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Supplemental information

Longitudinal analysis of serum neutralization of SARS-CoV-2 Omicron BA.2, BA.4, and BA.5 in patients receiving monoclonal antibodies

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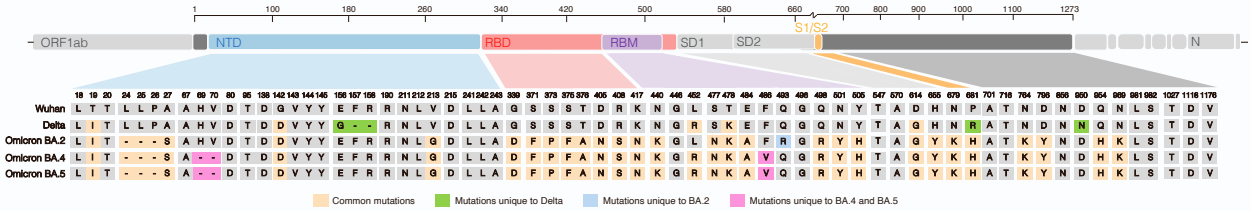
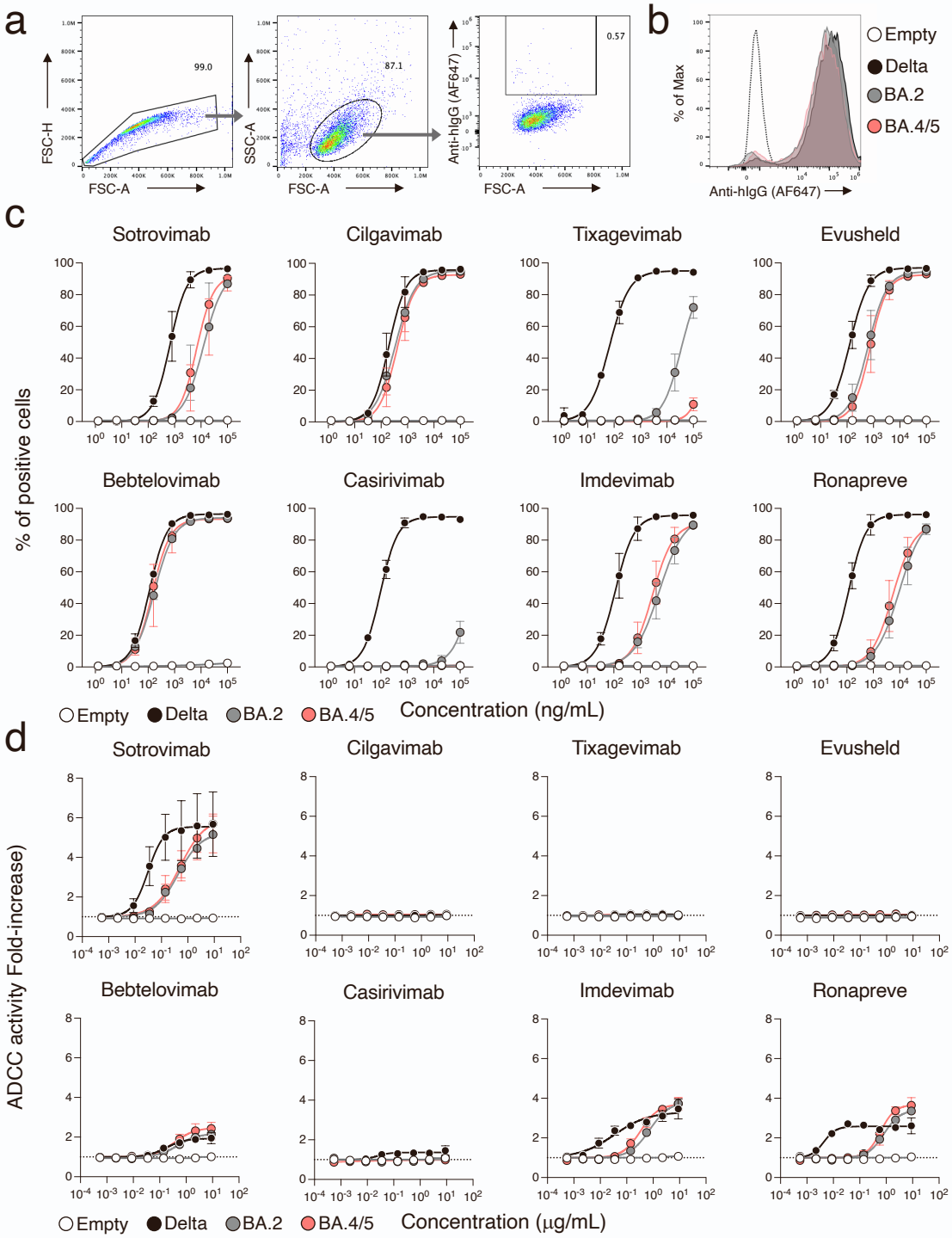


