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Supplemental information

Ad26.COV2.S priming provided a solid immunological base for mRNA-based COVID-19 booster vaccination

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Supplemental Appendix

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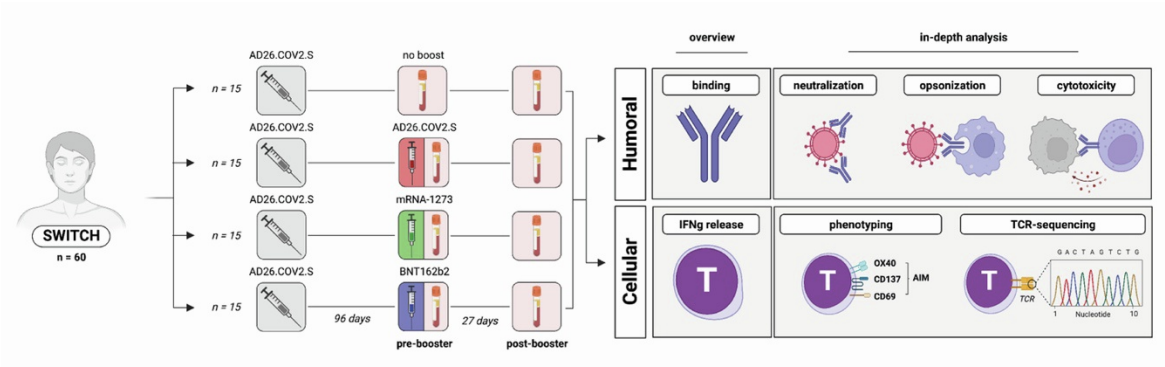

Figure S1: Study design and previously reported binding antibody levels and whole blood T-cell responses. Study design of the SWITCH immune profiling analysis. Immune responses in n=60 individuals that received either no (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) booster vaccination were assessed pre- and post-booster vaccination.

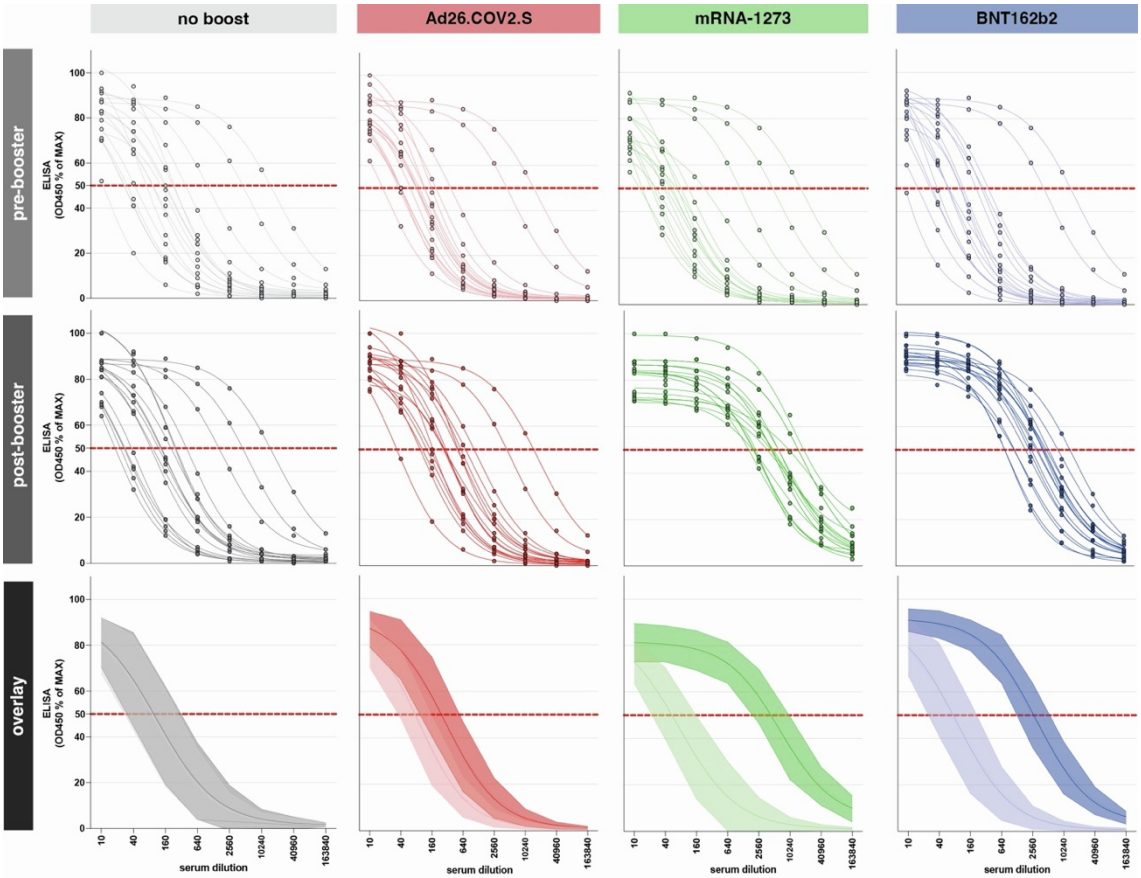

Figure S2: ELISA S-curves per vaccination regimen. Related to Figure 1. Log(inhibitor) versus response curves with four parameter variable slopes based on OD450 values % of max per vaccine group, pre-booster vaccination (top), post-booster vaccination (middle), and overlayed depicting the group mean with error bands representing the standard deviation (bottom). Red dotted line indicates the 50% endpoint.

