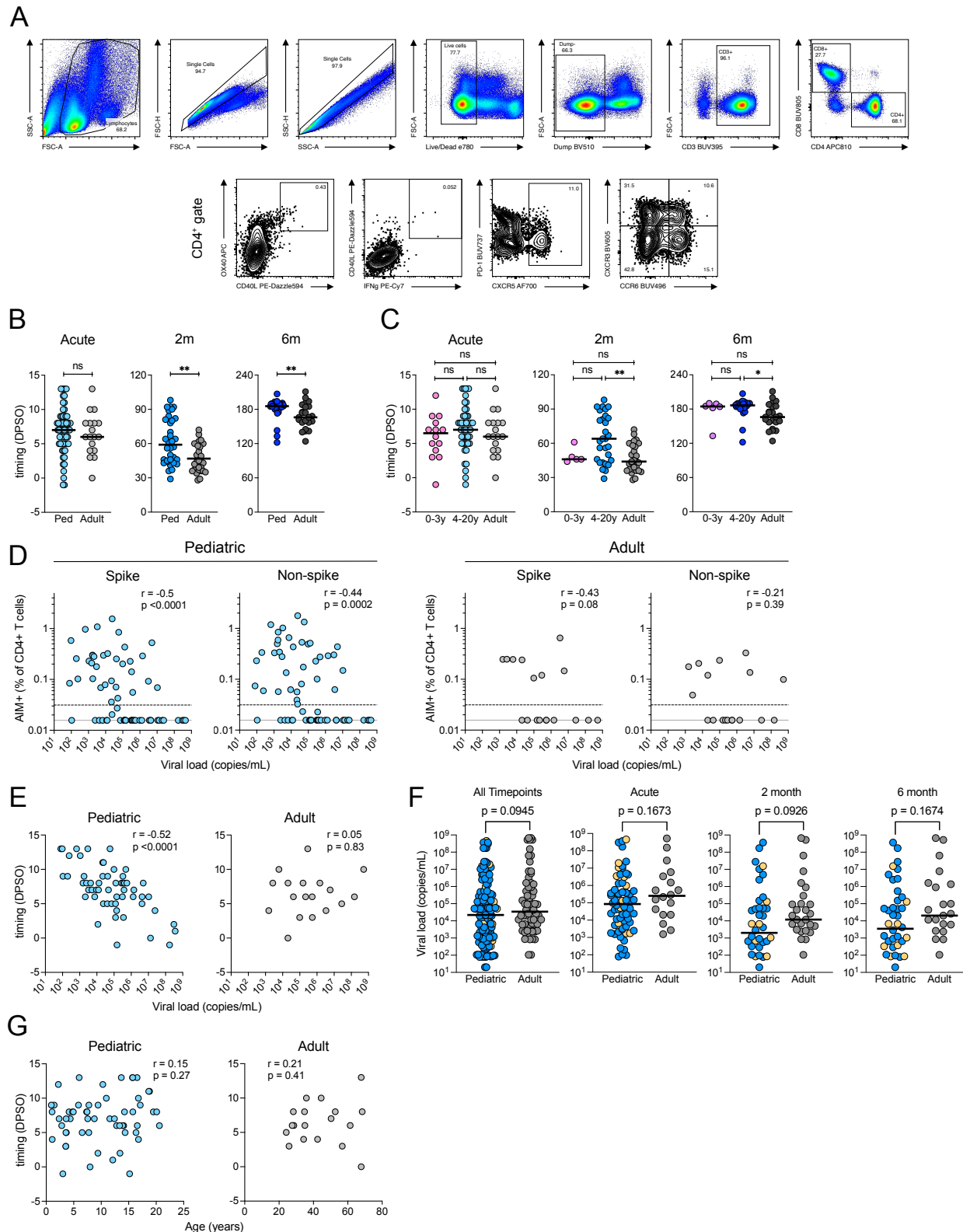
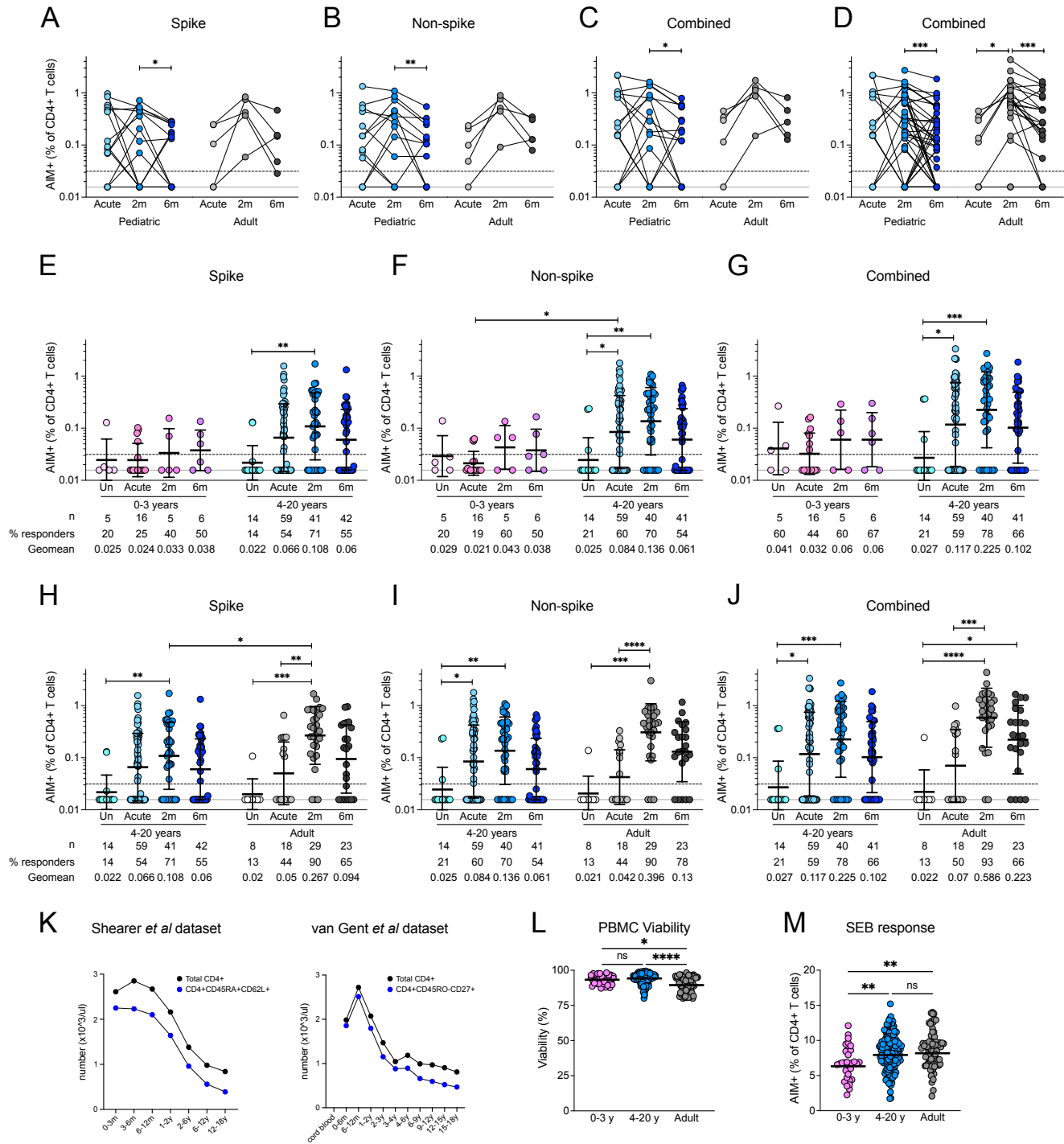




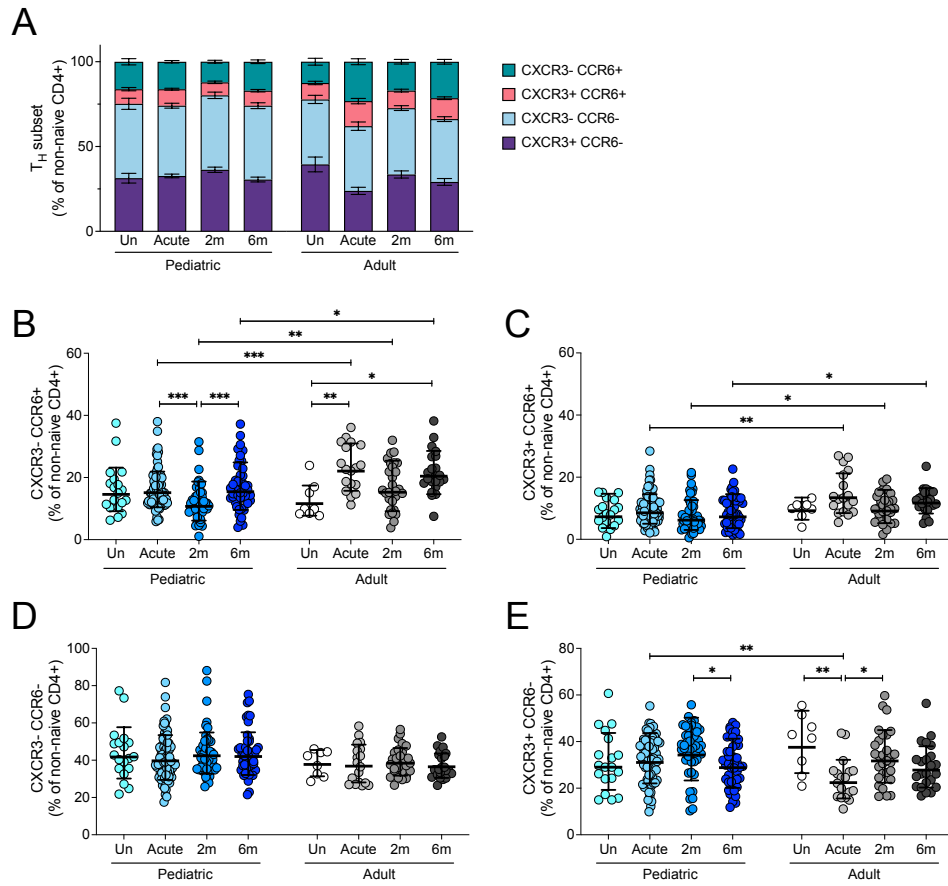
Supplemental Figure 1. Gating strategy for flow cytometry analysis, viral load and sample collection times

(A) Gating strategy for hybrid AIM and ICS analysis of SARS-CoV-2-specific CD4⁺ T cells. (B and C) Blood sample collection times indicated as days post symptom onset (DPSO) at the acute, 2-month ("2m"), and 6-month ("6m") timepoints. (D) Correlation between spike- and non-spike-AIM⁺ response in children and adults (as indicated) with viral load at the acute timepoint. (E) Correlation between blood sample collection times indicated as days post symptom onset (DPSO) with viral load in children and adults (as indicated). (F) Comparison of viral loads between children and adults; orange circles represent asymptomatic children. (G) Correlation between blood sample collection times indicated as days post symptom onset (DPSO) with donor age in children and adults (as indicated). Centerlines in B and C represent the median. Dotted line in D indicates the limit of quantification of the assay determined by calculating the geomean of the background (DMSO treated); solid grey line indicates the lower limit of detection. "AIM⁺" denotes the frequencies of OX40⁺CD40L⁺ cells as percentage of CD4⁺ T cells. "Ped" in B represents the pediatric group. 'P' values for B and F were calculated by Mann-Whitney test and for C by Kruskal-Wallis test with Dunn's correction, and are indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=non-significant. 'r' in D, E, and G indicates Spearman correlation coefficient.



