SUPPLEMENTAL MATERIALS

Supplemental Methods

Control cohorts

Data for anti-S IgG in healthy controls were obtained from a previous study of 20 asymptomatic adult health care workers who received two doses of the Moderna mRNA-1273 or Pfizer/BioNTech BNT162b2 vaccine. The same Roche Elecsys anti-SARS-CoV-2 S assay was used.

Data for T-cell responses in healthy controls were obtained from 20 adults who had previous SARS-CoV-2 infection and subsequently received two doses of the Moderna mRNA-1273 or Pfizer/BioNTech BNT162b2 vaccine a median of 370 days (interquartile range, 125 to 405 days) later. The same interferon-gamma enzyme-linked immune absorbent spot (ELISPOT) assay was used.

Specimens

Serum was isolated from whole blood collected in clot activator red top vacutainers and stored at -80^{o} Celsius. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Histopaque centrifugation from whole blood collected in acid citrate dextrose vacutainers, washed twice with phosphate-buffered saline, resuspended in a mixture of 90% fetal bovine serum and 10% dimethyl sulfoxide (DMSO), cooled at a controlled rate and stored in liquid nitrogen for flow cytometry.

Laboratory Testing

Abbott Architect anti-SARS-CoV-2 anti-Nucleocapsid (N) protein assay

The anti-N Architect SARS-CoV-2 IgG assays (Abbott, Chicago, IL) is a chemiluminescent microparticle immunoassay (CIMA) designed to measure IgG antibodies binding the N protein and was performed on an Architect i2000SR. Results from the anti-N SARS-CoV-2 IgG assay are reported as index values. The index value of 1.40 or greater was classified as positive per manufacturer's recommendation for the anti-N Abbott Abbott Architect SARS-CoV-2 IgG assay.

Roche Elecsys anti-SARS-CoV-2 anti-Spike (S) protein assay

The Elecsys Anti-SARS-CoV-2 S assay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is an electrochemiluminescence immunoassay which uses a double-antigen sandwich design for quantitative determination of immunoglobulins to the RBD domain of the S protein. Samples were prepared according to the manufacturer's instructions and analyzed on the Roche cobas e 411 platform. Using the manufacturer's guidelines, sample values ≥0.8 AU/mL were classified as positive for anti-SARS-CoV-2 antibodies. Samples above 250 AU/mL were automatically diluted (1:10 or 1:100) into the linear range of the assay, which allowed an upper limit of quantification of 25000 AU/mL for these analyses [1,2].

SARS-CoV-2 spike pseudotyped lentivirus neutralization assay

1.25E4 293T-ACE2 cells were seeded in 96-well plates and incubated in DMEM + 10% FBS for 16-18 hrs. The following day, 0.2E6-1.0E6 RLUs/well SARS-CoV-2 D614G Spike pseudotyped lentivirus was diluted 1:10 in DMEM with 10% FBS complete media. Sera was diluted 1:20 in DMEM with 10% FBS and seven three-fold serial dilutions were prepared. Equal parts diluted sera and pseudovirus were combined and incubated for one hour at 37°C. The mixture was added to the cells and incubated for fifty-two hours at 37°C. Following incubation, the media was removed and 30µl of luciferase substrate (Promega, Madison, WI, USA) was added. After two minutes of incubation, luminescence was measured on the VICTOR Nivo and IC50 was calculated from a standard curve using the CV30 monoclonal antibody (Absolute Antibody, Oxford, UK). A 50% neutralization dilution (ND50) ≥.004 IU/mL was considered positive and converted to IU/mL.

Total Immunoglobulin G

Total serum IgG was measured using turbidometry (University of Washington Immunology Laboratory, Seattle, WA).

T-cell analysis

T-cell responses were evaluated with the T-SPOT®*Discovery* SARS-CoV-2 (Oxford Immunotec, Abingdon, UK) IFN-gamma ELISPOT kit. PBMCs were rapidly thawed, counted, and plated at 3 X 10^5 cells/well, slightly above from package insert recommending 2.5 x 10^5 cells/well. Stimuli including negative control, proprietary Spike peptide pool, and positive control phytohemagglutinin provided by the manufacturer were added. After 18 hours of

incubation at 37°C, 5% CO_2, plates were developed using kit reagents and read on a S6 ELISPOT reader (CTL, Shaker Heights, OH) using a program optimized for the ELISPOT plate geometry and composition. An ELISPOT assay result of >20 sfu/mil PBMC was considered a positive result. Frozen PBMCs were used for testing rather than fresh samples, as outlined in the package insert.

