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Supplemental information

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mRNA vaccination in octogenarian nuns 15 and 20 months after recovery from COVID-19 elicits robust immune and antibody responses that include Omicron

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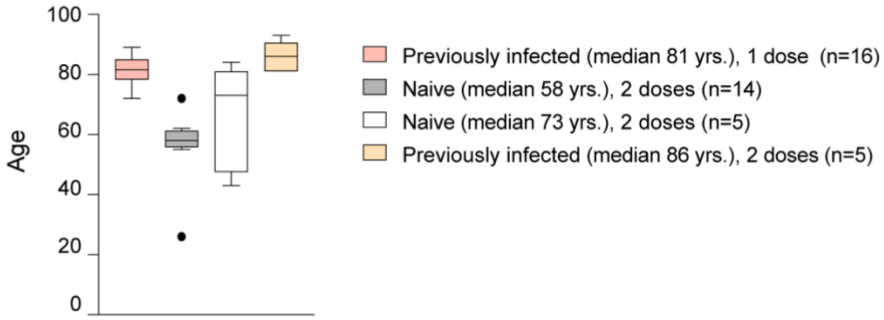

Figure S1. Comparison of ages between cohorts, related to Figures 1-3.

Median of age and the number sample in each group are within bracket. Median, middle bar inside the box; IQR, 50% of the data; whiskers, 1.5 times the IQR. The individual's age is listed in Table S1.

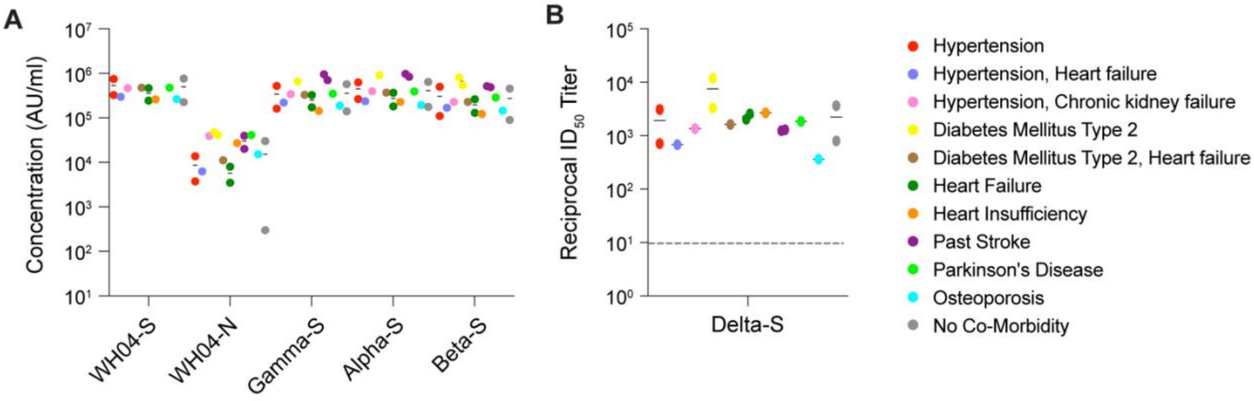

Figure S2. IgG antibody and Neutralizing antibody levels by comorbidities in the previously infected cohort, related to Figure 1.