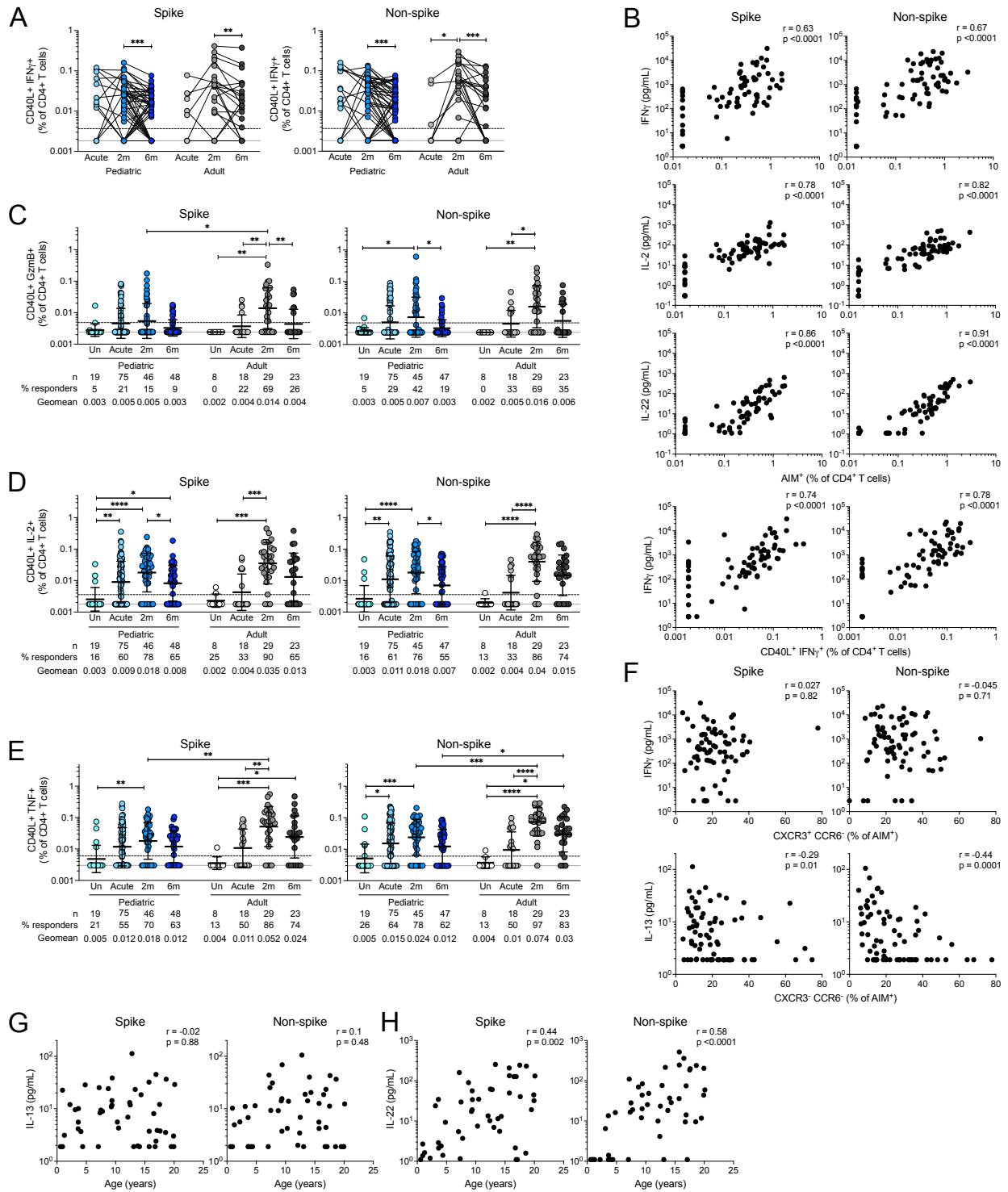
Supplemental Figure 2. SARS-CoV-2-specific CD4+ T cell response to SARS-CoV-2 increases with age

(A-C) Paired longitudinal (A) spike-specific, (B) non-spike specific, and (C) combined spike and non-spike-specific CD4+ T cell responses at indicated timepoints post-infection from donors that provided samples for all three timepoints. (D) Spike and non-spike combined CD4+ T cell responses at indicated timepoints post-infection. (E-G) Frequencies of SARS-CoV-2 (E) spike-specific, (F) non-spike-specific, and (G) combined spike- and non-spike-specific CD4+ T cells across indicated timepoints of infection in younger (0-3 years) and older (4-20 years) children. (H-J) Frequencies of SARS-CoV-2 (H) spike-specific, (I) non-spike-specific, and (J) combined spike- and non-spike- CD4+ T cells across indicated timepoints of infection in children and adults after removing the 0-3 age group from the pediatric cohort. (K) Data extracted from Shearer *et al* (DOI: 10.1016/j.jaci.2003.07.003) and van Gent *et al* (DOI: 10.1016/j.clim.2009.05.020) showing changes with age (months = "m", years = "y") in CD4+ T cell numbers per unit volume of blood. (L) Percent of viable PBMCs upon thawing, measured by a viability dye. (M) Frequencies of AIM+ T