B-Cell analysis

We immunophenotyped B-cells from PBMCs using flow cytometry as detailed below. Additionally, we identified SARS-CoV-2-specific B-cells in study participants by using fluorochrome conjugated tetramers of SARS-CoV-2 receptor binding domain (RBD) from the WA1 strain. To generate tetramers, avi-tagged RBD was biotinylated with BirA and mixed with fluorochrome conjugated streptavidin.

We thawed PBMCs quickly at 37°C and incubated them immediately for 30 minutes on ice in 100 µL of FACS buffer containing a cocktail of antibodies prior to washing and analysis on a FACSymphony (BD). FACS buffer consisted of 1× DPBS containing 1% newborn calf serum (Life Technologies, Thermo Fisher Scientific). Cells were labeled with antibodies including combinations of anti-CD4 BUV395 (clone SK3, BD), anti-IgM FITC (clone G20-127, BD), anti-CD8 PE (clone RPA-T8, BD), anti-CD19 BV421 (clone HIB19, BD), anti-CD45 BV510 (clone HI30, BD), anti-CD3 BV605 (clone UCHT1, BioLegend), anti-CD14 BV711 (clone M0P-9, BD), anti-CD16 BV711 (clone 3G8, BioLegend), anti-IgD BUV737 (clone IA6-2, BD), anti-CD27 PE-Cy7 (clone LG.7F9, Thermo Fisher Scientific), a fixable viability dye (FVD), a tetramer of SARS-CoV-2 RBD conjugated

to APC, and a tetramer of RSV PreF conjugated to APC-Dylight 755. The tetramer of RSV PreF allowed the exclusion of B-cells binding to either the RSV PreF or APC. B-cells were defined as CD19-expressing cells within the live, single $CD45^+$ $CD14^-$ $CD16^-$ lymphocyte population. The following B-cell populations were delineated: $CD27^-$ IgD^+ (naive B-cells), $CD27^+$ (memory B-cells), and $CD27^+$ IgM^- IgD^- (class-switched memory B-cells). SARS-CoV-2 S-specific B-cells were further delineated into the above-described B-cell populations. Absolute B- and T-cell counts were calculated by multiplying proportions from flow cytometry by absolute lymphocyte counts from complete blood cell count results. Analyses were performed using FlowJo Software version 10.7.1.

Supplemental Results

Three individuals received intravenous immunoglobulin G (IVIG) within 2 months of a sample collection date. One of these individuals did not demonstrate a humoral or cellular response, one developed intercurrent SARS-CoV-2 infection and was excluded from further data analyses, and one demonstrated an immune response in the absence of intercurrent SARS-CoV-2 infection. Of note, this last individual also demonstrated a cellular response in addition to the detection of circulating SARS-CoV-2 S-specific memory B-cells. Thus, the receipt of IVIG was unlikely to confound any of the reported data.

Supplemental Table

Table S1. Number of patients with a sample tested and evaluable with each assay at each time point.

Test and time point	Cohort	
	Pre-CAR-T N=21	Post-CAR-T N=24
Anti-spike IgG		
Post-second vaccine	20[1]	23[2]
Post-third vaccine	-	13[3]
Day 30 post-CARTx	5[4]	-
Day 90 post-CARTx	2[5]	-
Neutralization assay		
Post-second vaccine	20[1]	23[2]
Post-third vaccine	-	13[3]
Day 30 post-CARTx	5[4]	-
Day 90 post-CARTx	2[5]	-
ELISPOT		
Post-second vaccine	19[6]	21[7]
Post-third vaccine	-	12[8]
Day 30 post-CARTx	3[9]	-
Day 90 post-CARTx	2[10]	-
B Cell Analysis		
Post-second vaccine	20[1]	23[2]
Post-third vaccine	-	13[3]
Day 30 post-CARTx	2[11]	-
Day 90 post-CARTx	1[12]	-

