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## Intrinsic immunogenicity is a major determinant of type-specific responses in SARS-CoV-2 infections

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Author contribution statement

G.E.Q., M.W., and D.B. conceived the study, G.E.Q., M.V.S., K.L.P., J.L.U., B.L., and C-Y.L. designed and performed experiments, G.E.Q., M.V.S., C-Y.L., M.S.D., B.J.L., J.Z.N., R.S., M.W., and D.B. analyzed data and wrote the manuscript. J.L.B., K.E., S.B., J.R., K.L., A.F., A.B., H.L.T., A.J.C-M, A.N., M.G., S.Y., L.J.E., L.O., M.D., R.V., A.G., B.J.L., and D.B. designed human studies and recruitment. G.E.Q., B.J.L., R.S., and D.B. performed the data analyses. D.B. and M.W. directed the study and wrote the paper with G.E.Q.

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### Introductory paragraph:

Few type-specific antibodies that recognize drifted epitopes are made during post-vaccination exposures to SARS-CoV-2 variants<sup>1–12</sup>, perhaps due to suppression by prior immunity. We compared type-specific B cell responses in unvaccinated and vaccinated individuals with delta and omicron BA.1 SARS-CoV-2 variant infections. For both delta, which is antigenically similar to the vaccine strain, and the more distant BA.1 variant, neutralizing antibodies were greater in post-vaccination variant infections than in primary variant infections. Delta type-specific memory B cells were reduced in post-vaccination delta infections relative to primary variant infections. Yet some drifted epitopes in the delta variant elicited minimal responses even in primary infections. For BA.1 infections, type-specific antibodies and memory B cells were mostly undetectable, irrespective of prior immunity. Thus, poor intrinsic antigenicity of drifted epitopes in delta and BA.1 infections superseded the impact of prior immunity. Enhancing the immunogenicity of vaccine antigens may promote type-specific responses.

### Main text:

Pre-existing immunity can impede *de novo* type-specific responses against heterologous variant viruses, a phenomenon known as antigenic imprinting<sup>13–15</sup>. Antigenic imprinting has

been difficult to quantify directly in humans due to complex and often unknown immune histories<sup>14</sup>. Because the COVID-19 pandemic has allowed the tracing of infection histories in individuals, the development of monovalent vaccines that induce well characterized B cell responses, and the temporally defined emergence of viral variants<sup>11,16,17</sup>, it presents an opportunity to directly address the details of antigenic imprinting. Post vaccination heterologous infection and booster vaccine responses in humans are dominated by pre-existing SARS-CoV-2 Spike cross-reactive memory B cells, with few antibodies and memory B cells that uniquely recognize drifted epitopes<sup>1–12</sup>. Because most human studies lack the control represented by unvaccinated individuals infected for the first time with a SARS-CoV-2 variant, the degree of suppression of type-specific responses by antigenic imprinting, as opposed to inherent differences in the immunogenicity of type-specific epitopes, has been difficult to quantify. We draw from multiple testing studies and groups to recruit naïve, unvaccinated individuals that lacked initial antigenic exposure to ancestral SARS-CoV-2 and examined their responses to infections with the delta and BA.1 variants.

We recruited participants from the on-campus Test All, Test Smart (TATS) program at the University of Arizona who had tested positive for SARS-CoV-2 between July 1, 2021 and December 1, 2021, but had not tested positive prior to these dates. Participants had either received two doses of monovalent BNT162b2 or mRNA-1273 (n=37, female, 78%, median age = 24.5, age range =18–65; referred to hereafter as ‘post-vaccination delta infection’, Methods), or were unvaccinated at the time of infection (n=12, female, 58%, median age = 21.9, range = 19–42; referred to as ‘primary delta infection’). The types of symptoms were similar in post-vaccination delta infections compared to primary delta infections (Extended Data Fig. 1a). The median duration of symptoms was shorter in the post-vaccination delta infection group (7 days) compared to the primary delta infection group (15 days) (Extended Data Fig. 1b). For vaccination only controls, we included serum samples from 65 additional individuals vaccinated at least 150 days prior to blood collection (n = 65, female, 68%, median age = 39, range=22–70), hereafter “vaccinated only” (Extended Data Table 1), 36 of which had sufficient volume for virus neutralization assays. These participants were part of a separate statewide antibody testing initiative and had no self-reported SARS-CoV-2 infections prior to vaccination<sup>18</sup>. Blood samples were collected at a median of 90 days (interquartile range (IQR) 32, 99) after testing positive for SARS-CoV-2 for primary delta infection or 75 days (IQR 48, 84) for post-vaccination delta infection (Extended Data Table 1) and at a median of 204 days (IQR 179, 249) after the last vaccine dose for vaccinated only individuals and 286 days (IQR 249, 315) for post-vaccination delta infection (Extended Data Table 1). Virus neutralizing antibody titers were significantly higher for post-vaccination delta infection compared to primary delta infection against both early pandemic WA1/2020 (hereafter WA1) (43-fold, Fig. 1a) and the delta variant viruses (26-fold, Fig. 1a), indicating a robust recall response.

We next measured antibody responses by ELISA against Spike antigens from early pandemic Wuhan/Hu1/2019 (hereafter called WuHu1) and delta. RBD-binding antibody titers were elevated in post-vaccination delta infection against both WuHu1 and delta compared to primary delta infection and vaccination only (Fig. 1b). Vaccination-only had slightly elevated titers of WuHu1 RBD-binding antibodies compared to delta RBD antibodies (Fig. 1b), while primary delta infections had higher titers of delta RBD-binding

antibodies than vaccination only (Fig. 1b). Post vaccination delta infection samples had an even ratio of WuHu1/delta RBD binding antibodies (Fig. 1b). There was no correlation between the time post-vaccination and neutralizing or RBD-binding antibody titers, or time post-vaccination and the ratio of WuHu1/delta RBD-binding antibodies (Extended Data Fig. 1c). These data suggested that primary infection and vaccination generated type-specific serological responses.

Two non-synonymous mutations in the delta RBD, T478K and L452R, deviate from the vaccine sequence. The RBD epitope defined by L452 is immunodominant in WuHu1<sup>1,19,20</sup>, while the L452R mutation leads to escape from some neutralizing antibodies<sup>21–24</sup>. To estimate the epitope preferences of circulating antibodies, we produced a delta RBD protein in which R452 was reverted to L452 (hereafter delta-RBD<sup>L452</sup>). As measured by ELISA, vaccination only individuals had elevated antibody titers to delta-RBD<sup>L452</sup> compared to delta-RBD (Fig. 1c), consistent with prior reports<sup>25</sup>. However, primary delta infection cohorts did not reciprocally show elevated antibody titers against delta-RBD compared to delta-RBD<sup>L452</sup> (Fig. 1c), suggesting that the L452R mutation did not create an immunodominant neoepitope. Serum from post-vaccination delta infection donors had a relatively even ratio of antibodies that bound delta-RBD<sup>L452</sup> or delta-RBD (Fig. 1c). We also produced proteins encoding either the S1 domain of WuHu1 Spike (WuHu1-S1), or a version containing the N-terminal domain (NTD) mutations of delta Spike (T19R, G142D, E156-, F157-, R158G; hereafter WuHu1-S1<sup>delta-NTD</sup>)<sup>26–28</sup>. Vaccination only sera had a relatively even ratio of antibodies that bound WuHu1-S1 and WuHu1-S1<sup>delta-NTD</sup> (Fig. 1d), while circulating antibodies in primary delta infections were slightly elevated against the WuHu1-S1<sup>delta-NTD</sup> relative to WuHu1-S1 antibodies (Fig. 1d). Similar results were observed for WuHu-S1 and Delta S1 antibody titers (Fig. 1e). These data suggested a shift in immunodominance in delta infection away from L452R and towards NTD epitopes.