cells from the indicated sample groups upon stimulation with SEB. Centerlines and error bars in E-J represent the geometric mean and geometric standard deviation, respectively. Centerlines in L and M represent the geometric mean. Dotted line in A-J indicates the limit of quantification of the assay determined by calculating the geomean of the background (DMSO treated); solid grey line indicates the lower limit of detection. Uninfected participants are indicated as "Un"; 2-month and 6-month timepoints as "2m" and "6m", respectively. "AIM+" denotes the frequencies of OX40⁺CD40L⁺ cells as percentage of CD4+ T cells. 'P' values for A-D were calculated by Wilcoxon test, for E-J by Kruskal-Wallis test with Dunn's correction for comparisons within the pediatric and adult age groups and by Mann-Whitney test with Holm-Sidak correction for comparisons across the pediatric and adult age groups, and for L and M by Kruskal-Wallis test with Dunn's correction, and are indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; non-significant 'p' values are not shown or are indicated as 'ns' L and M.



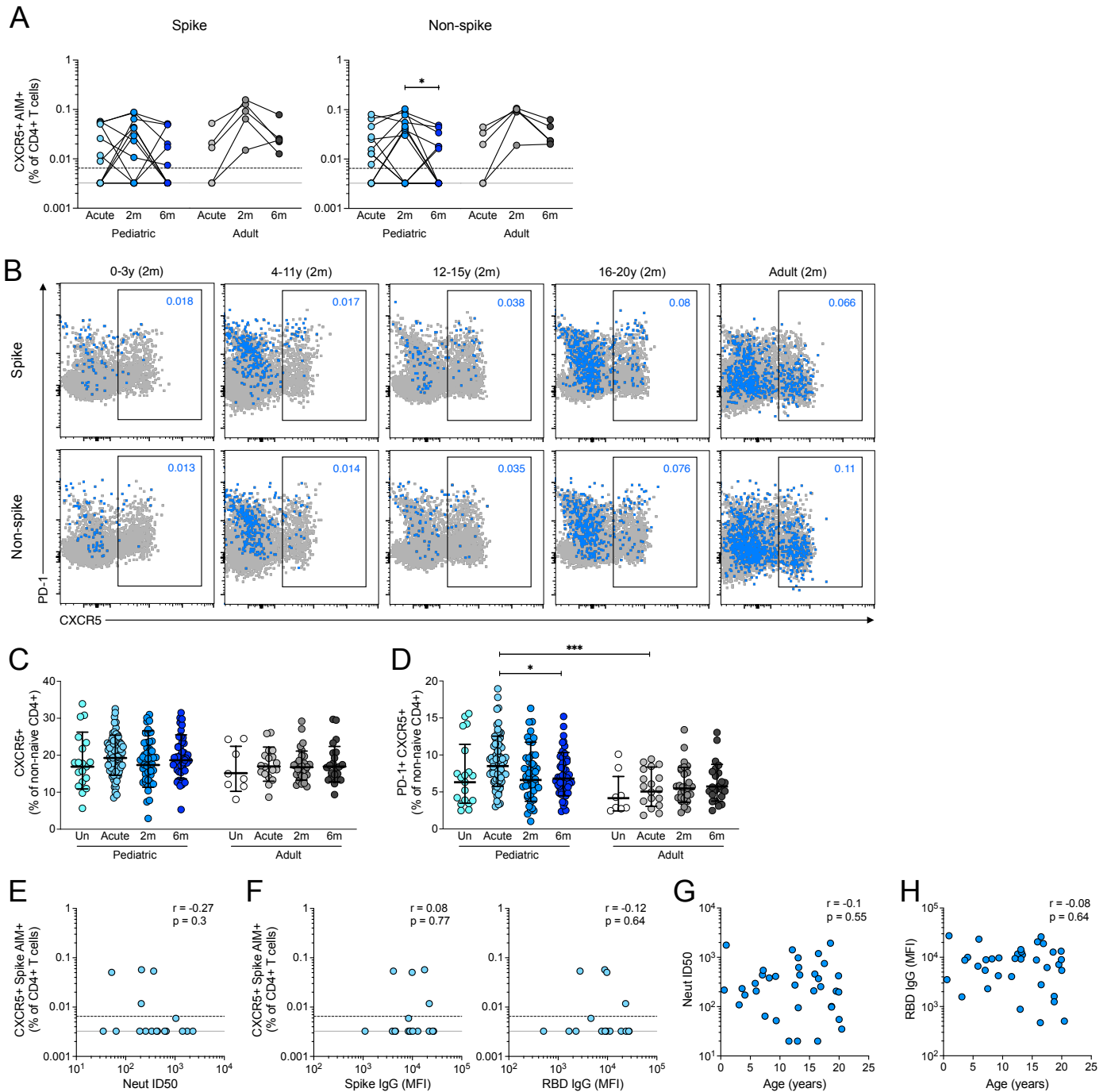
Supplemental Figure 3. T helper subset distribution in SARS-CoV-2 infected children and adults