Supplementary figure 1 (related to Figure 1): Mutational landscape of Delta and Omicron BA.2, BA.4 and BA.5 spike proteins.

Domains of the protein are color-coded: NTD, N-Terminal Domain; RBD, Receptor-Binding Domain; RBM, Receptor-Binding Motif; SD1, subdomain 1; SD2, subdomain 2, S1/S2, region proximal to the furin cleavage site. Mutations in the amino acid sequence are indicated in comparison to the ancestral Wuhan-Hu-1 sequence (GenBank: NC_045512). Colored boxes highlight unique and shared mutations.



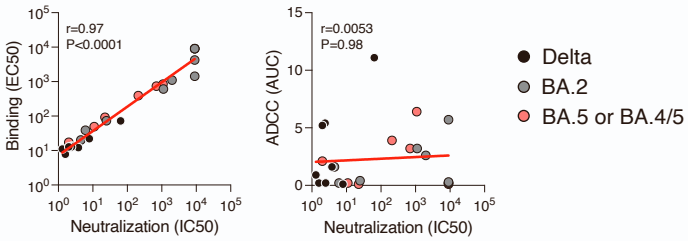
Supplementary figure 2 (related to Figure 1): Capacity of therapeutic antibodies to bind and elicit ADCC against the BA.4/5 spike.

a. Gating strategy of the binding assay. Raji cells stably expressing and empty transgene were incubated with bebtelovimab conjugated to biotin (200ng/mL), stained with a streptavidin coupled to AlexaFluor 647 (AF647) and analyzed by flow-cytometry. A representative example of the gating strategy is shown (MFI are 784, 97853, 71735, 68635 for Empty, Delta, BA.2 and BA.4/5, respectively).

b. An example of the fluorescence signal obtained with bebtelovimab (200ng/mL) on the Raji cells expressing Delta, Omicron BA.2 and Omicron BA.5 spikes. The Raji empty cells are used as control.

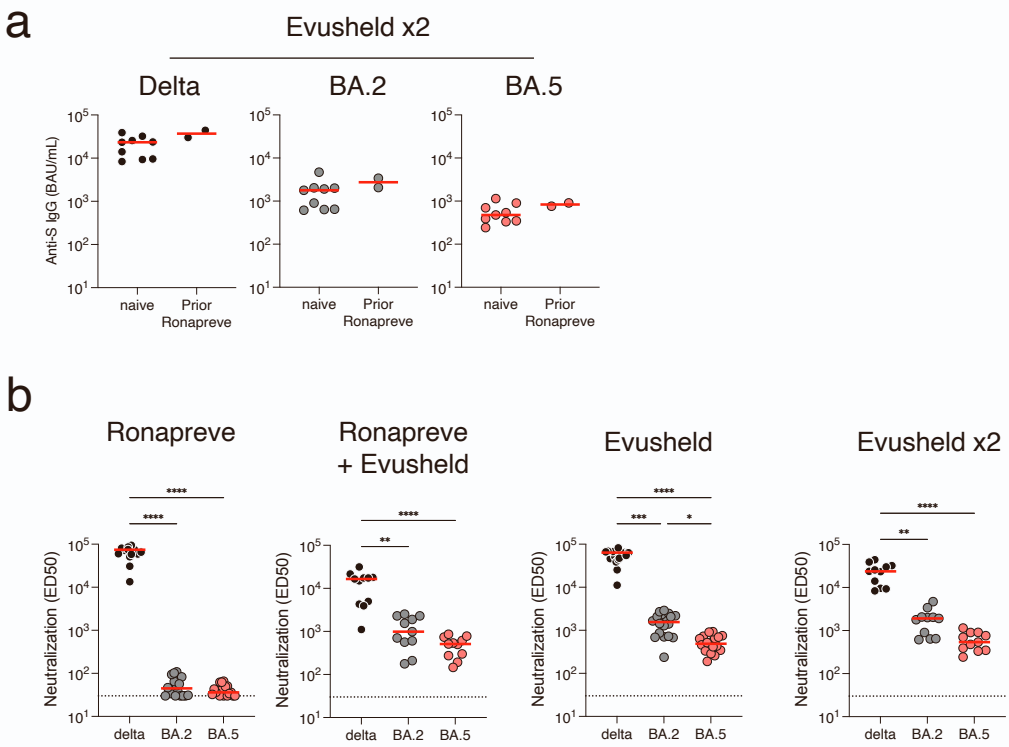
c. Dose–response analysis of the binding by the indicated antibodies and by Evusheld, a combination of cilgavimab and tixagevimab, and Ronapreve, a combination of casirivimab and imdevimab. The % of mAbs positive cells measured by flow cytometry against antibody C° in limiting-dilutions are depicted. Data are mean ± s.d. of 2 independent experiments. The EC50 values for each antibody are presented in Table 1.