We next used WuHu1 S1 and delta S1 antigen tetramers to quantify the frequency of type-specific CD19<sup>+</sup>IgD<sup>+</sup>IgM<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup> memory B cells (hereafter B<sub>MEM</sub> cells) in post-vaccination delta infection and primary delta infection using the same cohorts and timepoints as described above (Extended Data Fig. 2a). Additionally, we included a naïve cohort (n=13, female, 69%, median age = 36.7, range = 19–81; referred to hereafter as ‘naïve’) with PBMCs collected prior to vaccination in 2021<sup>29</sup>, and vaccinated only control cohort (n=62, female, 61%, median age = 31.9, range = 18–65; referred to hereafter as ‘vaccinated only<sup>TATS</sup>’), from which we collected PBMCs (Extended Data Table 1). Blood samples for vaccination only<sup>TATS</sup> were collected at a median of 143 days (IQR 117, 183) post-vaccination. We quantified the S1-specific B<sub>MEM</sub> cells, as S1 contains the RBD and NTD and almost all neutralizing epitopes in the Spike protein<sup>30</sup>, and generally does not react with pre-existing B<sub>MEM</sub> cells specific to other common coronavirus antigens<sup>31</sup>. Delta S1 type-specific B<sub>MEM</sub> cells were detected in primary delta infections and in post-vaccination delta infections compared to naïve and vaccinated only<sup>TATS</sup> individuals who had never been exposed to delta (Fig. 2a), suggesting *de novo* responses that recognized variant-unique epitopes. However, the numbers of delta S1 type-specific B<sub>MEM</sub> cells, calculated as frequencies of total PBMCs, were lower in post-vaccination delta infection (average of 0.00023%) than in primary delta infection (0.00049%) (Fig. 2a). After subtraction of the background percentage of delta S1 type-specific staining in vaccinated only<sup>TATS</sup> B<sub>MEM</sub>

cells, the difference between post-vaccination delta infection (0.000038%) and primary delta infection (0.00036%) was approximately 9.6-fold (Fig. 2a). The numbers of cross-reactive B<sub>MEM</sub> cells that bound both WuHu1 S1 and delta S1 were elevated in post-vaccination delta infections compared to primary delta infections (Fig. 2a), consistent with a robust recall response in vaccinated individuals. In both primary and post-vaccination delta infections, B<sub>MEM</sub> cells that bound delta S1 only were rare compared to cross-reactive cells that bound both WuHu1 and delta S1 (Fig. 2a). We observed a significant positive correlation ( $P = 0.0028$ ) between the number of cross-reactive and delta-specific B<sub>MEM</sub> cells in post-vaccination delta infections, but not in vaccination only individuals (Fig. 2b). These observations suggested that highly immunogenic infections promoted both cross-reactive and type-specific responses.

In mice, suppression of *de novo* responses by antigenic imprinting is inversely correlated to antigenic distance<sup>32,33</sup>. Because BA.1 is more antigenically distant to the monovalent vaccines than delta, we quantified the degree of suppression in post-vaccination BA.1 infections. We recruited 50 vaccinated individuals (female, 66%, median age = 40 years, range = 19–71) who tested positive for SARS-CoV-2 infection between January 1 and March 31, 2022 (hereafter ‘post-vaccination BA.1 infection’, Extended Data Table 1). Sixteen saline gargle samples from this cohort were sequenced and all were confirmed to be positive for the BA.1 variant. These individuals had no documented or reported prior positive tests for SARS-CoV-2 infections. We used samples from the vaccination only<sup>TATS</sup> cohort, which had no exposure to BA.1, as negative controls for BA.1 type-specific responses (Extended Data Table 1). Relative to both primary and post-vaccination delta infection, post-vaccination BA.1 infection generally led to a shorter median duration of respiratory symptoms (6 days) (Extended Data Fig. 1a, b). We were not able to recruit unvaccinated, primary BA.1 infection individuals on campus, but we obtained sera from 56 individuals (female 49%, median age = 44, range = 25–62) who met these criteria from the Centers for Disease Control and Prevention HEROES and RECOVER projects<sup>34,35</sup>, and PBMC samples from 6 of these individuals (hereafter ‘primary BA.1 infection’, Extended Data Table 1). Participants in the HEROES and RECOVER studies had baseline serology performed to confirm naïve serostatus and underwent weekly PCR testing for SARS-CoV-2 infections. A minimum of 80% compliance to the weekly testing was required to remain in the study, thereby minimizing the chances of undetected infections in this cohort. Blood samples were collected at a median of 37 days (interquartile range (IQR) 31, 45) after testing positive for SARS-CoV-2 for primary BA.1 infection or 63 days (IQR 42, 71) for post-vaccination BA.1 infection (Extended Data Table 1) and at a median of 143 days (IQR 117, 183) after the last vaccine dose for vaccination only<sup>TATS</sup> individuals and 132 days (IQR 101, 196) for post-vaccination BA.1 infection (Extended Data Table 1). Neutralizing antibody titers against WA-1 were 9.1-fold higher relative to those against BA.1 in vaccination only<sup>TATS</sup> individuals (Fig. 3a). Serum from post-vaccination BA.1 infections had a significantly higher neutralizing antibody titers based on PRNT<sub>90</sub> assays (plaque reduction neutralization titer 90%) against BA.1 than both vaccination only<sup>TATS</sup> (63-fold, Fig. 3a) and primary BA.1 infections (27-fold, Fig. 3a), consistent with a B<sub>MEM</sub> cell recall response during post-vaccination infection.

We next examined the specificities of the overall antibody responses. Because more than 90% of the neutralizing epitopes and 15 of the lineage-defining mutations are found in the RBD region of BA.1 Spike<sup>36</sup>, compared to only 2 such mutations in delta Spike, we evaluated the WuHu1 RBD- or BA.1 RBD-specific antibody titers in the same cohorts and timepoints as above. Post-vaccination BA.1 infection sera had increased WuHu1 and BA.1 RBD-binding antibodies compared to vaccination only<sup>TATS</sup> or primary BA.1 infection cohorts (Fig. 3b), while vaccination only<sup>TATS</sup> sera had greater RBD BA.1 antibody titers compared to primary BA.1 infections (Fig. 3b). Antibodies from vaccination only<sup>TATS</sup> individuals had a median WuHu1 RBD/BA.1 RBD antibody binding ratio of 1.27 (Fig. 3b). Antibodies from primary BA.1 infections had a median WuHu1 RBD/BA.1 RBD binding ratio of 0.47 (Fig. 3b). The WuHu1 RBD/BA.1 RBD-binding antibody ratios in post-vaccination BA.1 infections more closely resembled those of vaccination only<sup>TATS</sup> than those of primary BA.1 infections (Fig. 3b). There was no statistically significant impact of time post-infection on neutralizing or RBD-binding antibodies or WuHu1 RBD/BA.1 RBD antibody ratios (Extended Data Figure 3a,b). To directly quantify type-specific B cell responses, we used WuHu1 Spike bound to magnetic beads to deplete the WuHu1 Spike-reactive antibodies from primary BA.1 infection serum samples. Because of the low serum sample volumes in the primary BA.1 infection cohort, we obtained serum samples from the Immunity Associated with SARS-CoV-2 study<sup>37</sup> (IASO) from 10 unvaccinated individuals with confirmed primary BA.1 (n = 10, female, 70%, median age = 48.5, range = 33–65); hereafter ‘primary BA.1 infection<sup>IASO</sup>’ or primary WuHu1 (n = 15, female, 73%, median age = 44.7, range = 32–55); hereafter ‘primary WuHu1 infection<sup>IASO</sup>’ infections (Extended Data Table 1). Blood samples were collected at a median of 45 days (interquartile range (IQR) 37, 53) after testing positive for SARS-CoV-2 for primary WuHu1 infection<sup>IASO</sup> or 42 days (interquartile range (IQR) 27, 48) after testing positive for SARS-CoV-2 for primary BA.1 infection<sup>IASO</sup> (Extended Data Table 1). After WuHu1 Spike-reactive antibody depletion, BA.1 type-specific antibodies were detected in only 2 of the 10 primary BA.1 infection<sup>IASO</sup> by ELISA (Fig. 3c).

Next, we used RBD tetramers to identify antigen-specific CD19<sup>+</sup>IgD<sup>−</sup>IgM<sup>−</sup>CD27<sup>+</sup>CD38<sup>−</sup> B<sub>MEM</sub> cells in post-vaccination BA.1 infection, primary BA.1 infection, vaccination only<sup>TATS</sup> and naïve cohorts (Fig. 4a and Extended Data Fig. 4a,b). The frequency of WuHu1 and BA.1 RBD-cross-reactive and BA.1 RBD-binding B<sub>MEM</sub> cells in primary BA.1 infection was not statistically significantly different compared to B<sub>MEM</sub> cells in naïve cohorts (Fig. 4a). WuHu1 RBD-specific and WuHu1 and BA.1 RBD-cross-reactive B<sub>MEM</sub> cells were detected in vaccination only<sup>TATS</sup> and post-vaccination BA.1 infection groups (Fig. 4a). We next used tetramers specific for the full-length trimeric WuHu1 or BA.1 Spike protein to capture a greater breadth of B<sub>MEM</sub> cell specificities in these same cohorts, including non-RBD S1 specificities in post-vaccination BA.1 infection, primary BA.1 infection, vaccination only<sup>TATS</sup> and naïve cohorts. We could not consistently detect BA.1 Spike type-specific B<sub>MEM</sub> cells in primary BA.1 infection or post-vaccination BA.1 infection samples at frequencies higher than in naïve or vaccination only<sup>TATS</sup> individuals (Fig. 4b). Because type-specific responses could emerge later in the response<sup>1,2</sup>, we longitudinally quantified the frequency of RBD-specific B<sub>MEM</sub> cells ranging from day 21-day 422 post-BA.1 infection. We did not detect a consistent time-dependent increase in

the frequency of BA.1-specific B<sub>MEM</sub> cells in primary or post-vaccination BA.1 infection samples (Extended Data Fig. 4b). Thus, type-specific responses to BA.1 did not develop over time.