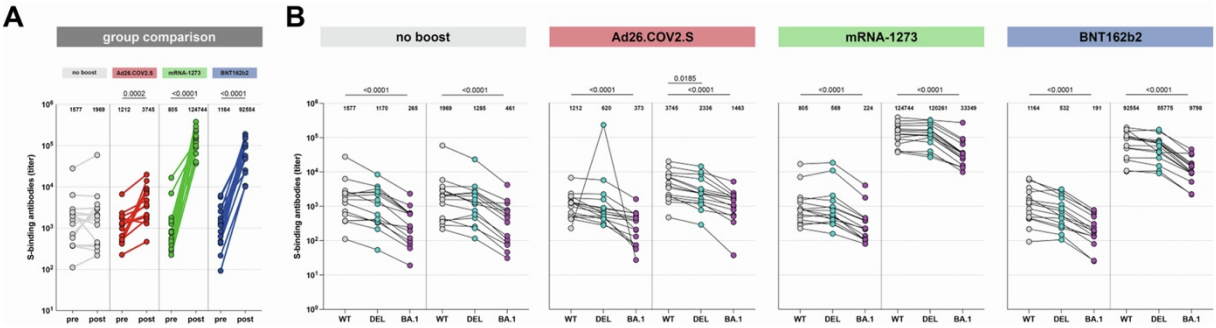

Figure S3: Binding antibody levels pre- and post-booster vaccination. Related to Figure 1. (A) S-specific binding antibody levels following no boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) vaccination following Ad26.COV2.S priming. **(B)** Cross-reactivity of S-specific binding antibodies against ancestral SARS-CoV-2 (grey), Delta (cyan), or Omicron BA.1 (pink) variants pre- and post-booster. WT = ancestral virus, delta = Delta variant, BA.1 = Omicron BA.1 variant. Symbols represent individual donors (n=15 per group). Wilcoxon rank test was performed for the comparison of pre- versus post-booster vaccination responses; a p-value of 0.05 was considered significant. Friedman test followed by Dunn's multiple comparisons was used to compare vaccine responses to variants within each group; only differences between ancestral SARS-CoV-2 and variants are shown in the figure. This supplemental figure is an alternative illustration of the data depicted in **Fig. 1B** and **1C**.