1. 1/21 had positive anti-N result post 2nd dose and was excluded from response summary
2. 1/24 not tested post 2nd dose, only tested post 3rd dose.
3. Of 15 individuals tested post 3rd dose, 2 had positive anti-N result and were excluded from response summary
4. Of 6 individuals tested at 30 days post-CAR-Tx, 1 had positive anti-N result and was excluded from response summary
5. Of 4 individuals tested at 90 days post-CAR-Tx, 2 had positive anti-N result and were excluded from response summary
6. 1/21 had positive anti-N result post 2nd dose and another individual had test results that were not evaluable. These two were excluded from response summaries.
7. 1/24 not tested post 2nd dose and another two individuals had test results that were not evaluable. These three were excluded from response summaries.
8. Of 15 individuals tested post 3rd dose, 2 had positive anti-N result and another patient had test results that were not evaluable. These three were excluded from response summary.
9. Of 6 individuals tested at 30 days post-CAR-Tx, 1 had positive anti-N result and two had test results that were not evaluable. These three were excluded from response summaries.
10. Of 4 individuals tested at 90 days post-CAR-Tx, 1 had positive anti-N result and another had test results that were not evaluable. These two were excluded from response summaries.
11. Of 3 individuals tested at 30 days post-CAR-Tx, 1 had positive anti-N result and was excluded from response summaries.
12. Of 3 individuals tested at 90 days post-CAR-Tx, 2 had positive anti-N result and were excluded from response summaries.

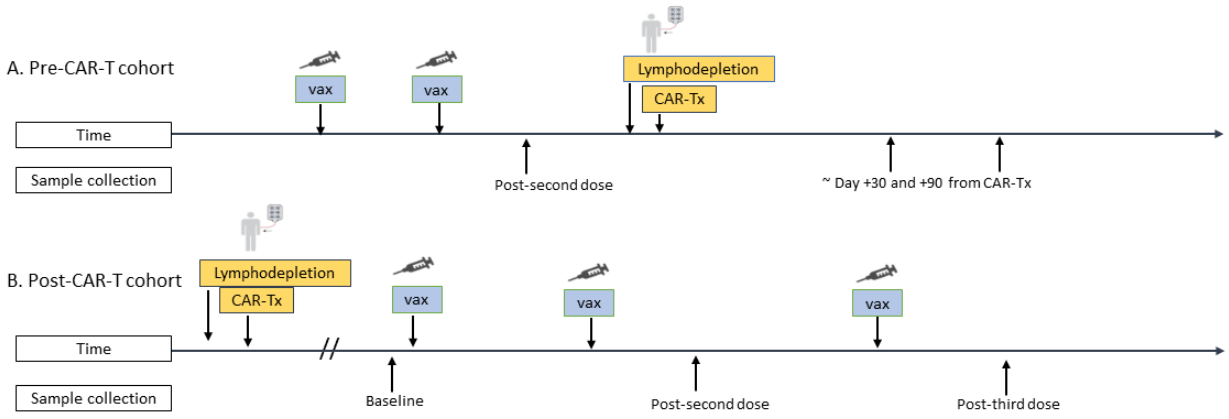

Figure S1. Vaccine administration and sample collection timelines.

Timelines demonstrating blood sample collection, SARS-CoV-2 mRNA vaccination, and CAR-Tx for the (A) pre-CAR-Tx cohort and (B) post-CAR-Tx cohort.