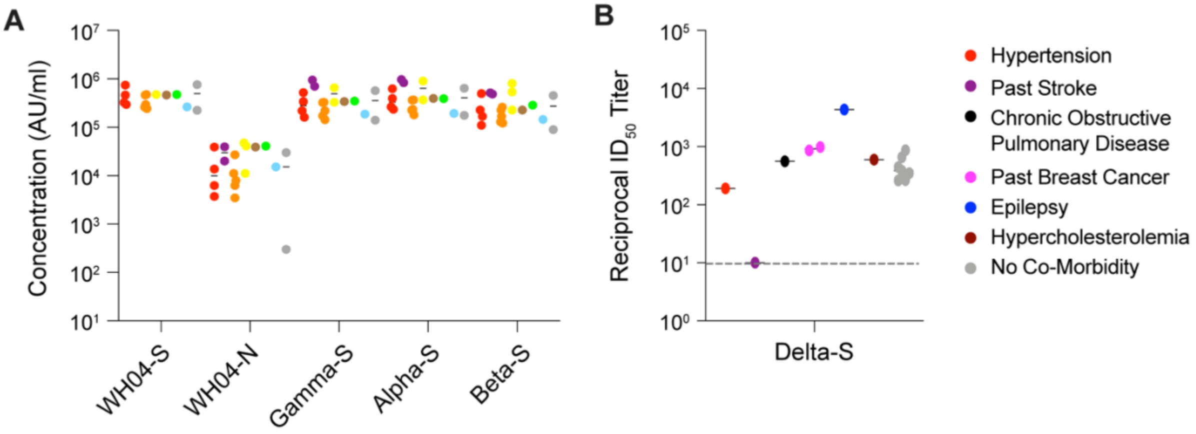

Figure S3. IgG antibody and Neutralizing antibody levels by comorbidities in the naïve cohort, related to Figure 2.