These data demonstrated a partial suppression of type-specific responses in post-vaccination delta infections compared to primary delta infections, while in the more antigenically distinct omicron BA.1 infections, where less imprinting was expected<sup>32</sup>, no type-specific responses were detected in both post-vaccination BA.1 infections and primary infections. Thus, intrinsic immunogenicity of the antigen superseded the impact of prior immunity on type-specific responses post-infection.

Suppression of omicron-specific responses by prior immunity was reported in a study that included unvaccinated primary omicron infection cohorts<sup>38</sup>. Although we did not detect a suppression of BA.1 responses, we observed a partial suppression of delta-specific memory B cell numbers in post-vaccination delta infections compared to primary infections. Because the overall BA.1 response was weak, it is possible that suppression did occur but was below the limit of detection of our assays. Additionally, the serum depletion assays conducted here may have depleted antibodies that bound BA.1 Spike better than WuHu1 Spike but were at least partially cross-reactive.

We found that the intrinsic immunogenicity of type-specific responses in delta and omicron BA.1 infections superseded the impacts of antigenic imprinting. While type-specific responses could be detected against some epitopes of the antigenically-similar delta variant, such responses were not observed against the more distant BA.1 variant across multiple assays, irrespective of prior immunity. Moreover, delta and BA.1 primary infection cohorts showed weak immune responses both at the level of specific drifted epitopes, such as R452 for delta, and the virus as a whole for BA.1. As one possible explanation, omicron BA.1 Spike RBD was reported to interact with inhibitory receptors like siglec-9 to suppress antibody responses<sup>39</sup>. There may also be selection against immunogenic neutralizing antibody epitopes during prolonged within-host viral evolution, which is likely how BA.1 emerged<sup>40</sup>. The immunogenicity of epitopes may be species-specific, as type-specific antibodies comprised ~20% of the response in mice vaccinated against BA.1<sup>11</sup>. Repeated exposures to relevant epitopes through boosting with updated vaccines<sup>41</sup>, perhaps incorporating glycan shielding or epitope masking of conserved immunodominant epitopes<sup>42–44</sup>, may counteract low intrinsic immunogenicity and promote type-specific responses<sup>41,45</sup>. Improving intrinsic immunogenicity and defining the mechanisms of antigenic imprinting and immune suppression of *de novo* responses will help guide vaccination strategies and overcome these barriers.

## Methods

### Human participants

All human studies conducted at The University of Arizona were approved by the Institutional Review Board for the Human Subjects Protection Program (STUDY00000314). Individuals who had participated in the voluntary on-campus saline gargle testing program and had either never tested positive or had tested positive during the delta or BA.1 waves



**Delta TATS cohort:** All recruited individuals who tested positive by RT-qPCR and had sufficient sequence coverage to assign a lineage had sequences confirmed as delta. During this period, the delta variant represented 100% of PCR<sup>+</sup> samples on campus that could be assigned a PANGO-lineage<sup>48</sup>, as determined through viral sequencing of all remnant samples below a Ct value of 35.

**Omicron BA.1 cohort:** We recruited individuals from our voluntary on-campus testing program who had tested positive for SARS-CoV-2 between January 1 and March 31, 2022. All individuals for this study who tested positive by PCR had sequences confirmed to be BA.1. Individuals with a SARS-CoV-2 infection caused by a delta variant or other omicron sublineages were excluded from the study. Viral genome sequencing of all remnant PCR<sup>+</sup> samples on campus during this period below a Ct value of 35 demonstrated that 93.7% of samples that could be assigned a PANGO-lineage<sup>48</sup> were caused by the BA.1 sublineage of Omicron.

### PBMC and plasma preparation

Twenty milliliters of blood was collected by venipuncture in heparinized Vacutainer tubes (BD). For PBMCs, 15ml of Ficoll-Paque PLUS (Thermo Fisher Scientific) was added to 50-ml Leucosep tubes (Greiner) and spun for 1min at 1,000g to transfer the density gradient below the filter. Twenty milliliters of blood from the heparinized tubes were then poured into the top of the Leucosep tube and spun at 1,000g for 10min at room temperature with the brake off. The top plasma layer was carefully collected and frozen at -20 °C, and the remaining supernatant containing PBMCs above the filter was poured into a new 50-ml conical tube containing 10mL of PBS and centrifuged at 250 × g for 10 min. Cell pellets were resuspended in RPMI media containing 10% FCS and counted on a Vi-Cell XR (Beckman Coulter). Cells were diluted to a concentration of 2 × 10<sup>7</sup> cells per mL in RPMI media containing 10% FCS. An equal volume of 80% FCS + 20% dimethyl sulfoxide was added dropwise and inverted once to mix. Suspensions were distributed at 1 ml per cryovial and frozen overnight at -80°C in Mr. Frosty freezing chambers (Nalgene). Vials were then transferred to storage in liquid nitrogen. Serum or PBMC sample requests should be sent to D.B. and will be made available pending sufficient remaining quantities and completion of a material transfer agreement.

### ELISA and quantification of antibody titers

Serological assays were performed as previously described<sup>18</sup>. WuHu1 RBD (cat. no. SPD-C52H3), WuHu1 S1 subdomain of the SARS-CoV-2 S glycoprotein (cat. no. S1N-C52H3), WuHu1 Spike (cat. no. SPN-C52H9), Delta RBD (cat. no. SPD-C52Hh), Delta S1 subdomain (cat. no. S1N-C52Hu), Omicron (BA.1) RBD (cat. no. SPD-C522e), Omicron (BA.1) Spike (cat. no. SPN-C52Hz) and Nucleocapsid (cat. no. NUN-C5227) were purchased from Acro Biosystems. Chimeric proteins (Delta RBD-L452 and Delta NTD-WuHu1 S1) were custom synthesized by GenScript. To obtain titers and single-dilution OD450 values, antigens were immobilized on high-adsorbency 384-well plates at 5 ng mL<sup>-1</sup>. Plates were blocked with 1% non-fat dehydrated milk extract (Santa Cruz Biotechnology, sc-2325) in sterile PBS (Thermo Fisher Scientific HyClone PBS, SH2035) for 1 h, washed with PBS containing 0.05% Tween-20 (PBS-Tween) and overlaid for 60

min with either a single 1:60 dilution or five serial 1:3 dilutions beginning at a 1:60 dilution of serum or plasma. Plates were then washed and incubated for 1 h in 1% PBS and milk containing anti-human Pan-Ig HRP-conjugated antibody (Jackson ImmunoResearch, 109-035-064) at a concentration of 1:2,000 for 1 h. Plates were washed with PBS-Tween solution followed by PBS wash. To develop, plates were incubated in tetramethylbenzidine (Fisher Scientific) before quenching with 2 N H<sub>2</sub>SO<sub>4</sub>. Plates were read for 450-nm absorbance on CLARIOstar Plus from BMG Labtech. All samples were also read at 630 nm to detect any incomplete quenching. Any samples above background 630-nm values were re-run. Area under the curve (AUC) values were calculated in GraphPad Prism (v9). All antigens were confirmed to have equivalent binding to the control anti-SARS-CoV-2 CR3022 monoclonal antibody (Creative BioLabs).

### Serum depletion and quantification

Streptavidin magnetic beads (BioLabs, S1420S) were washed twice with PBST and incubated with biotinylated recombinant spike proteins from Wuhan-1 or BA.1 strains of SARS-CoV-2 in PBS at 30 µg per mg beads for 30 min at room temperature on an end-over-end mixer. Beads were washed twice with PBS and incubated with diluted sera samples for 30 min at room temperature with agitation. Depleted sera were collected on the KingFisher Flex extraction robot (ThermoFisher), stored at 4 °C, and quantified as previously described<sup>11</sup>. Serum antibody titers are shown as ng/mL equivalents of monoclonal antibody CV3-25.

### Virus neutralization assays

All authentic virus assays were performed at Biosafety Level 3 and were approved by the University of Arizona Institutional Biosafety Committee. SARS-CoV-2, isolate USA-WA1/2020, was deposited by Dr Natalie J. Thornburg at the Centers for Disease Control and Prevention and obtained from the World Reference Center for Emerging Viruses and Arboviruses. Stocks of WA1/2020 SARS-CoV-2 were generated as a single passage from received stock vial on mycoplasma-negative Vero cells (ATCC CCL-81). B.1.617.2 (Delta) was received from WRCEVA, strain designation GNL-1205. B.1.1.529 (Omicron) originated from a nasopharyngeal swab collected at the University of Arizona. It was passaged once on Calu-3 cells and then once on Vero cells to generate a master stock. Viral PANGO-lineage, BA.1.1<sup>48</sup>, was confirmed by Illumina sequencing (Deposited on GISAID with the accession number: EPI\_ISL\_17886211) of the master stock.