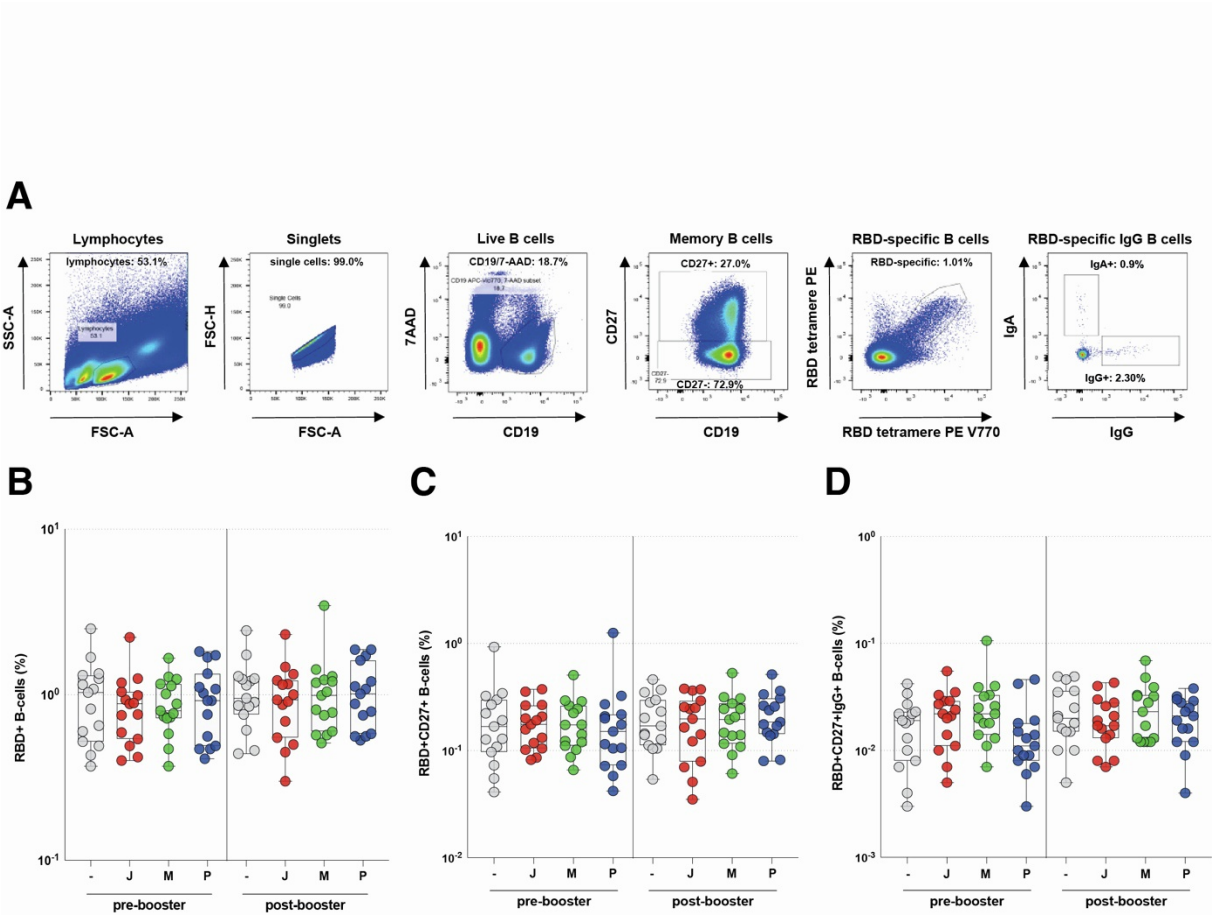

Figure S4: Gating strategy and detection of RBD-specific B-cells. Related to Figure 1. (A) Lymphocytes were gated based on the SSC-A and FSC-A and single cells were selected based on FSC-H and FSC-A. Next, LIVE CD19+ cells were selected for the analysis of RBD-specific B cells. In addition, the CD27+ subpopulation of the CD19+ B-cells (memory B cells) and the CD27+ IgG+ subpopulation was selected. In each subpopulation, the proportion of RBD-specific B cells was determined by gating on RBD-tetramer-PE and RBD-tetramer-PE-Vio770 double positive cells. **(B)** Percentage of total RBD-specific B cells, **(C)** RBD-specific memory B cells, and **(D)** RBD-specific IgG memory B cells of total B cells in whole blood after no-boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) booster vaccination pre- and post-booster vaccination. - = no boost, J = Ad26.COV2.S, M = mRNA-1273, P = BNT162b2. Symbols represent individual donors (n=15 per group). Kruskal-Wallis test followed by Dunn's multiple comparisons was performed for comparison of vaccine responses between groups; only differences between Ad26.COV2.S and mRNA-1273, or Ad26.COV2.S and BNT162b2 are shown in the figure.

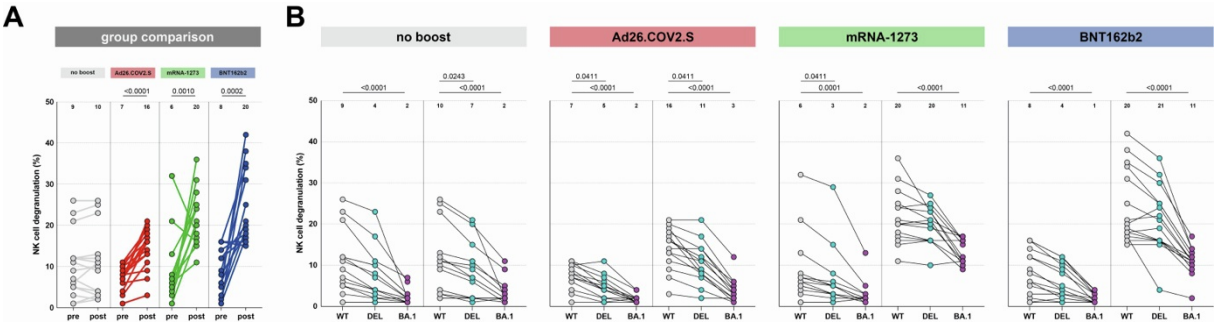

Figure S5: ADCC-mediating antibody levels pre- and post-booster vaccination. Related to Figure 2. (A) S-specific ADCC-mediating antibody levels following no boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) vaccination following Ad26.COV2.S priming. **(B)** Cross-reactivity of S-specific ADCC-mediating antibodies against ancestral SARS-CoV-2 (grey), Delta (cyan), or Omicron BA.1 (pink) variants pre- and post-booster. WT = ancestral virus, delta = Delta variant, BA.1 = Omicron BA.1 variant. Symbols represent individual donors (n=15 per group). Wilcoxon rank test was performed for the comparison of pre- versus post-booster vaccination responses; a p-value of 0.05 was considered significant. Friedman test followed by Dunn's multiple comparisons was used to compare vaccine responses to variants within each group; only differences between ancestral SARS-CoV-2 and variants are shown in the figure. This supplemental figure is an alternative illustration of the data depicted in **Fig. 2B** and **2C**.