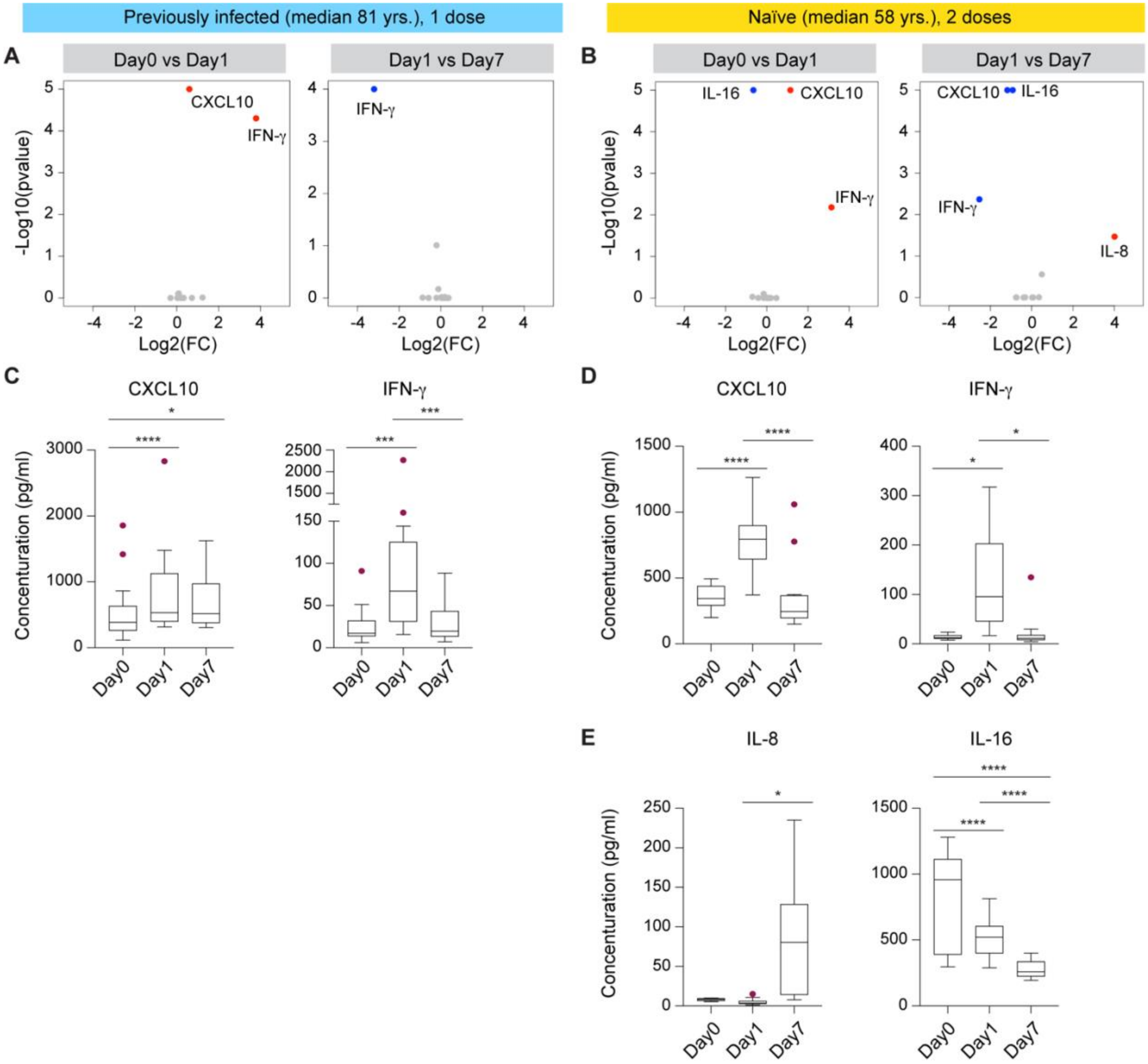

Figure S4. Serum cytokine and chemokine levels, related to Figures 1-2.

(A-B) Volcano plots depict differentially expressed cytokines and chemokine at day 0, 1 and 7 of 1 dose vaccination of previously infected nuns (A, Day0, *n* = 16; Day1, *n* = 16; Day7, *n* = 15) and 2 dose vaccination of naïve community (B, Day0, *n* = 9; Day1, *n* = 13; Day7, *n* = 14). Red dots indicate significant upregulation; blue dots indicate significant downregulation (p-value < 0.05). (C-D) Cytokine and chemokine concentrations in serum prior to and post vaccination (pg/ml) of CXCL10 and IFN-g. (E) Concentrations of IL-8 and IL-16 in serum before and after vaccination. Boxes show median, 25th and 75th percentiles and whiskers show the range. *p*-value are from 2-way ANOVA with Tukey's multiple comparisons test and Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli method and listed in Data S2. **p* < 0.05, ****p* < 0.001, *****p* < 0.0001.