Supernatant and cell lysate were combined, subjected to a single freeze–thaw and then centrifuged at 1,800 × g for 10 min to remove cell debris. For PRNTs for SARS-CoV-2, Vero cells (ATCC, CCL-81) were plated in 96-well tissue culture plates and grown overnight. Vero cells were confirmed by PCR to be free of mycoplasma using the Universal Mycoplasma Detection Kit (ATCC). Serial 1:3 dilutions of serum or plasma samples were performed in duplicate and incubated with 100 plaque-forming units of SARS-CoV-2 for 1h at 37 °C. Plasma/serum dilutions plus virus were transferred to the cell plates and incubated for 2h at 37 °C in 5% CO<sub>2</sub> and then overlaid with 1% methylcellulose. After 72h, plates were fixed with 10% neutral buffered formalin for 30min and stained with 1% crystal violet. Plaques were imaged using an ImmunoSpot Versa plate reader. The most dilute serum or

plasma concentration that led to ten or fewer plaques was designated as the PRNT<sub>90</sub> titer. Input PFU for each experiment was confirmed by plaque assay.

### Flow cytometry

One milliliter of pre-warmed FBS was added to a frozen cryovial of PBMCs, which was rapidly thawed in a 37°C water bath. Samples were poured into 15 mL conical tubes containing 5 mL of pre-warmed RPMI with 5% FBS and 1% Antibiotic-Antimycotic (Gibco). Tubes were centrifuged at  $250 \times g$  for 5 min at room temperature.

**Delta:** Supernatants were removed and cell pellets were resuspended in 200  $\mu$ L of staining buffer containing 1  $\mu$ L each of anti-CD38-APC (BioLegend, clone HIT2), anti-CD13-PE-Cy7 (BioLegend, clone WM15), anti-CD21-PE-Dazzle (BioLegend, clone Bu32), anti-CD19-APC-eFluor-780 (Invitrogen, clone HIB19), anti-IgD-PerCP-Cy5.5 (Biolegend, clone IA6-2), anti-IgM-FITC (Biolegend, clone MHM-88), anti-CD27-BV510 (Biolegend, clone M-T271), anti-CD11c-Alexa700 (BioLegend, clone Bu15). Staining buffer also contained Delta-S1-PE and S1-BV421 B cell tetramers.

**BA.1:** Supernatants were removed and cell pellets were resuspended in 200  $\mu$ L of staining buffer containing 1  $\mu$ L each of anti-CD38-BV421 (BioLegend, clone HB-7), anti-CD13-PE-Dazzle 594 (BioLegend, clone WM15), anti-CD21-PerCP Cy 5.5 (BioLegend, clone Bu32), anti-CD19-APC-eFluor-780 (Invitrogen, clone HIB19), anti-IgD-BV510 (Biolegend, clone 11-26c.2a), anti-IgM-FITC (Biolegend, clone MHM-88), anti-CD27-PE Cy 7 (Biolegend, clone M-T271), anti-CD11c-Alexa700 (BioLegend, clone Bu15). Cells were stained with live-dead marker, Zombie Yellow (BioLegend) according to manufacturer's recommendations. Staining buffer also contained BA.1-Spike-PE and Spike-Alexa Fluor 647 tetramers (Extended Data Fig. 4).

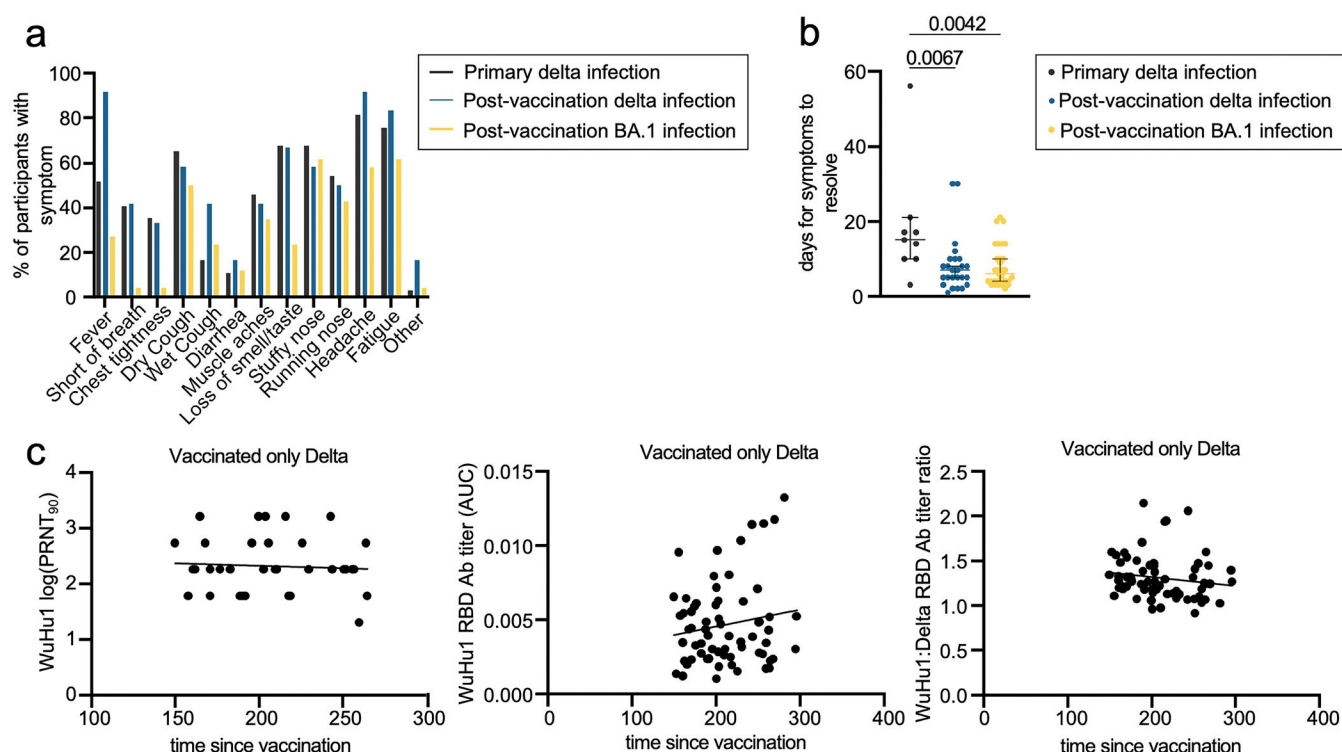
The following antibodies were used for flow cytometry: CD19 (APC-Cy-7, clone SJ25C1, BioLegend cat # 363009), CD13 (PE-Cy-7, clone WM13, Biolegend cat # 301712), IgD (PerCP-Cy-5.5, clone IA6-2, Biolegend cat # 348208), IgM (Alexa Fluor 488, clone MHM-88, Biolegend cat # 314506), Streptavidin-BV421 (Biolegend cat # 405226), Streptavidin-PE (Biolegend cat # 405204), CD27 BV510, clone M-T271, CD38 (Alexa Fluor 647, clone HB-7, CytexBio cat # SKU 356631), CD19 (Alexa eFluor 780, clone HIB19, Thermo Scientific cat # 47-0199-42), Zombie Yellow (Biolegend cat # 423104), CD13 (PE-Dazzle 594, clone WM15, Biolegend cat # 301720), IgD (BV510, clone IA6-2, Biolegend cat # 348220), IgM (FITC, clone MHM-88, Biolegend cat # 314506), CD27 (PE-Cy-7, clone O323, Biolegend cat # 302838), CD38 (BV421, clone HB-7, Biolegend cat # 356618), Streptavidin-Brilliant Violet 650 (Biolegend cat # 405231), Streptavidin - Brilliant Violet 711 (Biolegend cat # 405241), Streptavidin - Alexa Fluor 647 (Biolegend cat # 405237). All antibodies were validated by the manufacturer on human PBMCs and used at 1:200 dilution. Tetramer reagents were assembled by mixing 100  $\mu$ g  $\text{ml}^{-1}$  of C-terminal AviTagged S1, Delta S1, WuHu1 Spike, or BA.1 Spike (ACROBiosystems) with 100  $\mu$ g  $\text{ml}^{-1}$  of streptavidin-PE(BioLegend), streptavidin-BV421 (BioLegend), or streptavidin-Alexa Fluor 647 (BioLegend), respectively, at a 6:1 molar ratio for S1 or 4:1 molar ratio for Spike, in which  $\frac{1}{5}$  of the final volume of streptavidin was added every 10 min. S1 and Spike

tetramers were validated by staining Lenti-X 293T cells(Takara Bio) as a negative control or 293T-hACE2-expressing cells (BEI Resources, NR-52511) as a positive control. Lenti-X 293T cells were confirmed to be free of mycoplasma; 293T-hACE2 cells were maintained in media containing 1% pen/strep to minimize chances of contamination. PBMC samples were stained for at least 20 min, washed and filtered through 70-µm nylon mesh. Data were analyzed on either a BD LSR2 (tetramer validation only), a Fortessa cytometer (Delta), or BD Cytex Aurora (BA.1). Data were analyzed using FlowJo software.