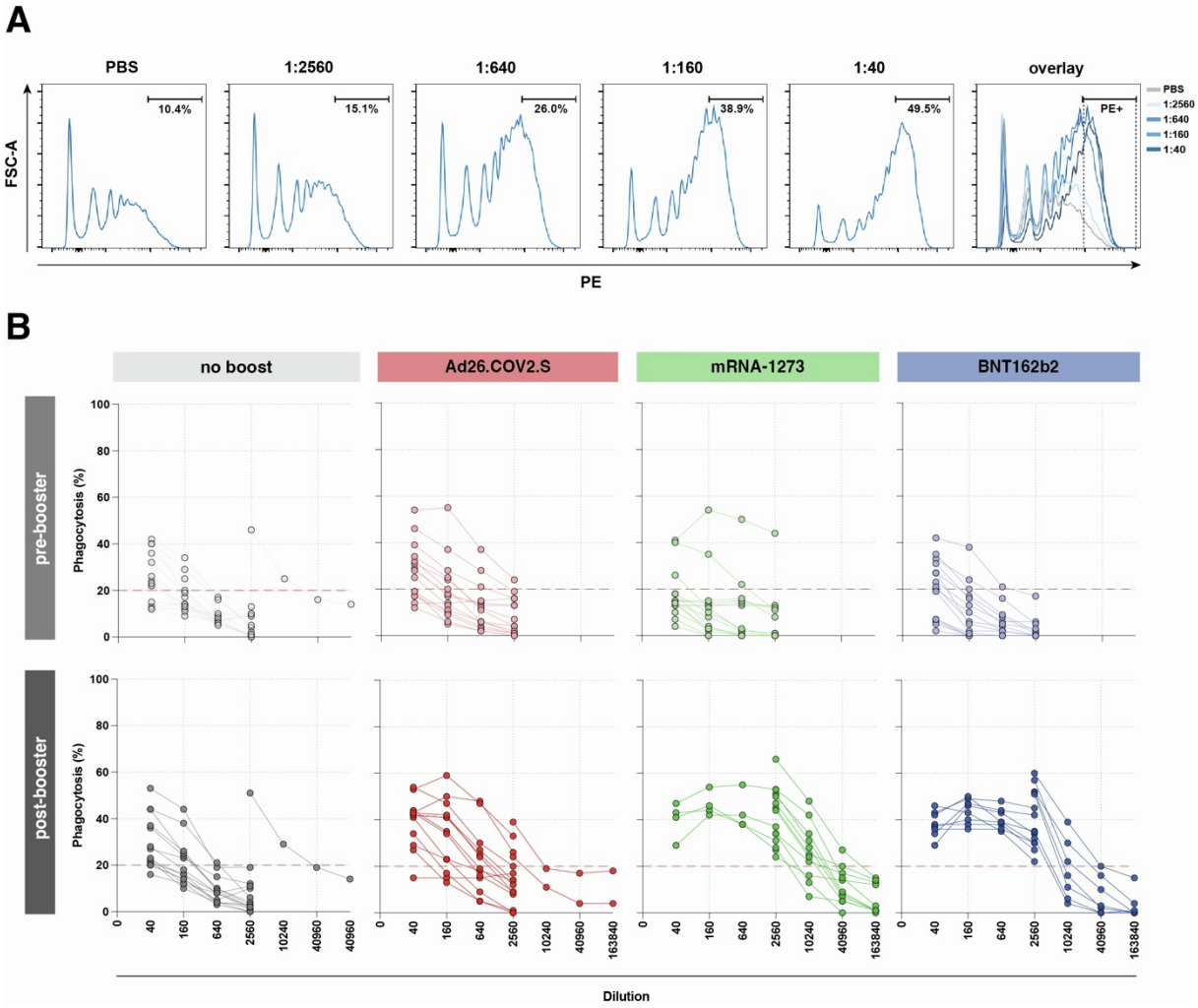

Figure S6: ADCP selection of PE+ cells in a dilution series from one representative sample and individual ADCP S-curves per vaccine regimen. Related to Figure 2. (A) Selection of phagocytosing cells. PE signal in PBS control was set at 10% background phagocytosis, and positive sera diluted 1:2560, 1:640, 1:160, and 1:40 from left to right. Overlay depicts PBS control in grey and different dilutions of sera in shades of blue. **(B)** Individual dilution series in ADCP pre- and post-booster vaccination per group: 'no boost' (grey), Ad26.COV2.S boost (red), mRNA-1273 boost (green), and BNT162b2 boost (blue). Red dotted line indicates the 20% endpoint dilution, which was used for calculation of ADCP-mediating antibody titers.

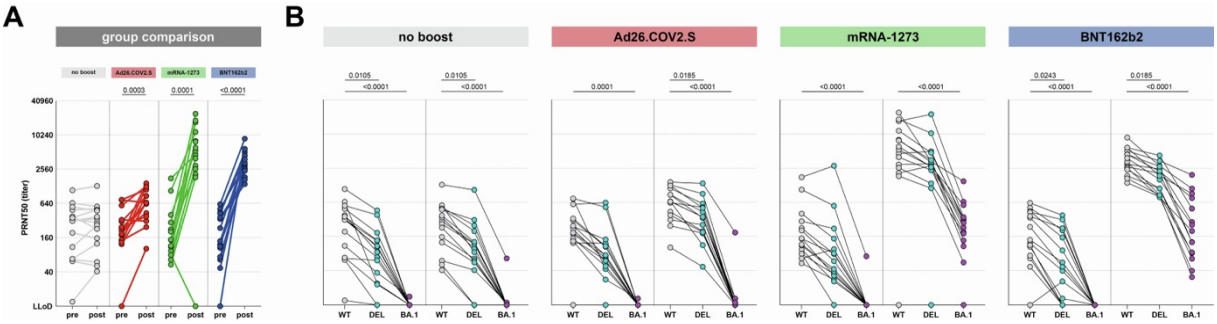

Figure S7: Neutralizing antibody levels pre- and post-booster vaccination. Related to Figure 3. (A) Neutralizing antibody levels following no boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) vaccination following Ad26.COV2.S priming. **(B)** Cross-reactivity of neutralizing antibodies against ancestral SARS-CoV-2 (grey), Delta (cyan), or Omicron BA.1 (pink) variants pre- and post-booster. WT = ancestral virus, delta = Delta variant, BA.1 = Omicron BA.1 variant. Symbols represent individual donors (n=15 per group). Wilcoxon rank test was performed for the comparison of pre- versus post-booster vaccination responses; a p-value of 0.05 was considered significant. Friedman test followed by Dunn's multiple comparisons was used to compare vaccine responses to variants within each group; only differences between ancestral SARS-CoV-2 and variants are shown in the figure. This supplemental figure is an alternative illustration of the data depicted in **Fig. 3B** and **3C**.