(A) Proportion plot showing distribution of T helper subsets among total non-naïve CD4⁺ T cells in children and adults at indicated stages of SARS-CoV-2 infection. (B-E) Percentage of CXCR3⁻CCR6⁺ (B), CXCR3⁺CCR6⁺ (C), CXCR3⁻CCR6⁻ (D), and CXCR3⁺CCR6⁻ (E) cells among total non-naïve CD4⁺ T cells across indicated time points post-infection in children and adults. Centerlines and error bars in B-E represent the geometric mean and geometric standard deviation, respectively. Uninfected participants in A-E are indicated as "Un"; 2-month and 6-month timepoints as "2m" and "6m", respectively. 'P' values were calculated for B-E by Kruskal-Wallis test with Dunn's correction for comparisons within the pediatric and adult age groups and by Mann-Whitney test with Holm-Sidak correction for comparisons across the pediatric and adult age groups and are indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; non-significant 'p' values are not shown.



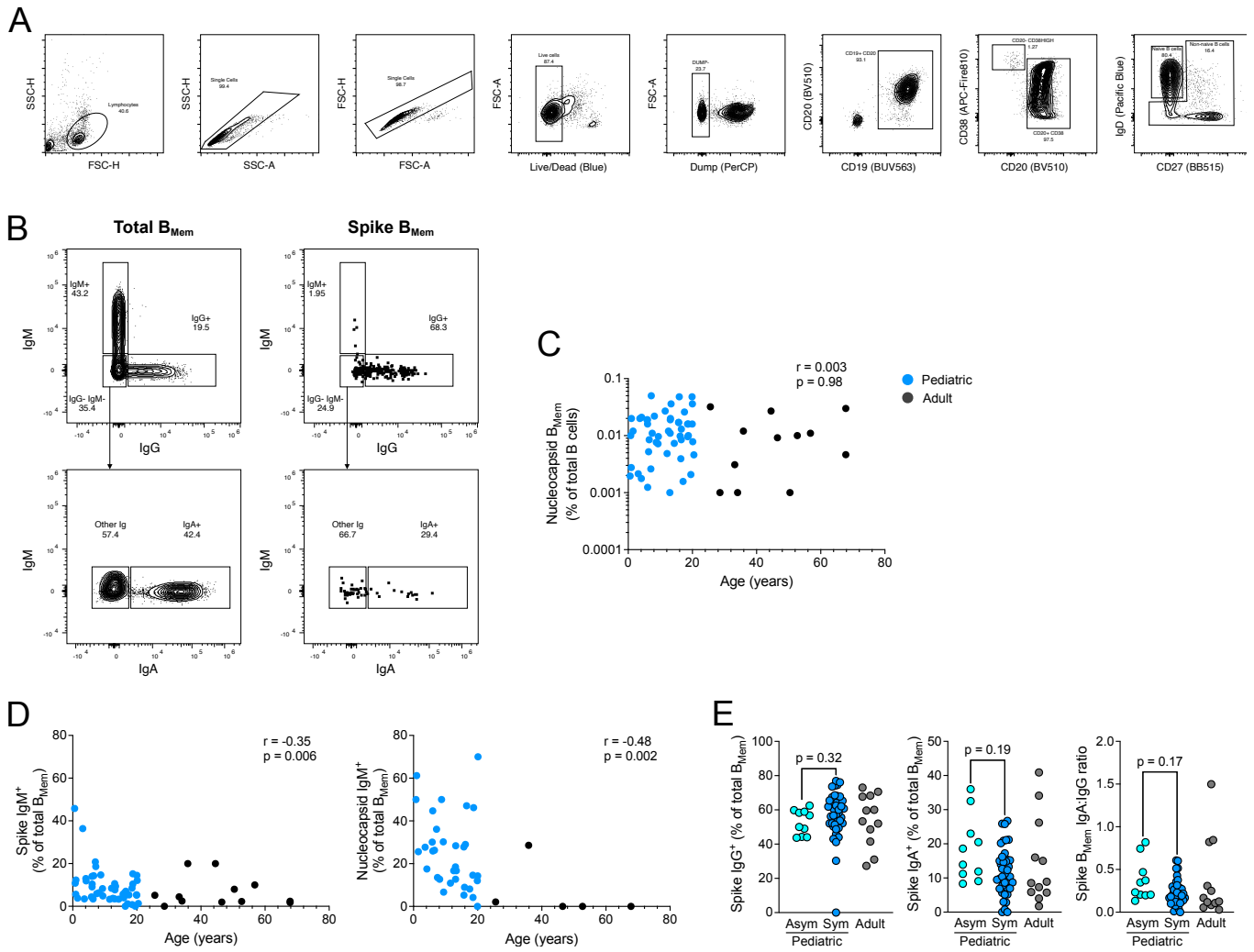
Supplemental Figure 4. SARS-CoV-2-specific CD4 $^{+}$ T cell cytokine response to SARS-CoV-2 in children

(A) Paired longitudinal spike- (left) and non-spike- (right) specific IFN γ producing CD4 $^{+}$ T cell frequencies at indicated time points post-infection from donors that provided samples for two or three time points. (B) Correlation