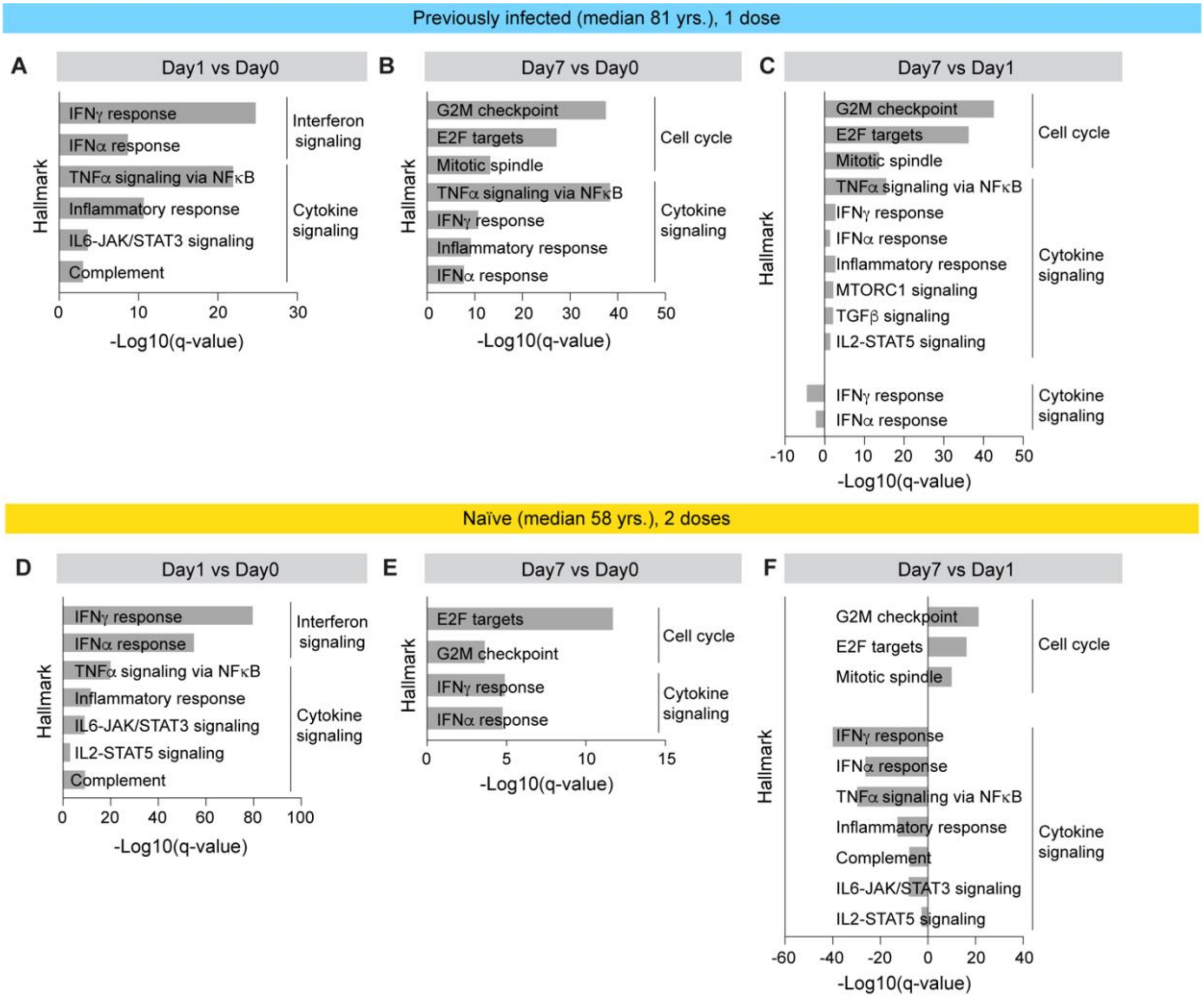

Figure S5. Genes expressed at significantly higher levels between days were significantly enriched in Hallmark gene sets, related to Figure 4.

X-axis denotes statistical significance as measured by minus logarithm of FDR q-values. Y-axis ranked the terms by q values.

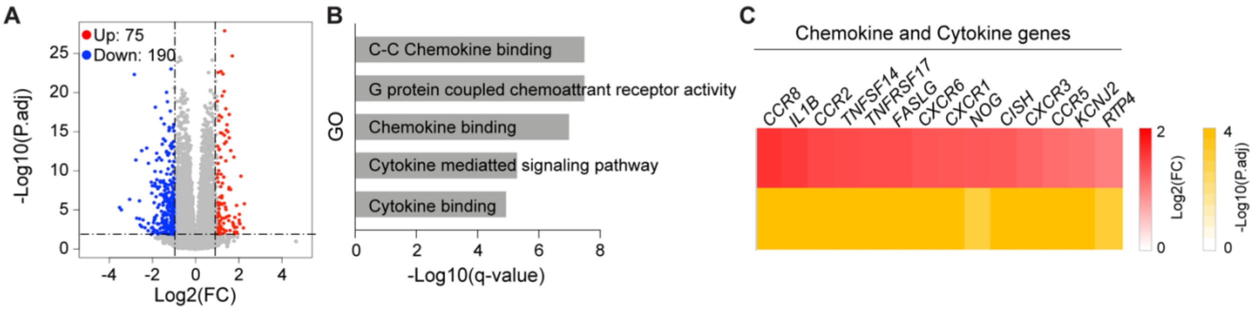

Figure S6. Immune feature of 1 dose vaccination in SARS-CoV-2 naïve cohort, related to Figure 4. Transcriptional signatures of 1st vaccination in Naïve cohort. (A) Volcano plot of DEGs comparing Day0 versus Day10. DEGs (adjusted p-value, P.adj < 0.05) with a log2 fold change (FC) of more than 1 or less than -1 are indicated in red and blue, respectively. Non-significant DEGs are indicated in gray. The numbers of upregulated and downregulated genes are listed in Data S9. (Day0, *n* = 14; Day10, *n* = 14) (B) Genes expressed at significantly higher levels at Day10 were significantly enriched in Gene Ontology (GO) gene sets. X-axis denotes statistical significance as measured by minus logarithm of FDR q-values. Y-axis ranked the terms by q values. (C) Heatmaps showing log2 FC (top) and corresponding P.adj (bottom) of gene sets from B.