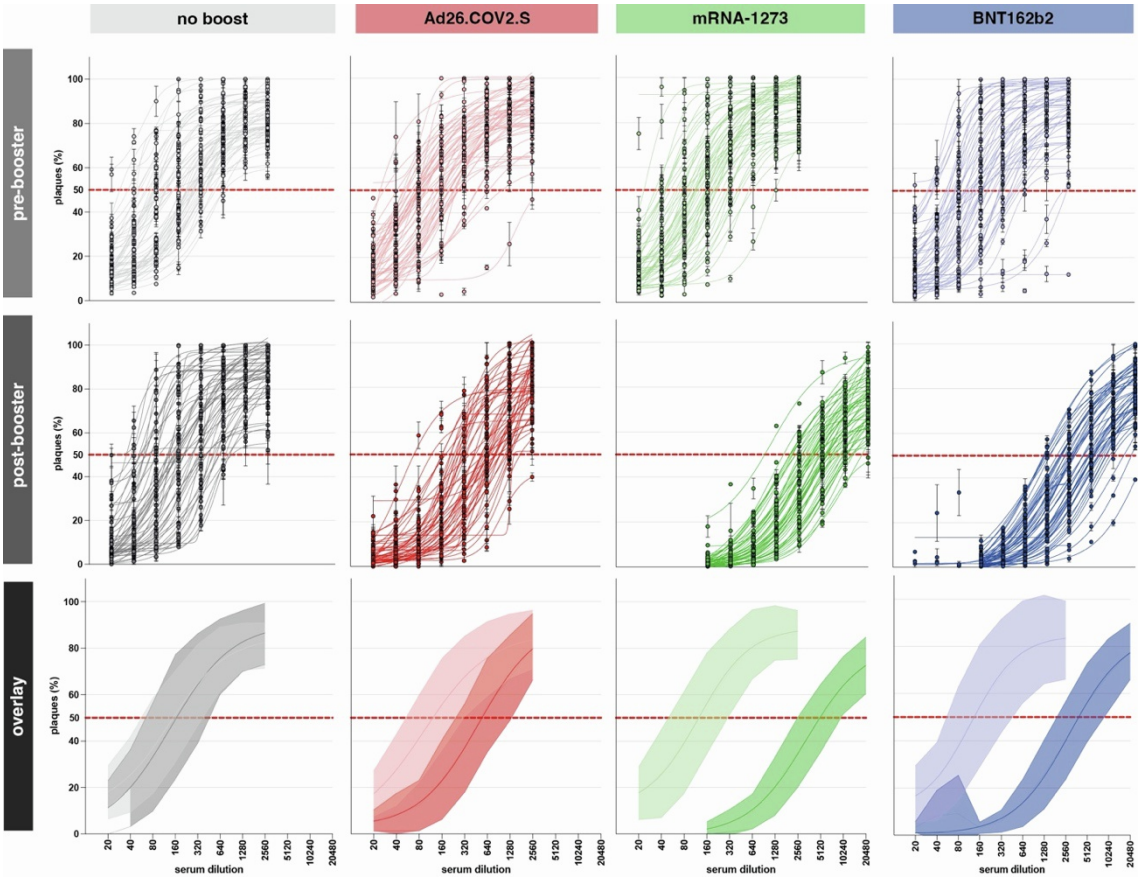

Figure S8: Individual PRNT S-curves per vaccine regimen. Related to Figure 3. log(inhibitor) versus response curves with four parameter variable slopes based on plaque counts compared to the virus control per vaccine group, pre-booster vaccination (top), post-booster vaccination (middle), and overlayed depicting the group mean with error bands representing the standard deviation (bottom). Red dotted line indicates the 50% endpoint.