### Statistical methods

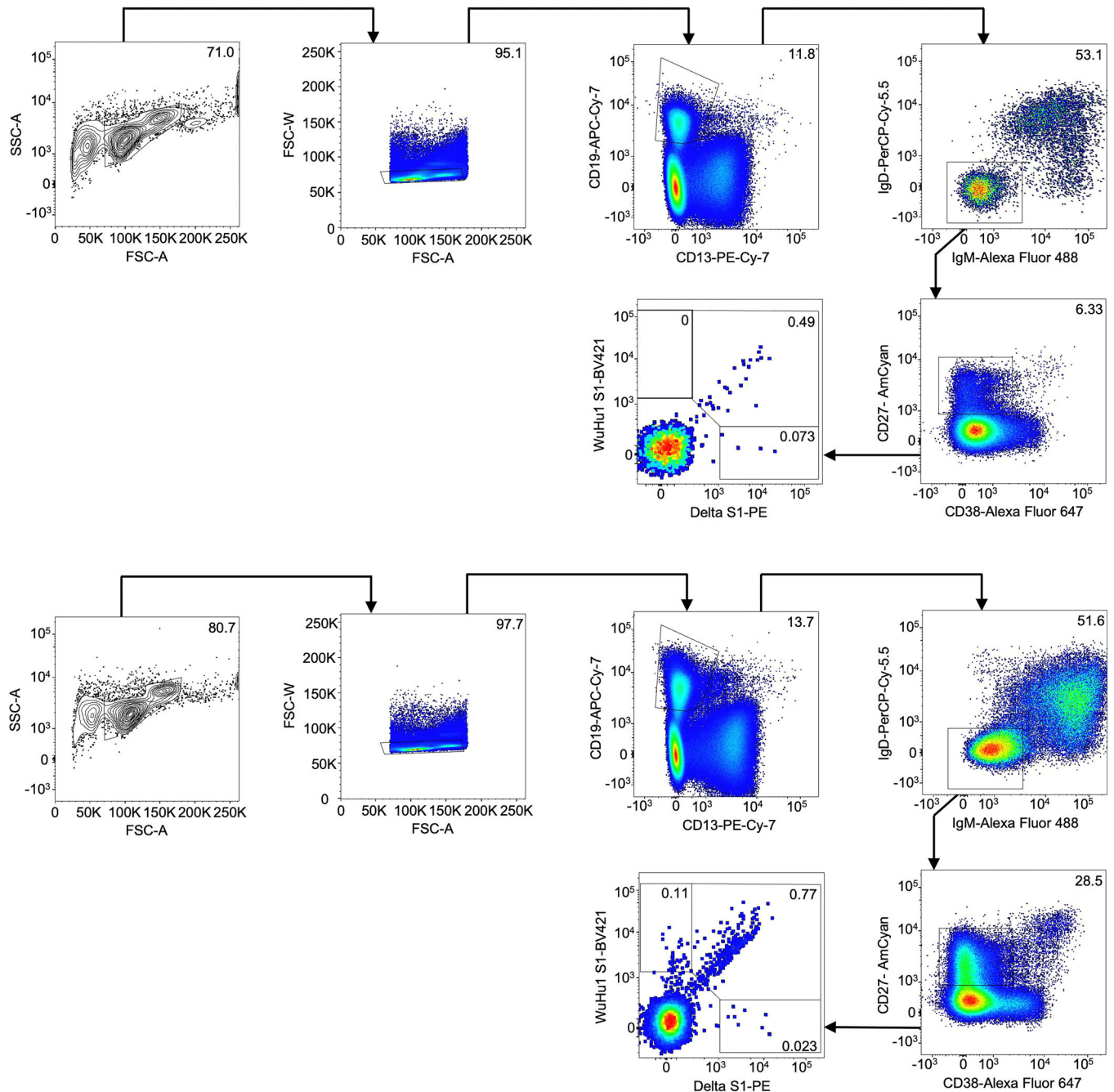
All analyses are listed in the figure legends and were performed in GraphPad Prism 10 and/or the R programming language (v4.0.5). For antibody AUC tests, we targeted 85% power to detect a minimum overall effect size of 0.256 with a sample size of at least 60 vaccination only, 60 post-vaccination infections, and 30 primary infections for total sample of 150 subjects and 80% power to detect a difference of at least 0.506 using the Tukey-Kramer (Pairwise) multiple comparison test at a 0.05 significance level and SD of no more than 0.554 (estimated from our previous studies to be a conservative estimate). For epitope-specific memory B-cell analyses we estimated effects size of 0.256 for the overall test and pairwise detectable differences of 1.12; these tests assume a common standard deviation of no more than 0.851 (on the log scale), and 80% power. We note that we did not reach these numbers for most of our cohorts. Nonetheless, the magnitudes of the effect sizes were large enough such that statistically significant differences could be detected in most experiments. Data distribution was assumed to be normal but this was not formally tested. Participants were allocated to distinct cohorts based on self-reported vaccination and infection status, and for the HEROES/RECOVER cohorts, based on serology and PCR results. The experiments were not randomized. Investigators conducting ELISAs were blinded to the experimental group. All other data collection and analysis were not performed blind to the conditions of the experiments. No data were excluded.

## Extended Data



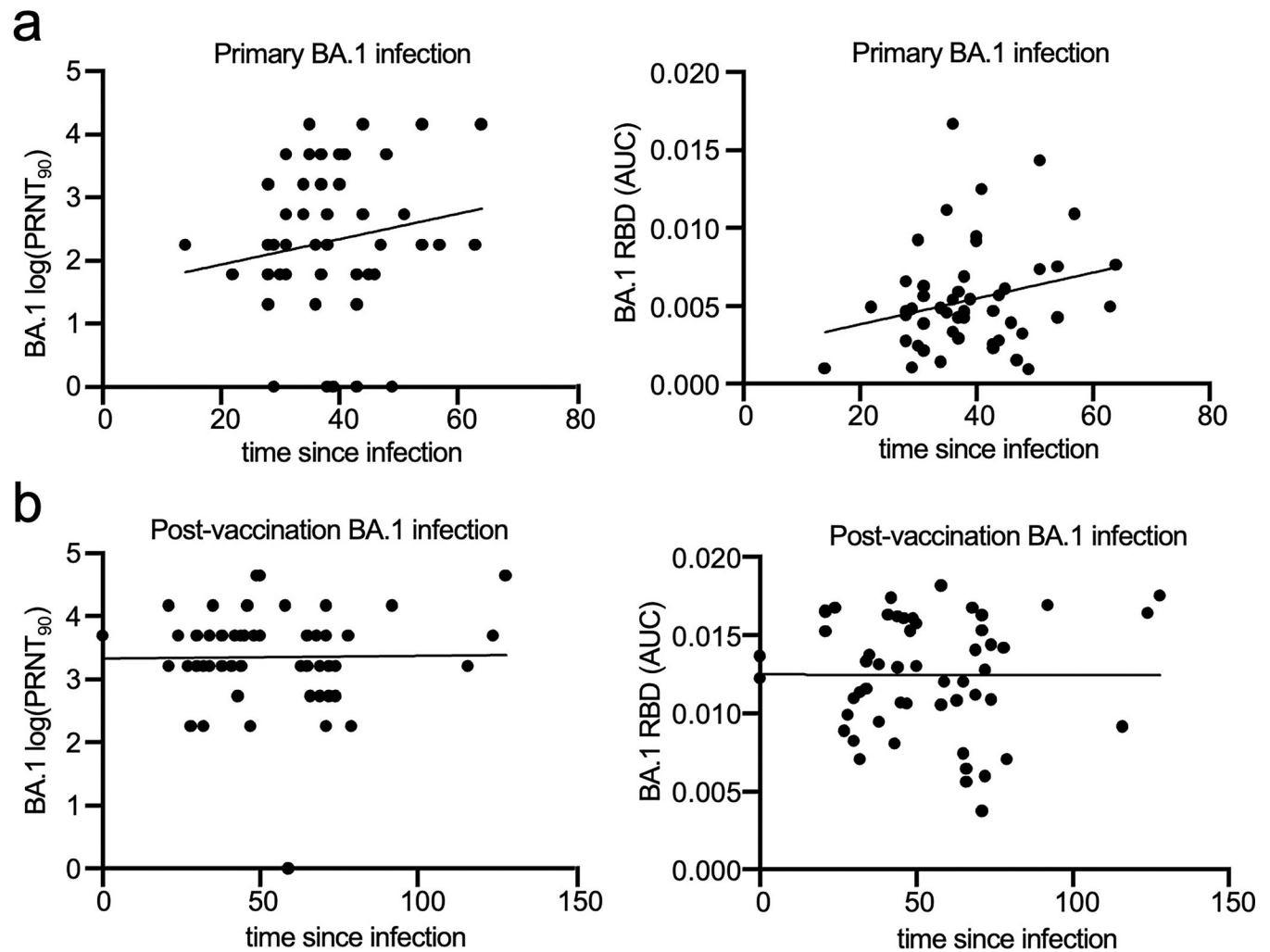
**Extended Data Fig. 1. Post-vaccination delta and BA.1 infections report symptoms lasting for a shorter duration compared to primary delta infections.**