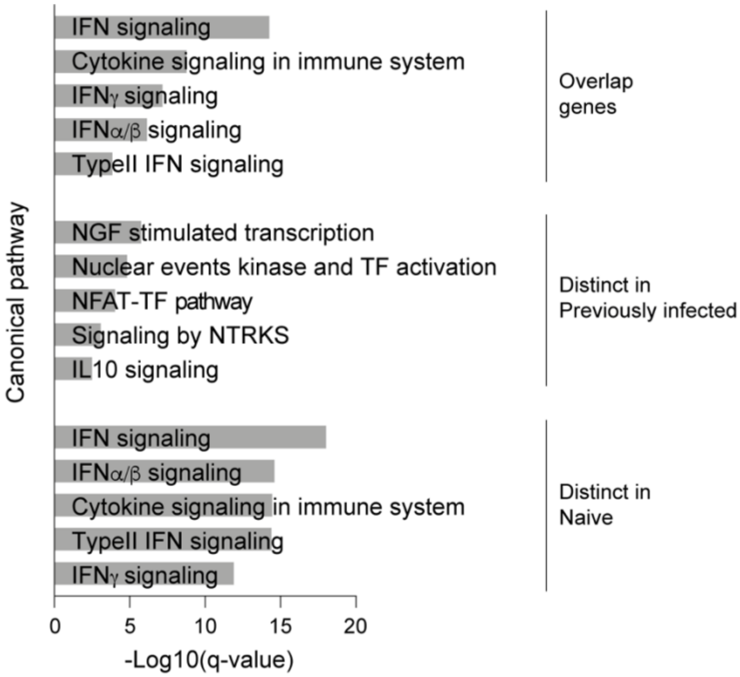

Figure S7. Genes from each compartment in Figure 5B were significantly enriched in Hallmark gene sets, related to Figure 5.

X-axis denotes statistical significance as measured by minus logarithm of FDR q-values.