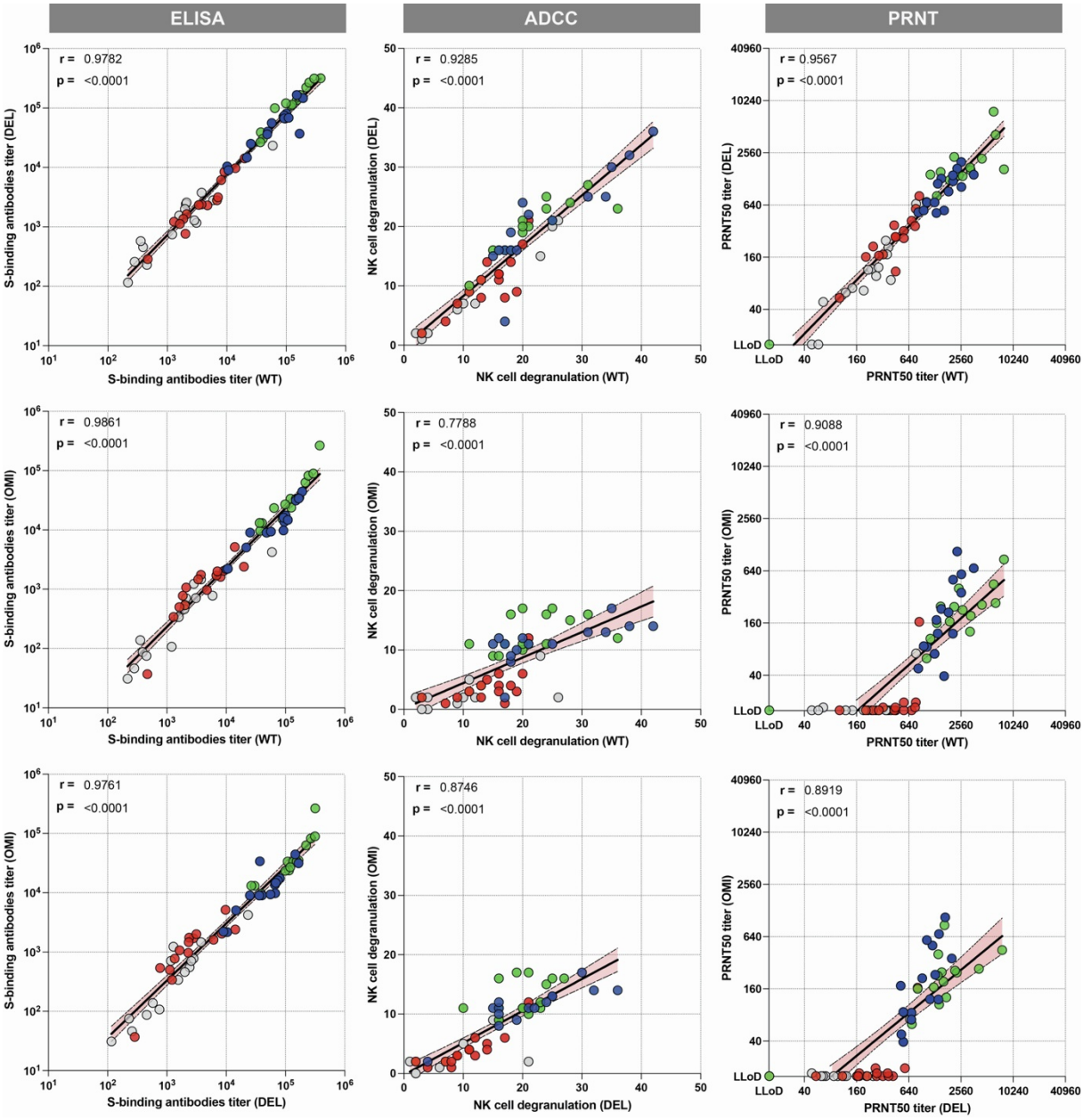

Figure S9: Binding and functional antibody responses to variants are correlated. Related to Figure 4. Correlation between S-specific binding, ADCC-mediating and neutralizing antibodies between ancestral (WT) and delta (DEL), ancestral and omicron BA.1 (OMI), or delta and omicron BA.1. Simple linear regression analysis on log-transformed data was used to calculate Spearman's correlation coefficient and p-values. Colors represent different booster groups: no boost (grey), Ad26.COV2.S boost (red), mRNA-1273 boost (green), and BNT162b2 boost (blue).