**a.** Bar plot showing percentage of individuals from each TATS cohort (primary delta infection ( $n = 12$ ), post-vaccination delta infection ( $n = 37$ ), and post-vaccination BA.1 infection ( $n = 50$ )) that reported experiencing respiratory symptoms in study entry survey. **b.** Dot plot showing reported days until symptoms resolved for each TATS cohort (primary delta infection ( $n = 12$ ), post-vaccination delta infection ( $n = 37$ ), and post-vaccination BA.1 infection ( $n = 50$ )). Each symbol represents an individual. Medians  $\pm$  95% CI are shown. Two-sided P values from t-test statistics were calculated for pairwise differences using one-way ANOVA. Post hoc Tukey's multiple comparisons test was applied. P values greater than 0.05 are not depicted. **c.** Correlation of time since vaccination with PRNT<sub>90</sub> values against WuHu1 (left), antibody titers against WuHu1 RBD (middle), and the ratio of WuHu1:Delta RBD binding antibodies in vaccinated only ( $n = 62$ ) cohort. Each symbol represents an individual. Medians  $\pm$  95% CI are shown. Two-sided Pearson correlation analysis was performed (left ( $p = 0.72$ ), middle ( $p = 0.22$ ), right ( $p = 0.21$ )).



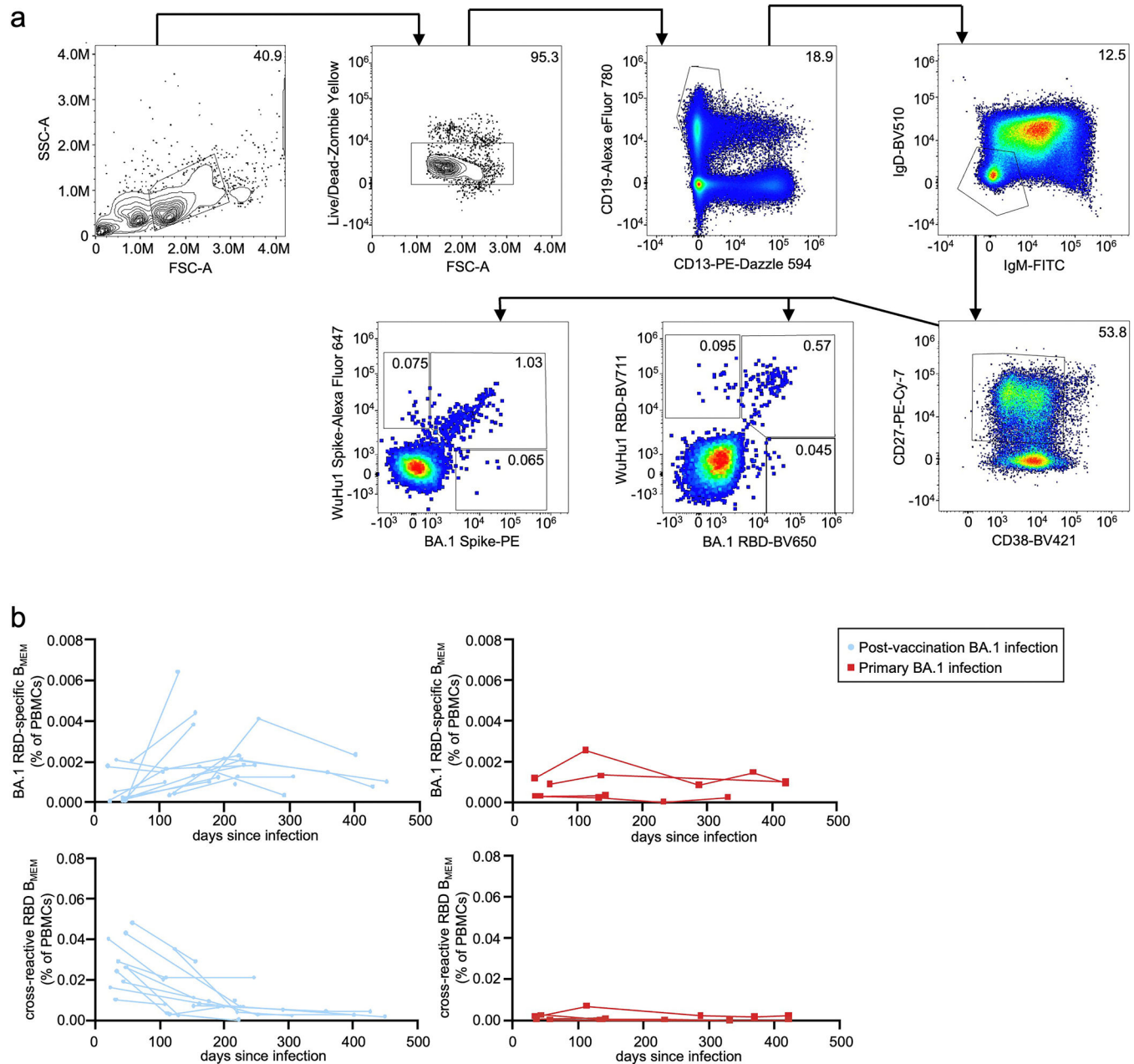
**Extended Data Fig. 2. Flow cytometric gating strategy and quantification with Delta S1 and WuHu1 S1 tetramers.**

Flow cytometry plot showing gating strategy for B<sub>MEM</sub> from a primary delta infection (top) and post-vaccination delta infection (bottom).



**Extended Data Fig. 3. No significant correlation is observed between antibody titers and time since infection in primary BA.1 and post-vaccination BA.1 infections.**

a. Correlation of time since infection and  $\text{PRNT}_{90}$  values against BA.1 (left) and antibody titers against BA.1 RBD (right) in primary BA.1 infection ( $n = 56$ ) cohort. Each symbol represents an individual. Two-sided Pearson correlation analysis was performed (left ( $p = 0.22$ ), right ( $p = 0.08$ )). b. Correlation of time since infection and  $\text{PRNT}_{90}$  values against BA.1 (left) and antibody titers against BA.1 RBD (right) in post-vaccination BA.1 infection ( $n = 50$ ) cohort. Each symbol represents an individual. Two-sided Pearson correlation analysis was performed (left ( $p = 0.91$ ), right ( $p = 1.0$ )).



**Extended Data Fig. 4. Cross-reactive and BA.1-specific  $B_{MEM}$  do not consistently increase over time post-infection.**

a. Flow cytometry plot showing gating strategy for  $B_{MEM}$  from a post-vaccination BA.1 infection. b. Plot showing quantification of WuHu1 and BA.1 RBD-specific CD19+IgD-IgM-CD27+CD38-  $B_{MEM}$  in primary BA.1 infection ( $n = 4$ ) and post-vaccination BA.1 infection ( $n = 15$ ) cohorts over time. Cells that bind both WuHu1 Spike and BA.1 RBD are quantified as cross-reactive Spike+, whereas cells that bind only BA.1 RBD are quantified as BA.1 RBD+.  $B_{MEM}$  are quantified as a percentage of total PBMCs. Each symbol represents an individual. Lines connect timepoints from the same individual.

**Extended Data Table. 1 Characteristics of cohorts**

Median values and interquartile range (IQR) is listed unless otherwise stated in the table.

