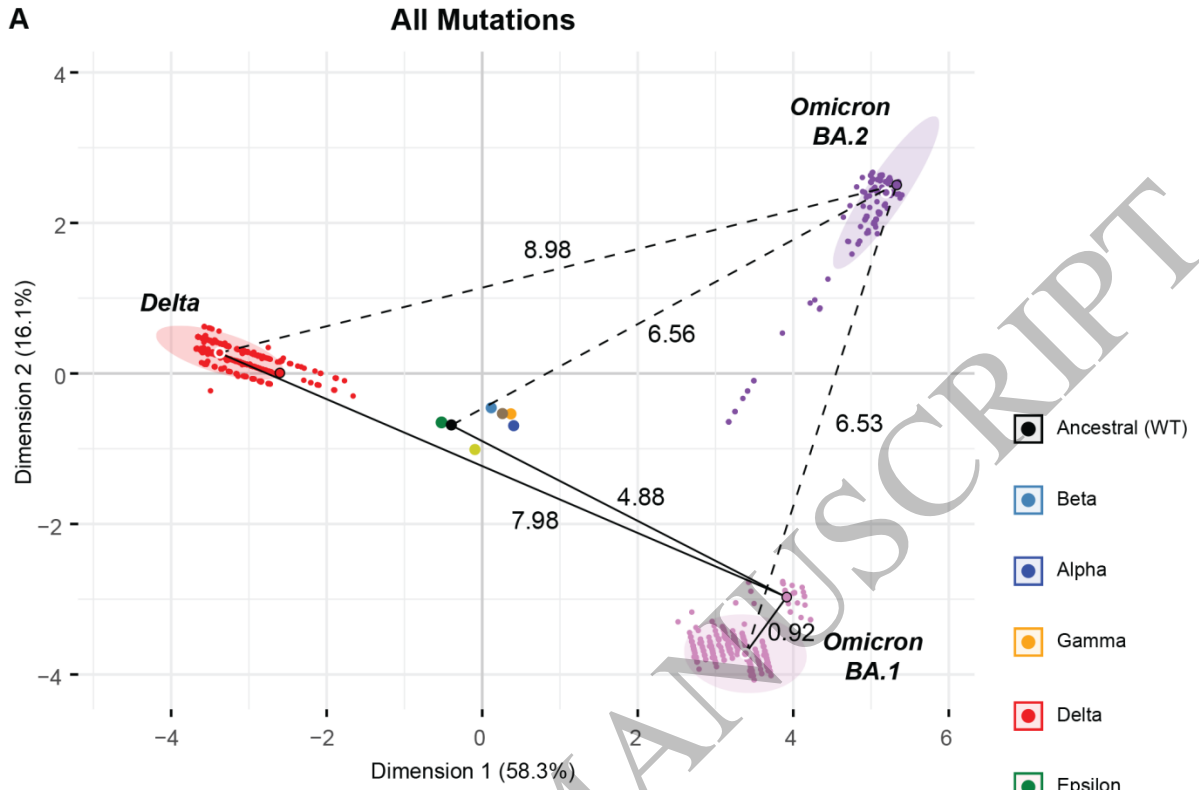


Figure 2
195x254 mm (x DPI)

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Figure 3
180x236 mm (x DPI)