d. Dose–response analysis of the ADCC activity by the indicated antibodies and by Evusheld, a combination of cilgavimab and tixagevimab, and Ronapreve, a combination of casirivimab and imdevimab. The fold-increase in CD16 activation as compared to the "no Raji" condition is indicated for each concentration of mAb. Data are mean ± s.d. of 2 independent experiments. Areas under the curve are scored and summarized in Table 1. The dashed line indicates the limit of detection.



Supplementary figure 3 (related to Figure 1): Correlation between neutralization, binding and ADCC activity of therapeutic mAbs.

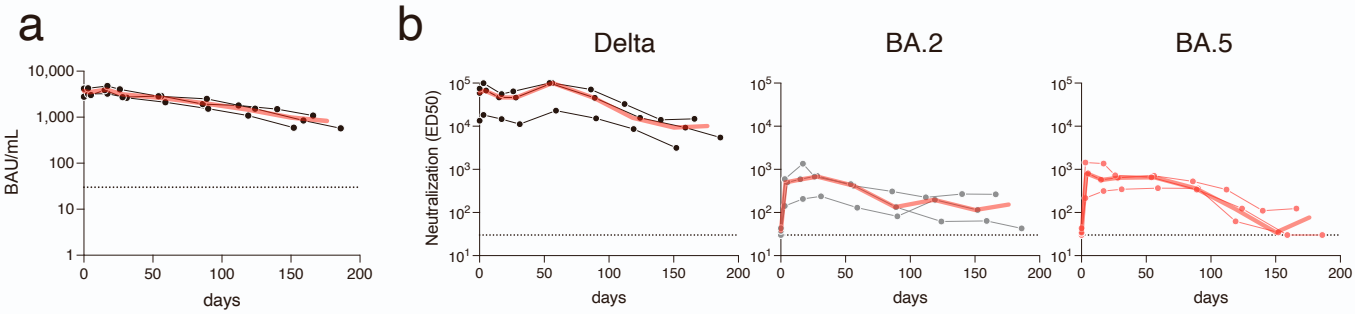
Correlation of neutralizing activity (IC50; ng/mL) of individuals therapeutic mAbs (Bamlanivimab, Casirivimab, Etesevimab, Imdevimab, Cilgavimab, Tixagevimab, Sotrovimab, Bebtelovimab) and recommended combinations (Ronapreve and Evusheld) and their binding capacity (left, EC50; ng/mL) and their ADCC activity (right; AUC). Colors indicate the viral strains. The analysis was performed using neutralization data from Delta, BA.2 and BA.5 and binding and ADCC data from Delta, BA.2 and BA.4/5 (n=24 pairs). All data are available in table 1. Correlation r and p values were calculated using a Spearman correlation test.