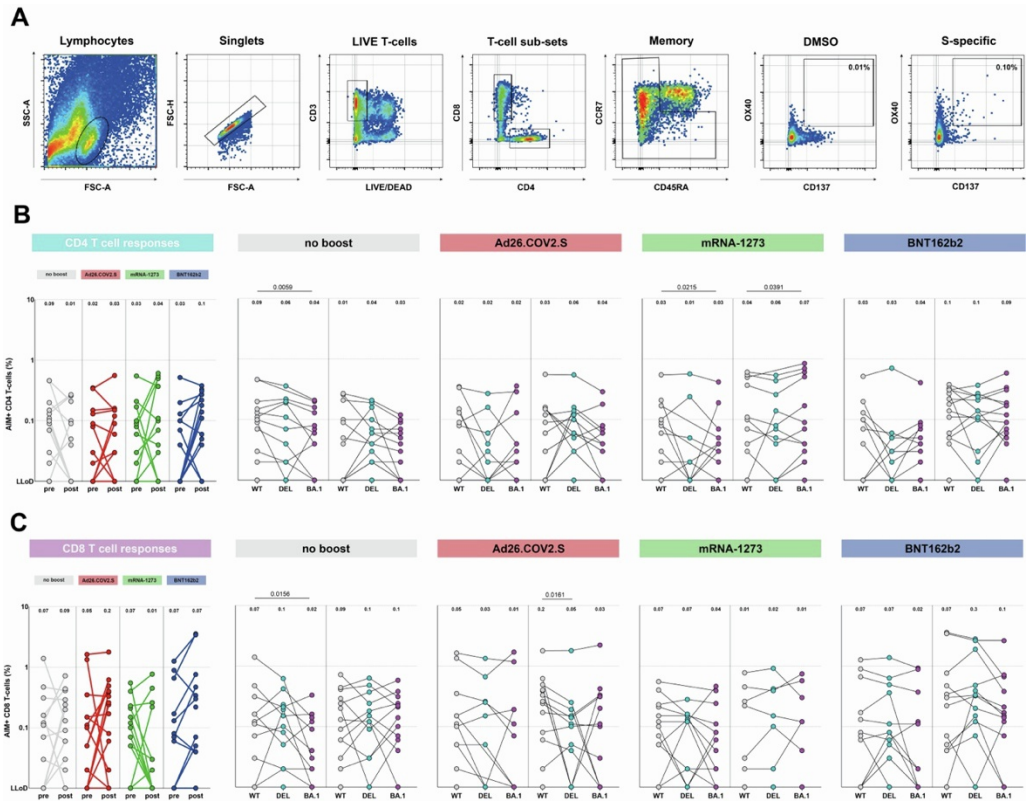

Figure S10: T cell responses pre- and post-booster vaccination. Related to Figure 5. (A) Lymphocytes were gated based on the SSC-A and FSC-A and single cells were selected based on FSC-H and FSC-A. Next, LIVE CD3+ cells were selected and sub-divided into CD4+ or CD8+ T-cells. From each sub-population naïve T-cells are excluded from further analysis based on their expression of CCR7 and CD45RA. Within the memory T-cell population OX40+CD137+ or CD69+CD137+ CD4 or CD8 T-cells, respectively, are defined as activated. A representative DMSO stimulated and S stimulated sample is shown. **(B)** CD4 T-cell responses following no boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) vaccination following Ad26.COV2.S priming. Cross-reactivity of CD4 T-cells against ancestral SARS-CoV-2 (grey), Delta (cyan), or Omicron BA.1 (pink) variants pre- and post-booster (right panels). **(C)** CD8 T-cell responses following no boost (grey), Ad26.COV2.S (red), mRNA-1273 (green), or BNT162b2 (blue) vaccination following Ad26.COV2.S priming. Cross-reactivity of CD8 T-cells against ancestral SARS-CoV-2 (grey), Delta (cyan), or Omicron BA.1 (pink) variants pre- and post-booster (right panels). WT = ancestral virus, delta = Delta variant, BA.1 = Omicron BA.1 variant. Symbols represent individual donors. Wilcoxon rank test was performed for the comparison of pre- versus post-booster vaccination responses for both CD4 and CD8 T-cells; a p-value of 0.05 was considered significant. Wilcoxon rank test was performed for the comparison of variant-specific T-cell responses between ancestral SARS-CoV-2 and variants; a p-value of 0.025 was considered significant after Bonferroni correction. Supplemental figure 10B is an alternative illustration of the data depicted in **Fig. 5C** and **5D**.

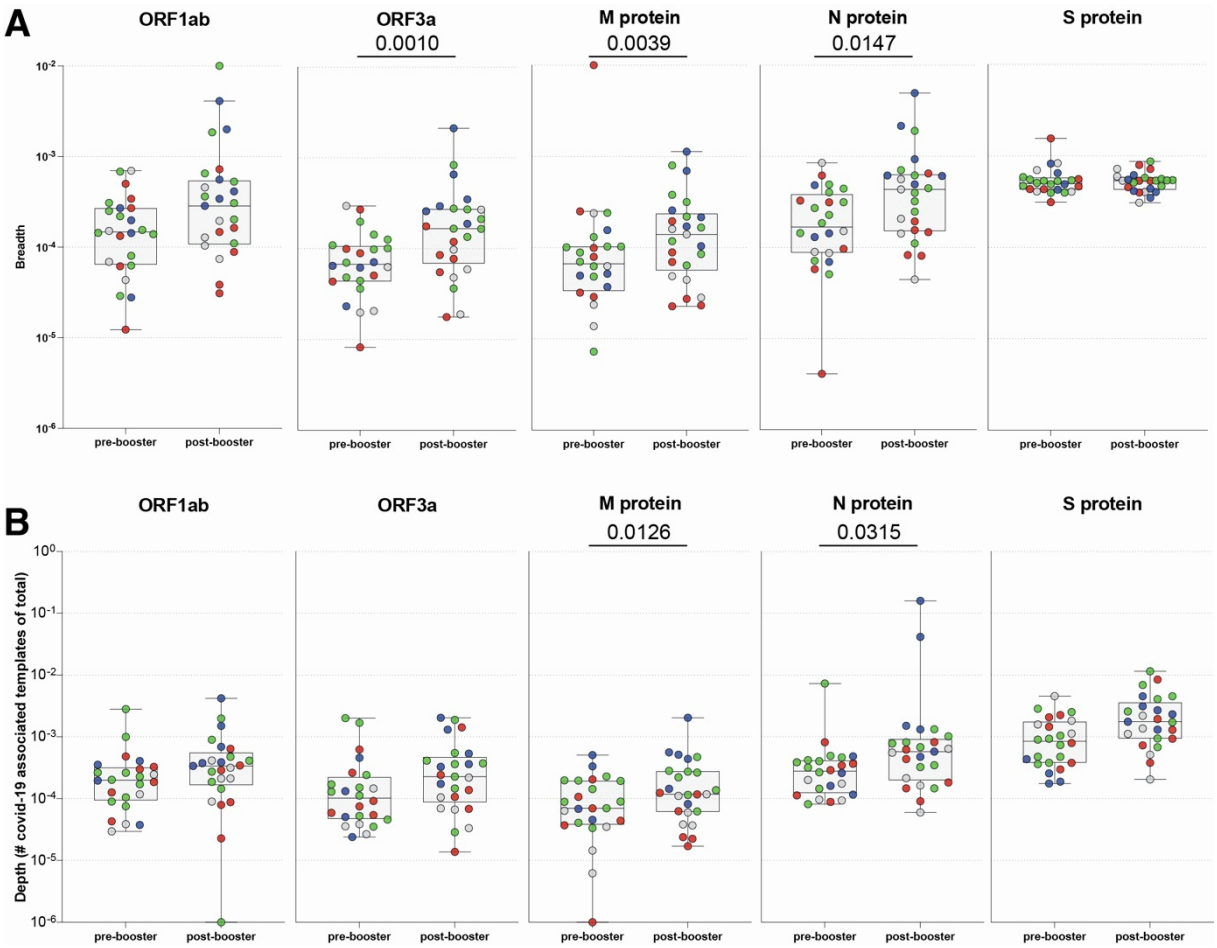

Figure S11: Breadth and depth of SARS-CoV-2-specific T-cell response. Related to Figure 6. (A) Breadth and **(B)** depth of the T-cell response to ORF1ab, ORF3a, M protein, N protein, and S protein pre- and post-booster vaccination. - = no boost (n=4 pre-boost / n=5 post-boost), J = Ad26.COV2.S (n=6), M = mRNA-1273 (n=10 pre-boost / n=8 post-boost), P = BNT162b2 (n=4 pre-boost / n=6 post-boost). Symbols represent individual donors. Box plot depicts the median with range (min to max). Wilcoxon rank test was performed for the comparison of T-cell breadth and depth pre- and post-booster vaccination.