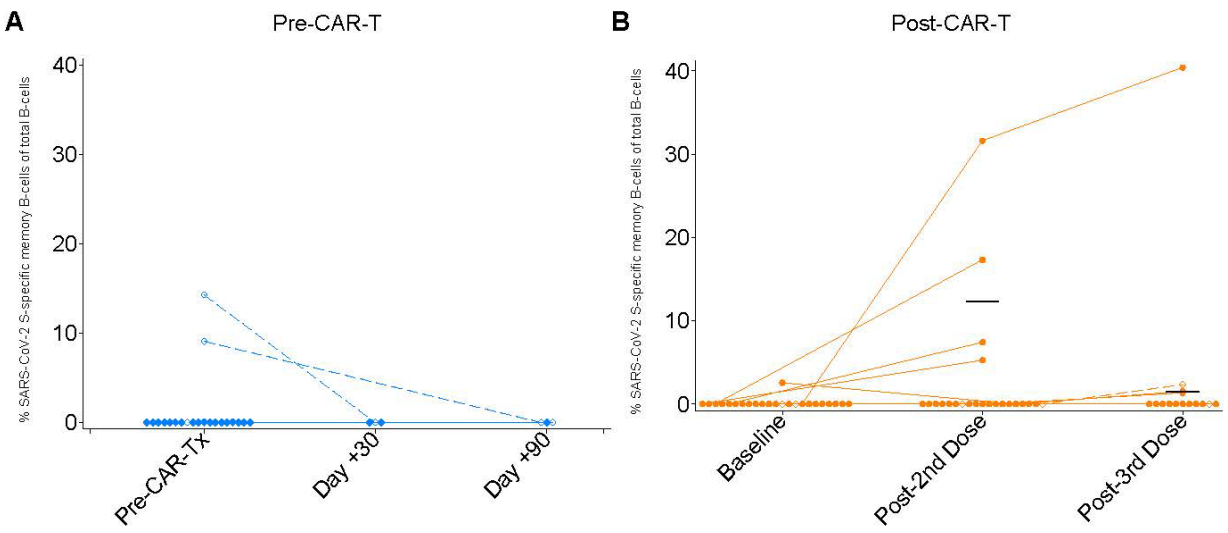

Figure S2. SARS-CoV-2-S-specific B-cell response by cohort.

Panel A depicts the percentage of peripheral blood B-cell lymphocytes that were SARS-CoV-2-S-specific memory B-cells after two mRNA vaccine doses pre-CAR-Tx, 30 days post CAR-Tx, and 90 days post CAR-Tx. **Panel B** depicts the percentage of peripheral blood B-cell lymphocytes that were SARS-CoV-2-S-specific memory B-cells in the post-CAR-Tx cohort prior to any vaccine administration (baseline), after two mRNA vaccine doses, and after three mRNA vaccine doses.

Figure Legend: The blue diamonds represent the pre-CAR-Tx cohort. The orange circles represent the post-CAR-Tx cohort. Black horizontal bars represent the median percentage among those with a positive result. Open symbols and dashed lines represent individuals who developed anti-nucleocapsid (N) antibodies and were excluded from median percentage analysis upon detection of anti-N antibodies. In the pre-CAR-Tx cohort, one individual was anti-N positive pre-CAR-Tx, one at 30 days post-CAR-Tx, and one at 90 days post-CAR-Tx. In the post-CAR-Tx cohort, two individuals became anti-N positive after the third vaccine dose. Pre-CAR-Tx N=21, Day +30 N=3, Day +90 N=3; Post-CAR-Tx baseline N=24, post-2nd dose N=23, Post-3rd dose N=15.