Supplementary figure 4 (related to Figure 2): Antibody levels and neutralization of delta, BA.2 and BA.5 in sera of immunocompromised individuals receiving mAbs.

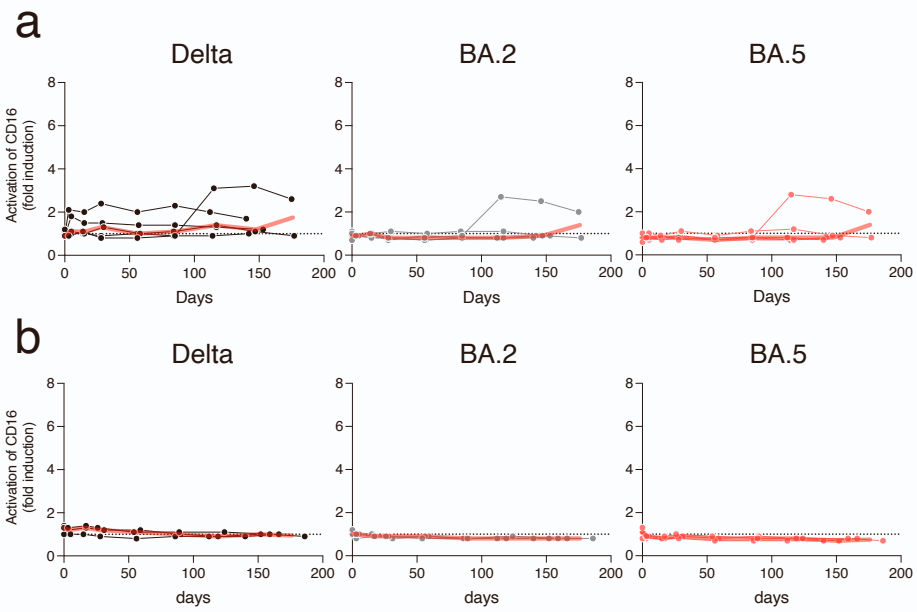
a. Serum neutralization of Delta, Omicron BA.2 and BA.5 in individuals who received 600 mg of Evusheld (Evusheld x2). Two of them received 1200 mg of Ronapreve >160 days prior to Evusheld administration (Prior Ronapreve). Indicated are Effective dilution 50% (ED50; titers) as calculated with the S-Fuse assay. Mann-Whitney test; non-significant comparisons are not indicated. Each dot is an individual. Red bars indicate median. The dashed line indicates the limit of detection.

b. Serum neutralization of Delta and Omicron BA.2 and BA.5 in the same individuals as in Figure 2. Indicated are Effective dilution 50% (ED50; titres) as calculated with the S-Fuse assay. Two-sided Kruskall–Wallis test with Dunn's multiple comparison correction. Each dot is an individual. Red bars indicate median. The dashed line indicates the limit of detection.



Supplementary figure 5 (related to Figure 2): Longitudinal evaluation of antibody levels and neutralization in 3 individuals who switched from Ronapreve to Evusheld.

Serum neutralization of Delta and Omicron BA.2 and BA.5 in the 3 individuals who switched from Ronapreve to Evusheld for their SARS-CoV-2 PrEP. Indicated are Effective dilution 50% (ED50 ; titers) as calculated with the S-Fuse assay. Two-sided Kruskall–Wallis test with Dunn's multiple comparison correction. Each dot is an individual. Red bars indicate median. The dashed line indicates the limit of detection.



Supplementary figure 6 (related to Figure 2): Longitudinal evaluation of the capacity of sera from Evusheld- and Ronapreve+Evusheld-treated individuals to activate the CD16 pathway.

Activation of the CD16 pathway was used as a surrogate of the capacity of the sera to elicit antibody-dependent cellular cytotoxicity (ADCC). The fold-increase in CD16 activation at a serum dilution of 1:100, using target cells expressing the indicated spike proteins, are shown. Data are normalized to cells transduced with an empty vector. All individuals and sampling points are depicted. **a.** Sera of 5 immunocompromised individuals who initiated an Evusheld PrEP. **b.** Sera of 3 immunocompromised individuals who initiated an Evusheld PrEP after receiving Ronapreve. The dashed line indicates a value of 1, meaning no activation. The red lines indicate the median.