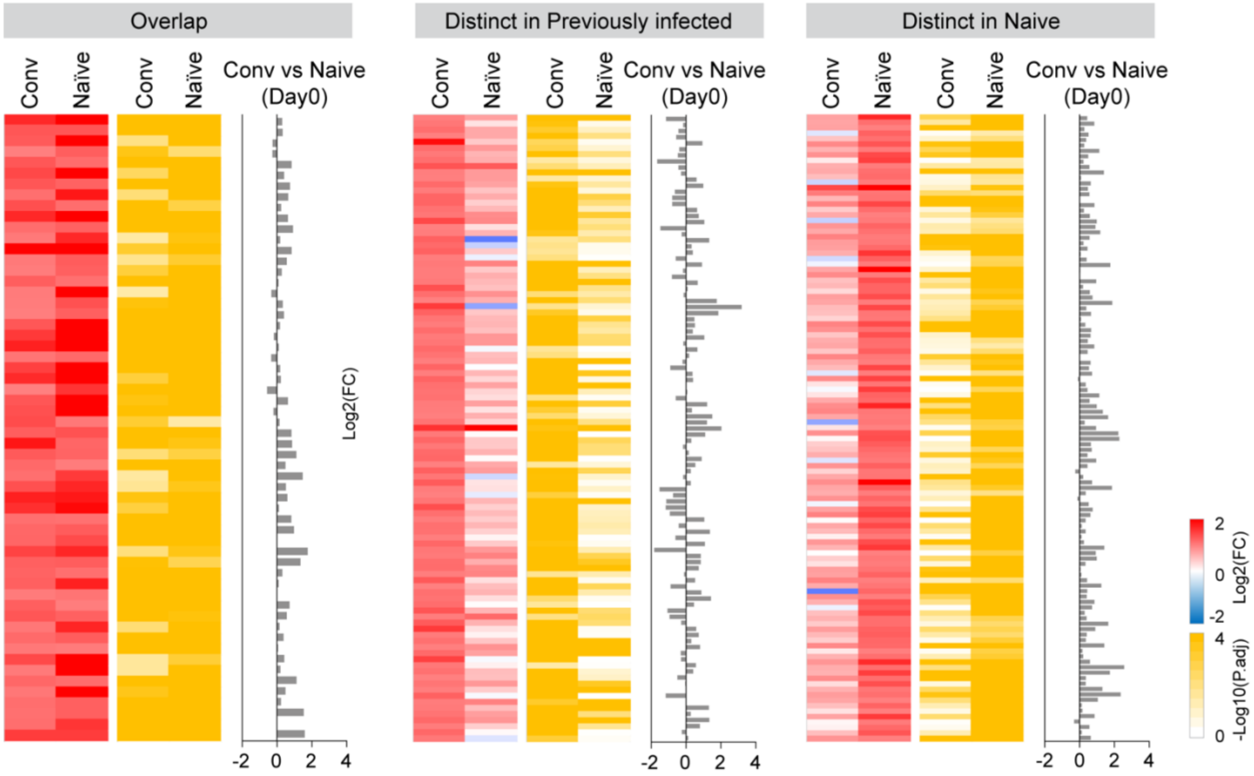

Figure S8. Transcriptional feature of SARS-CoV-2 naïve cohort to significantly regulated genes in previously infected cohort, related to Figure 5.

Heatmaps showing log2 FC (top) and corresponding P.adj (bottom) of genes from Figure 5C in both cohorts. Bar graphs show difference of basal gene expression level between both cohorts.

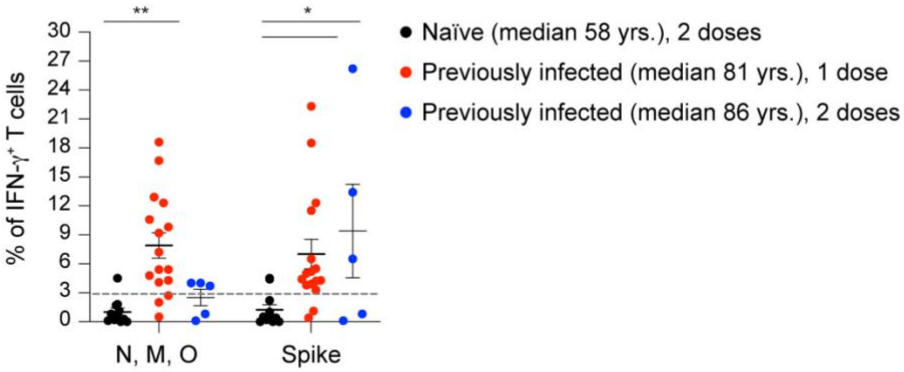

Figure S9. T-cell response, related to Figures 1-3.

Relative frequencies of active T cells after IFN-g stimulation. The data shown are active T-cell level of 4-month samples from previously infected nuns (19mo in Figure 1A) and naïve community (5.5mo in Figure 2A) with 1 dose or 2 dose vaccinations (Fisher's exact p<0.05). 2-way ANOVA with Tukey's multiple comparisons was used for the statistically significant. *$p < 0.05$, **$p < 0.01$. (Naïve, $n = 11$; Previously infected 1 dose, $n = 16$; Previously infected 2 doses, $n = 5$)