|                                       | vaccinated only (statewide antibody testing initiative) (n=65) | primary delta (n=12) | post-vaccination delta (n=37) | vaccinated only <sup>TATS</sup> (n=62) | primary BA.1 (n=56) | post-vaccination BA.1 (n=50) | primary WuHu1 infection <sup>IASO</sup> (n=15) | primary BA.1 infection <sup>IASO</sup> (n=10) | naive (n=13)      |
|---------------------------------------|----------------------------------------------------------------|----------------------|-------------------------------|----------------------------------------|---------------------|------------------------------|------------------------------------------------|-----------------------------------------------|-------------------|
| <b>Age</b><br>Median (min, max)       | 39 (22, 70)                                                    | 21.9 (19, 42)        | 24.5 (18, 65)                 | 31.9 (18, 65)                          | 44 (25, 62)         | 40 (19, 71)                  | 44.7 (32, 55)                                  | 48.5 (33, 65)                                 | 36.7 (19.5, 81.5) |
| <b>Sex</b>                            |                                                                |                      |                               |                                        |                     |                              |                                                |                                               |                   |
| Male                                  | 21 (32%)                                                       | 5 (42%)              | 8 (22%)                       | 24 (38.7%)                             | 26 (46.4%)          | 8 (16%)                      | 4 (27%)                                        | 3 (30%)                                       | 4 (31%)           |
| Female                                | 44 (68%)                                                       | 7 (58%)              | 29 (78%)                      | 37 (59.6%)                             | 25 (44.6%)          | 16 (32%)                     | 11 (73%)                                       | 7 (70%)                                       | 9 (69%)           |
| not reported                          |                                                                |                      |                               | 1 (1.6%)                               | 5 (8.9%)            | 26 (52%)                     |                                                |                                               |                   |
| <b>Prior SARS-CoV-2 infection</b>     |                                                                |                      |                               |                                        |                     |                              |                                                |                                               |                   |
| Yes                                   |                                                                | 12 (100%)            | 37 (100%)                     |                                        | 56 (100%)           | 50 (100%)                    | 15 (100%)                                      | 10 (100%)                                     |                   |
| No                                    | 65 (100%)                                                      |                      |                               | 62 (100%)                              |                     |                              |                                                |                                               | 13 (100%)         |
| Time since SARS-CoV-2 infection (IQR) |                                                                | 90 days (32, 99)     | 75 days (47.5, 83.75)         |                                        | 37 days (31, 44.5)  | 63 days (42, 71)             | 45.2 days (37, 53)                             | 41.9 days (26.5, 47.5)                        |                   |
| <b>COVID-19 Vaccination</b>           | 65                                                             | 0                    | 37                            | 62                                     | 0                   | 50                           | 0                                              | 0                                             | 0                 |
| <b># of shots</b>                     |                                                                |                      |                               |                                        |                     |                              |                                                |                                               |                   |
| 2                                     | 65 (100%)                                                      |                      | 37 (100%)                     | 16 (26%)                               |                     | 13 (26%)                     |                                                |                                               |                   |
| 3                                     |                                                                |                      |                               | 46 (74%)                               |                     | 15 (30%)                     |                                                |                                               |                   |
| 4                                     |                                                                |                      |                               |                                        |                     | 3 (6%)                       |                                                |                                               |                   |
| not reported                          |                                                                |                      |                               |                                        |                     | 19 (38%)                     |                                                |                                               |                   |
| time since vaccination (IQR)          | 204 days (178.5, 248.5)                                        |                      | 286 days (249, 315)           | 143.3 days (117.25, 182.5)             |                     | 132 days (100.5, 196)        |                                                |                                               |                   |

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## Data availability:

Virus sequences are available on GISAID (accession number: EPI\_ISL\_17886211).

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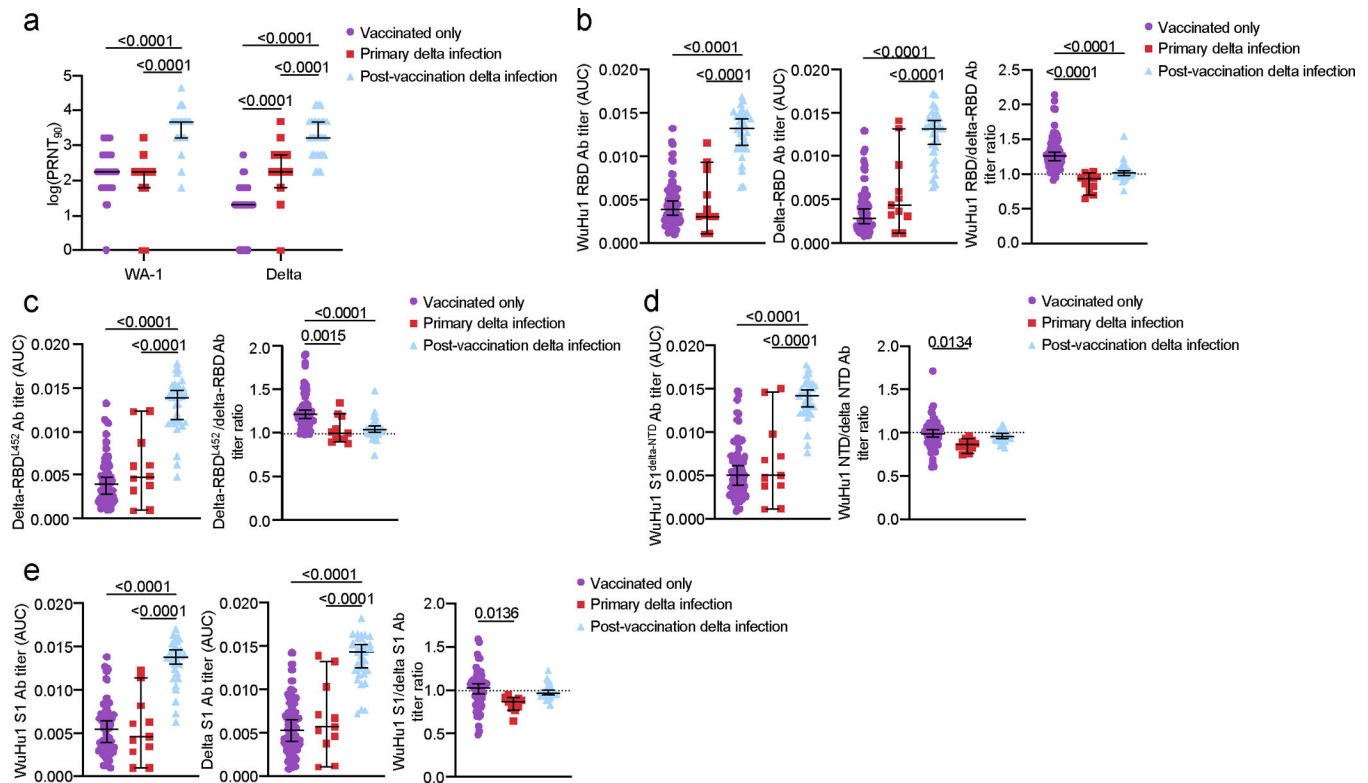


**Figure 1. Type-specific antibody responses are generated following delta infection.**

**a.** WA.1 and delta virus neutralization assays showing PRNT<sub>90</sub> values for vaccinated only (n = 36), primary delta infection (n = 12) and post-vaccination delta infection (n = 37) samples. Each symbol represents an individual. Medians ± 95% confidence intervals (CI) are shown. Two-sided P values were calculated for pairwise differences using two-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 not depicted. **b.** ELISA area under the curve (AUC) values for antibody binding to WuHu1-RBD and delta-RBD and the WuHu1 RBD/delta-RBD ratios in vaccinated only (n = 65), primary delta infection (n = 12) and post-vaccination delta infection (n = 37) cohorts. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values were calculated for pairwise differences using one-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 not depicted. **c.** ELISA of serum antibodies for delta-RBD<sup>L452</sup> and delta-RBD<sup>L452</sup>/delta-RBD titer ratio in vaccinated only (n = 65), primary delta infection (n = 12) and post-vaccination delta infection (n = 37) cohorts. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values were calculated for pairwise differences using one-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 not depicted. **d.** ELISA of antibodies for WuHu1-S1<sup>delta-NTD</sup> and WuHu1-S1/WuHu1-S1<sup>delta-NTD</sup> titer ratio in vaccinated only (n = 65), primary delta infection (n = 12) and post-vaccination delta infection (n = 37). Dotted line, WuHu1-S1/WuHu1-S1<sup>delta-NTD</sup> antibody ratio of 1. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values were calculated for pairwise differences using one-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 not depicted. **e.** ELISA of antibodies against WuHu1-S1, delta-S1

and WuHu1-S1/delta S1 binding ratios in vaccinated only (n = 65), primary delta infection (n = 12) and post-vaccination delta infection (n = 37). Each symbol represents an individual. Medians  $\pm$  95% CI are shown. Two-sided P values were calculated for pairwise differences using one-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 not depicted.