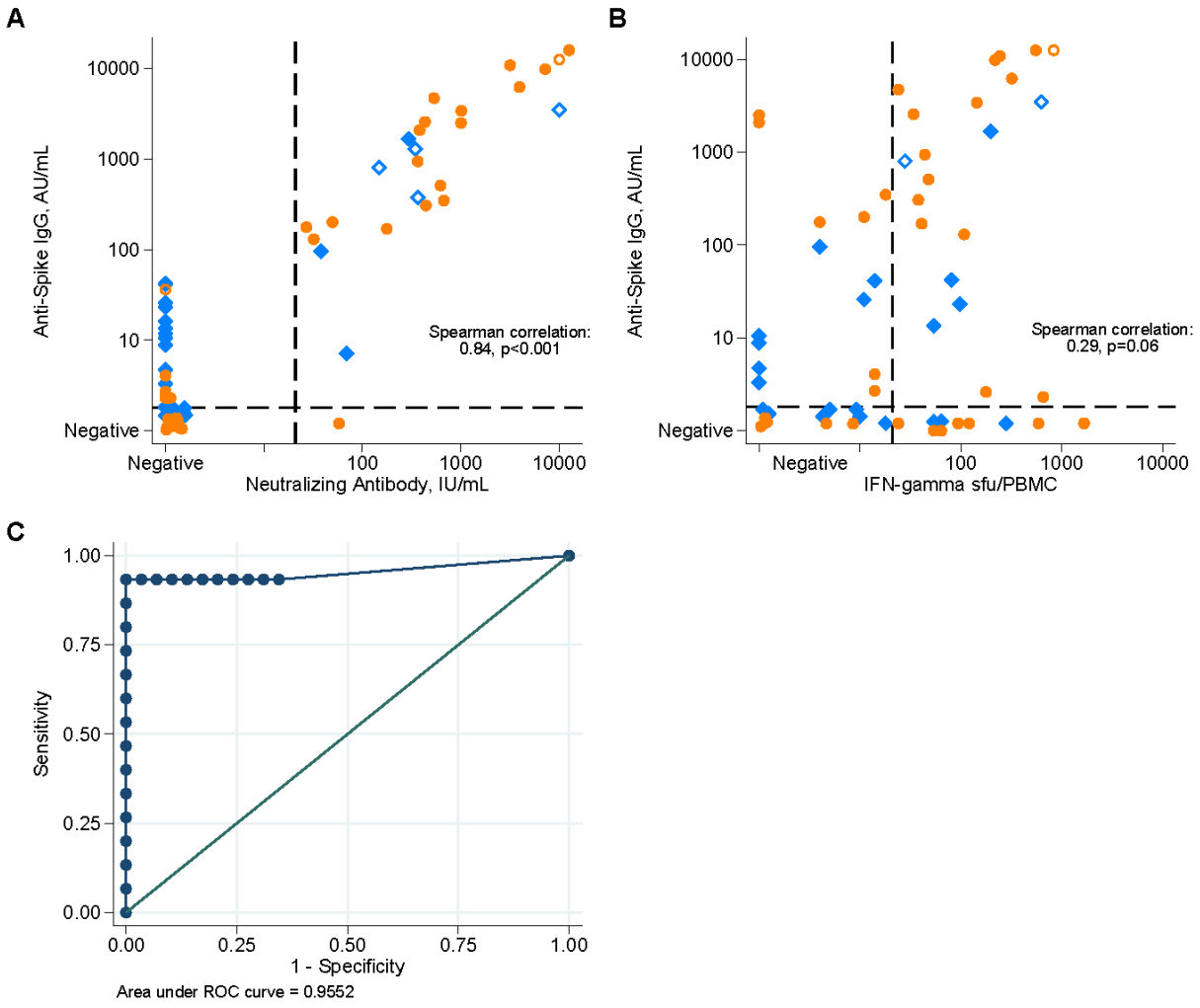

Figure S3. Humoral and cellular correlations among responses and ROC curve analysis of anti-S IgG for classifying neutralizing antibody production.

Panel A shows the correlation between SARS-CoV-2 anti-S IgG results with neutralizing antibody results. **Panel B** shows the correlation of SARS-CoV-2 anti-S IgG results with ELISPOT assay results. In **Panels A-B**, all time points with results from both tests were included; individuals may appear more than once. Spearman correlations were computed using per-patient mean $\log_{10}$ values. **Panel C** shows a receiver operating characteristic (ROC) curve for anti-S IgG levels

as a predictor of having neutralizing antibodies using results after second vaccination from both cohorts (AUC = 0.96; 95% confidence interval, 0.85- 0.99).

Figure Legend: The blue diamonds represent the pre-CAR-Tx cohort. The orange circles represent the post-CAR-Tx cohort. Open symbols represent individuals who developed anti-nucleocapsid (N) antibodies and were excluded from calculations. Dashed lines in black represent the positive cutoff values for each test. Results were $\log_{10}(x + 1)$ transformed for figures and prior to analyses.