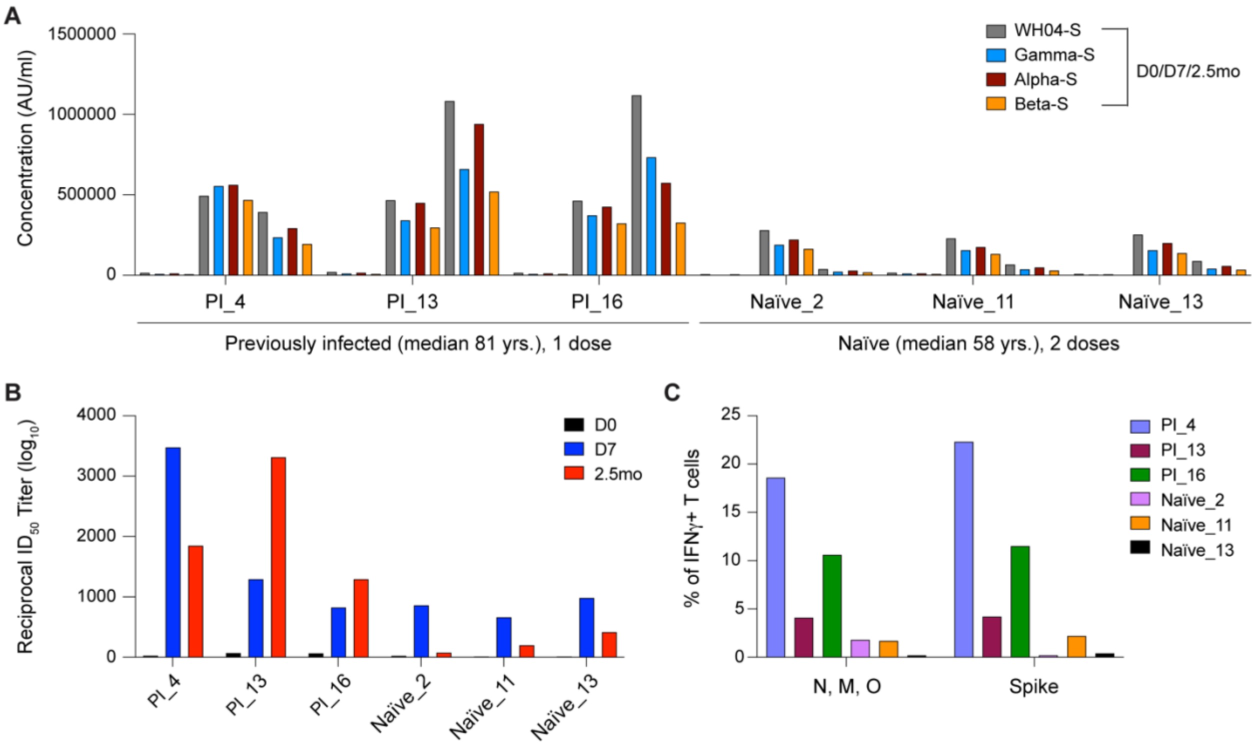

Figure S10. Bar graphs were shown the level of antibody to spike protein of four variants (A), neutralizing antibody (B) and T-cell activation (C) of three previously infected after the sole vaccination and in three naïve persons after the second vaccination, related to Figure 6.

Figure S11. Specific VH and VL genes give rise after vaccination were compared for VH and VL gene usage, related to Figure 6.

The V gene usage was assigned for all paired heavy (A), kappa (B), and lambda (C) chains in Previously infected nuns and Naïve community. The number in the inner circle indicates the number of sequences analyzed for the individual whose identifier is denoted above the circle. Pie slice size is proportional to the number of clonally related sequences. Colored slices indicate persisting clones to RBD, and white slices indicate singlets found at both time points. Full list is in Data S13.

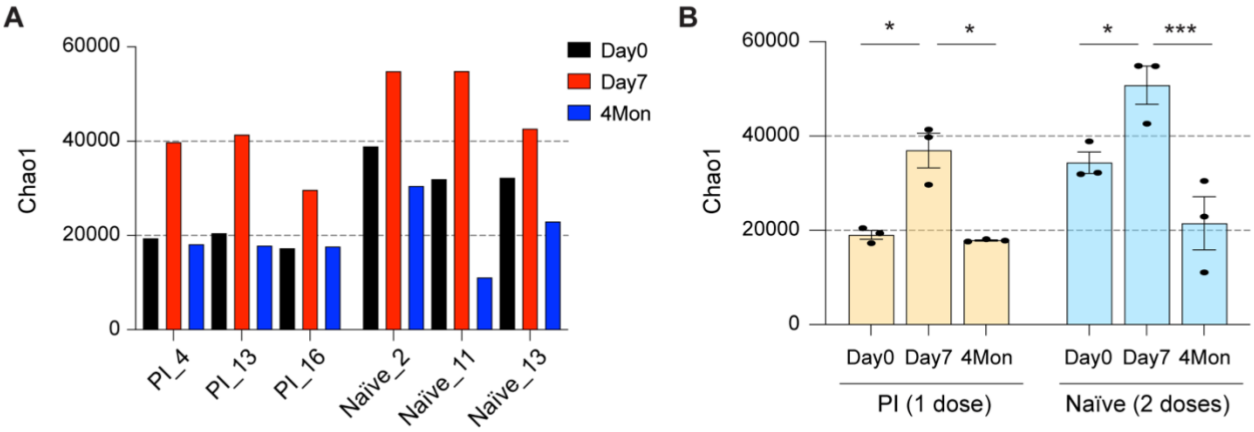

Figure S12. Chao1 diversity estimation of BCR/TCR genes by individual and timepoint (A) as well as group and timepoint (B), related to Figure 6.

p-value are from 2-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, ***$p < 0.001$.