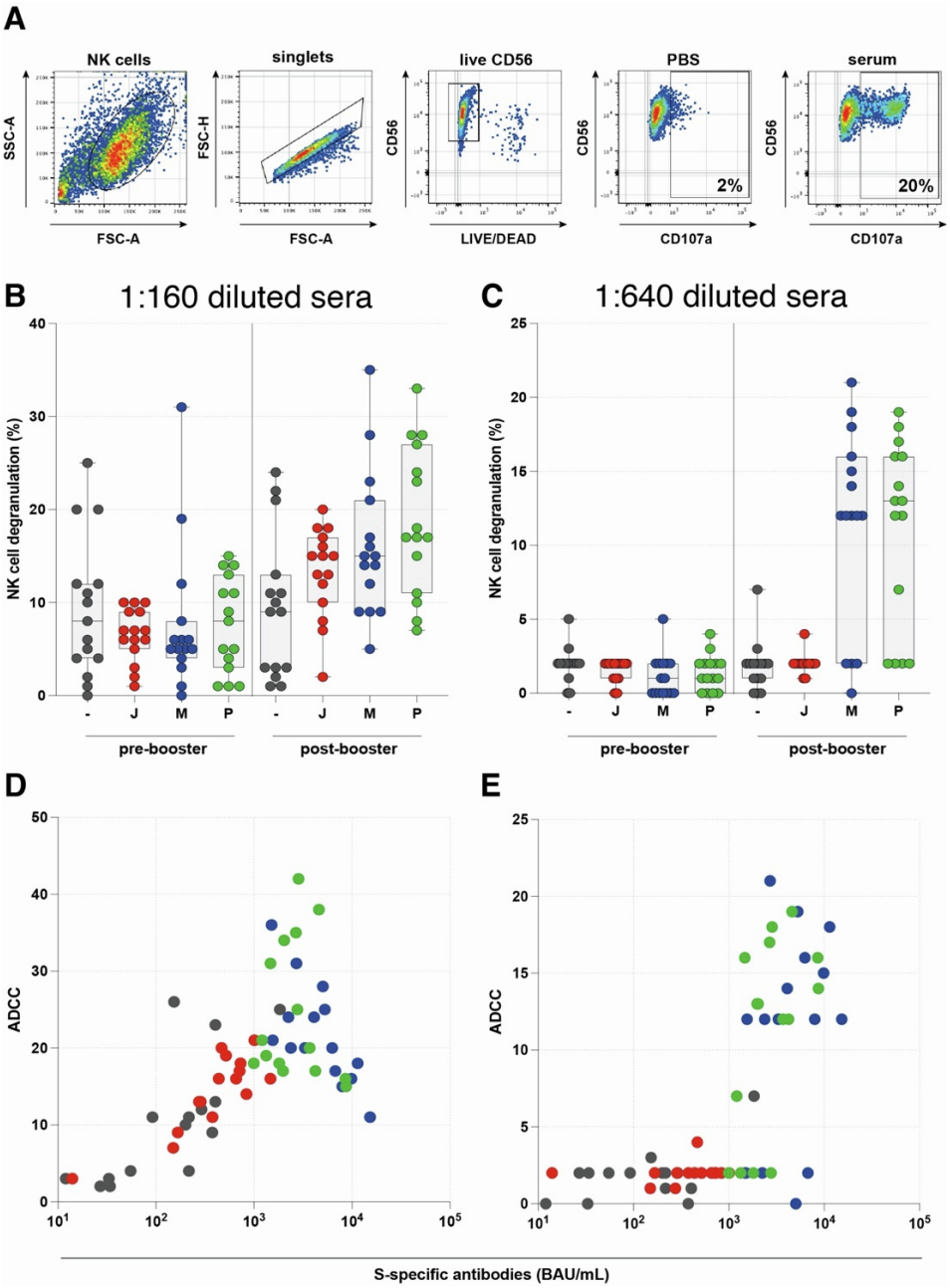

Figure S12: ADCC gating strategy and measurements from two independent experiments at two different serum dilutions. Related to Figure 2. (A) Gating strategy for ADCC assay, NK cells were gated on FSC-A and SSC-A, next singlets were selected by FSC-H and FSC-A and LIVE CD56^{+} cells were gated to assess CD107a expression. Representative figure for a PBS and positive serum sample after booster vaccination with mRNA-1273 are shown. **(B)** ADCC data with serum samples diluted 1:160 and **(C)** 1:640. NK cell degranulation is depicted as a % after PBS subtraction pre- and post-booster vaccination after 'no boost' (grey), Ad26.COV2.S boost (red), mRNA-1273 boost (green), or BNT162b2 boost (blue). **(D)** correlation of ADCC inducing antibodies and S1-specific binding antibodies at 1:160 dilution and **(E)** 1:640 dilution. Symbols represent individual donors (n=15). Box plot depicts the median with range (min to max).