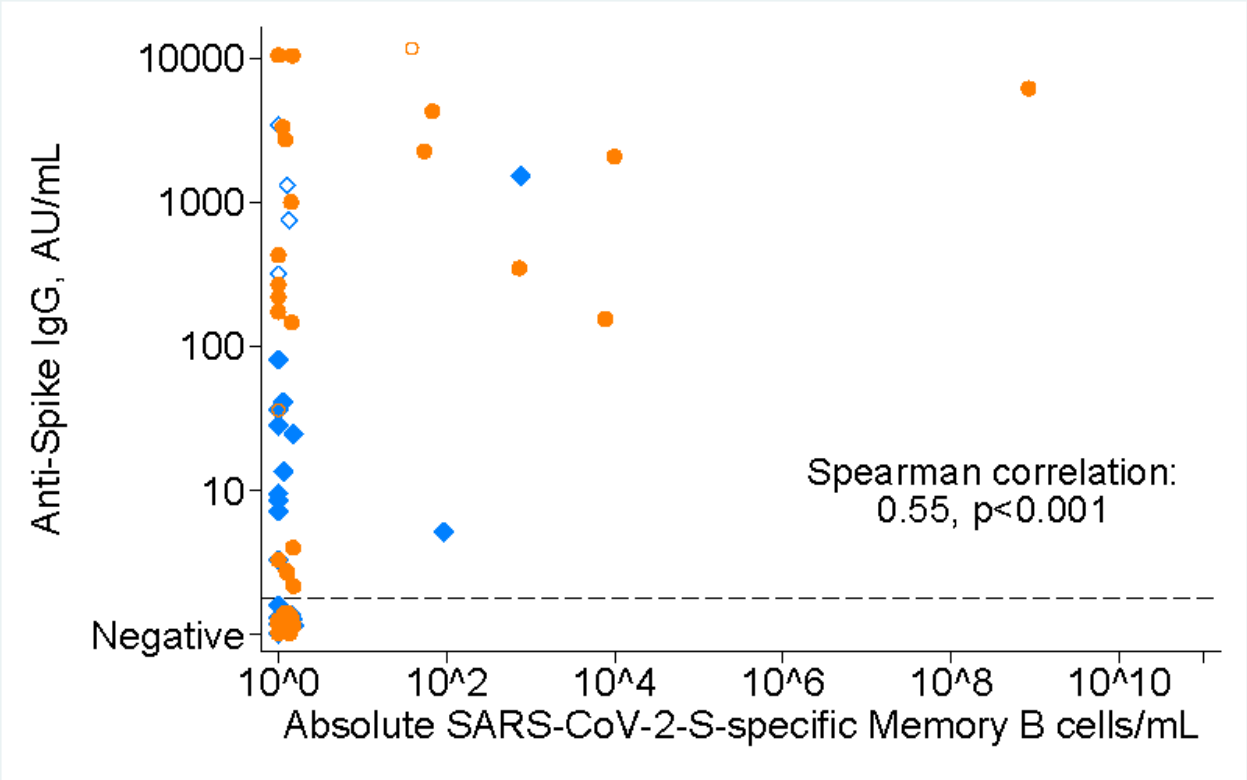

Figure S4. Correlation between anti-S IgG and and SARS-CoV-2-S-specific memory B-cell detection.

The blue diamonds represent the pre-CAR-Tx cohort. The orange circles represent the post-CAR-Tx cohort. Dotted lines represent the positive cutoff values. Each sample time point tested from an individual was included in this graph. Open symbols represent individuals who developed anti-nucleocapsid antibodies. Anti-Spike IgG results were $\log_{10}(x+1)$-transformed, and SARS-CoV-2-S-specific memory B-cells were $\log_{10}$ transformed for analysis.