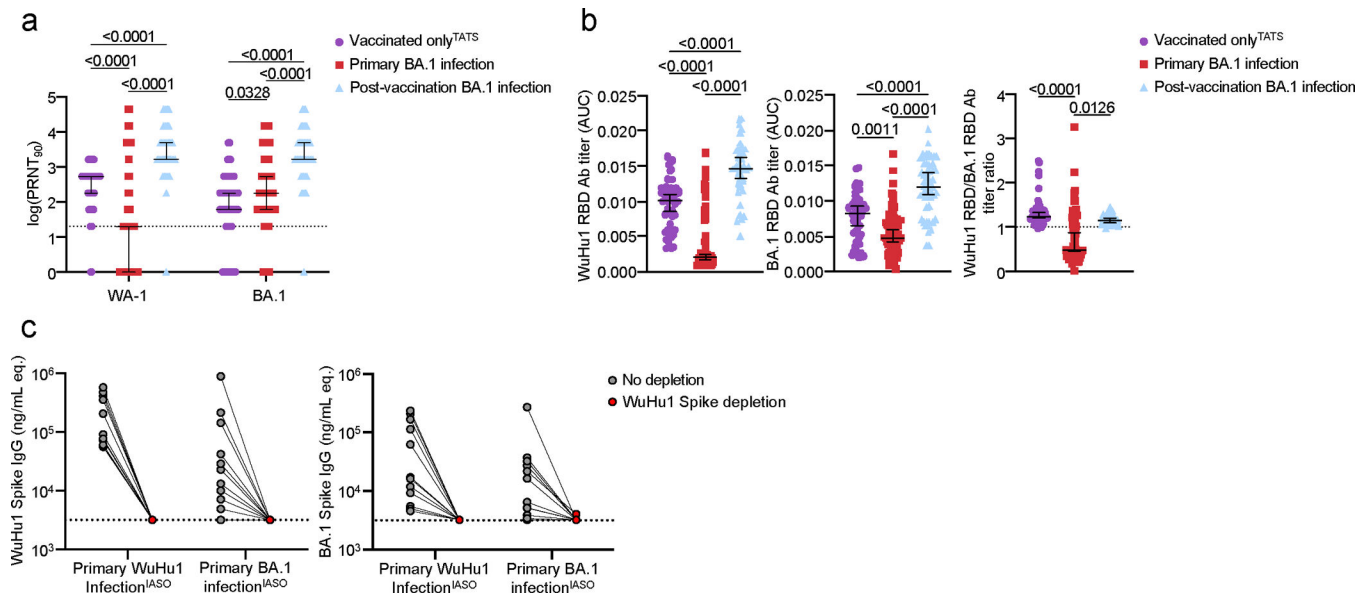


**Figure 3. Type-specific antibodies are minimally elicited by BA.1 infection.**

**a.** Virus neutralization assays performed with WA-1 and BA.1 isolates of SARS-CoV-2 showing PRNT<sub>90</sub> values in vaccinated only<sup>TATS</sup> (n = 62), primary BA.1 infection (n = 56) and post-vaccination BA.1 infection (n = 50) cohorts. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values were calculated for pairwise differences using two-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 are not depicted. **b.** ELISA showing area under the curve (AUC) values for antibody binding to WuHu1 RBD and BA.1 RBD (left and middle) and the WuHu1/BA.1 antibody binding ratios calculated by dividing WuHu1 AUC by the BA.1 AUC titer (right) in vaccinated only<sup>TATS</sup> (n = 62), primary BA.1 (n = 56), post-vaccination BA.1 (n = 62) cohorts. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values from t-test statistics were calculated for pairwise differences using one-way ANOVA with post hoc Tukey's multiple comparisons test. P values greater than 0.05 are not depicted. **c.** ELISA showing endpoint titer for antibody binding to WuHu1 Spike (left) and BA.1 Spike (right) in WuHu1 Spike-depleted serum from primary WuHu1 infection<sup>IASO</sup> (n = 15) and primary BA.1 infection<sup>IASO</sup> (n = 10) cohorts. Each symbol represents an individual and the solid line connects a sample before and after depletion. Dotted lines indicate the lower limit of detection.

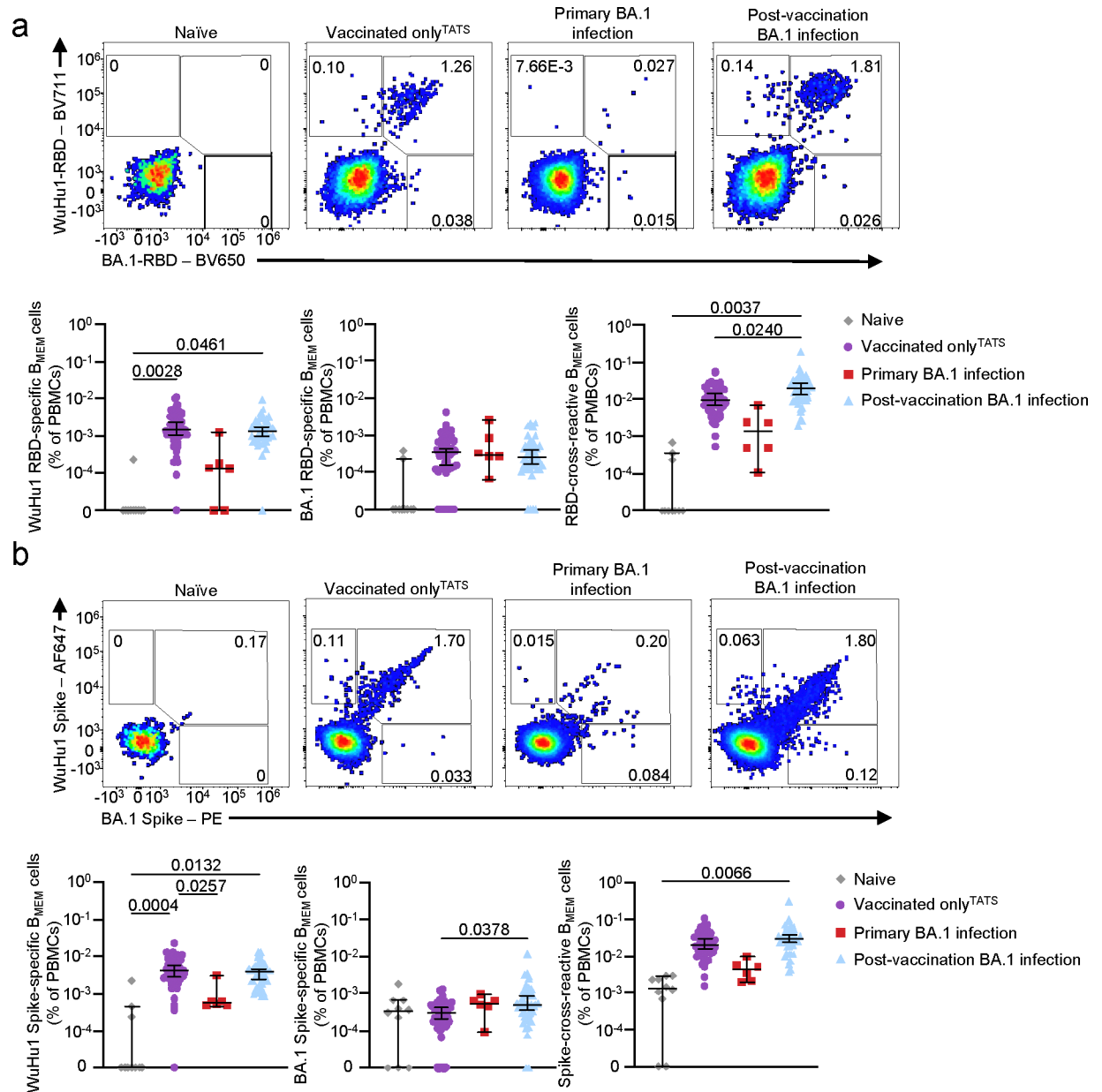


**Figure 4. BA.1-specific RBD and Spike B<sub>MEM</sub> cells are not detected above background levels in primary and post-vaccination infections.**

**a.** Representative flow cytometry plots (top) and quantification (bottom) of WuHu1 and BA.1 RBD-specific CD19<sup>+</sup>IgD<sup>+</sup>IgM<sup>+</sup>CD27<sup>+</sup>CD38<sup>+</sup> B<sub>MEM</sub> cells in naïve (n = 10), vaccinated only<sup>TATS</sup> (n = 62), primary BA.1 infection (n = 56) and post-vaccination BA.1 infection (n = 50) cohorts. Cells that bound both WuHu1 RBD and BA.1 RBD were quantified as RBD-cross-reactive B<sub>MEM</sub> cells, whereas cells that bound only WuHu1 RBD or BA.1 RBD were designated as WuHu1 RBD-specific B<sub>MEM</sub> cells or BA.1 RBD-specific B<sub>MEM</sub> cells, respectively. B<sub>MEM</sub> cells were quantified as a percentage of total PBMCs. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values from t-test statistics were calculated for pairwise differences using one-way ANOVA. Post hoc Tukey's multiple comparisons test was applied. P values greater than 0.05 are not depicted. **b.**

Representative flow cytometry plots (top) and quantification (bottom) of WuHu1 and BA.1 Spike-specific CD19<sup>+</sup>IgD<sup>-</sup>IgM<sup>-</sup>CD27<sup>+</sup>CD38<sup>-</sup> B<sub>MEM</sub> cells in naïve (n = 10), vaccinated only (n = 62), primary BA.1 infection (n = 56) and post-vaccination BA.1 infection (n = 50) cohorts. Cells that bound both WuHu1 Spike and BA.1 Spike were quantified as Spike-cross-reactive B<sub>MEM</sub> cells, whereas cells that bound only WuHu1 Spike or BA.1 Spike were quantified as WuHu1 Spike-specific B<sub>MEM</sub> cells or BA.1 Spike-specific B<sub>MEM</sub> cells, respectively. B<sub>MEM</sub> cells were quantified as a percentage of total PBMCs. Each symbol represents an individual. Medians ± 95% CI are shown. Two-sided P values from t-test statistics were calculated for pairwise differences using one-way ANOVA. Post hoc Tukey's multiple comparisons test was applied. P values greater than 0.05 are not depicted.