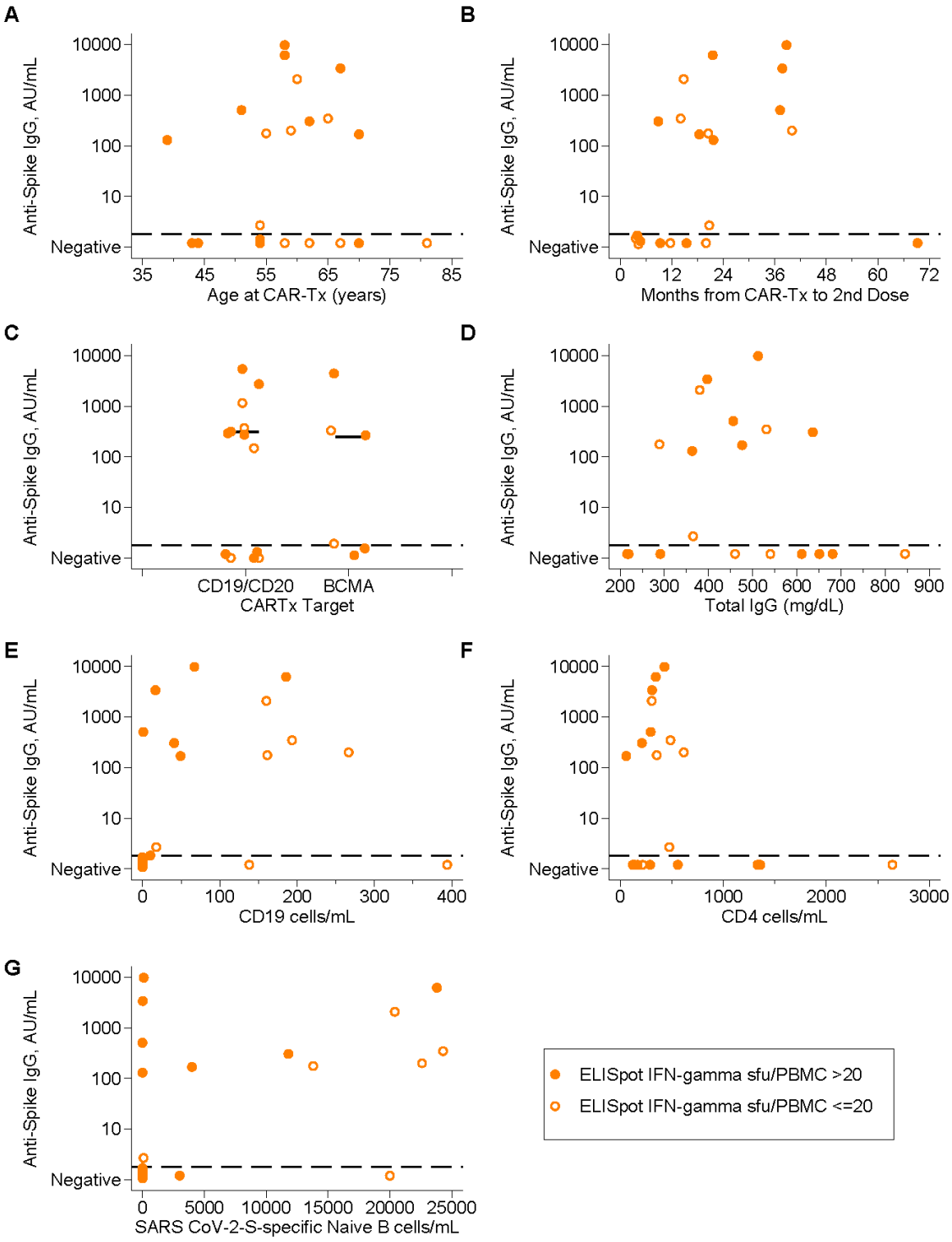

Figure S5. Anti-S IgG and ELISPOT T-cell responses in the post-CAR-Tx cohort stratified by baseline variables.

The panels show the distribution of anti-S IgG and ELISPOT T-cell responses (positive or negative) by pre-vaccine age (**Panel A**), months from CAR-Tx (**Panel B)**, CAR-Tx target (**Panel C**), total IgG (**Panel D**), absolute CD19+ B-cells/mL (**Panel E**), absolute CD4+ T-cells cells/mL (**Panel F**), and absolute SARS-CoV-2-S-specific naïve B-cells/mL (**Panel G**).

Filled versus open circles represent samples with positive versus negative ELISPOT T-cell results, respectively. Dashed lines represent the positive cutoff values. Only post-second mRNA vaccination sample results were included in this graph. Anti-Spike IgG results were $\log_{10}(x+1)$-transformed prior to analysis.

References

1. Bradley BT, Bryan A, Fink SL, Goecker EA, Roychoudhury P, Huang ML, Zhu H, Chaudhary A, Madarampalli B, Lu JYC, Strand K, Whimbey E, Bryson-Cahn C, Schippers A, Mani NS, Pepper G, Jerome KR, Morishima C, Coombs RW, Wener M, Cohen S, Greninger AL. Anti-SARS-CoV-2 Antibody Levels Measured by the AdviseDx SARS-CoV-2 Assay Are Concordant with Previously Available Serologic Assays but Are Not Fully Predictive of Sterilizing Immunity. J Clin Microbiol. 2021 Aug 18;59(9):e0098921. doi: 10.1128/JCM.00989-21. Epub 2021 Aug 18. PMID: 34165323; PMCID: PMC8373027.
2. Jochum, Simon et al. “Clinical utility of Elecsys Anti-SARS-CoV-2 S assay in COVID-19 vaccination: An exploratory analysis of the mRNA-1273 phase 1 trial.” *medRxiv : the preprint server for health sciences* 2021.10.04.21264521. 19 Oct. 2021, doi:10.1101/2021.10.04.21264521.