between the indicated secreted cytokines measured by CBA and the frequency of OX40 $^{+}$ CD40L $^{+}$ CD4 $^{+}$ T cells (top six panels) or CD40L $^{+}$ IFN γ^{+} CD4 $^{+}$ T cells (bottom two panels) measured by AIM ICS assay. (C-E) Frequencies of SARS-CoV-2 spike- (left) and non-spike- (right) specific, GzmB-producing (C), IL-2-producing (D), and TNF-producing (E) CD4 $^{+}$ T cells across indicated time points of infection in children and adults. (F) Correlation between secreted cytokine measured by CBA and the frequency of T helper subset determined by hybrid AIM assay. Indicated populations shown as percent of OX40 $^{+}$ CD40L $^{+}$ CD4 $^{+}$. (G) Correlations of secreted IL-13 by spike- (left) and non-spike- (right) specific T cells at the acute phase of infection with age. (H) Correlations of secreted IL-22 by spike- (left) and non-spike- (right) specific T cells at the acute phase of infection with age. Centerlines and error bars in C-E represent the geometric mean and geometric standard deviation, respectively. Dotted line in A and C-E indicates the limit of quantification of the assay determined by calculating the geomean of the background (DMSO treated); solid grey line indicates the lower limit of detection. Uninfected participants are indicated as "Un"; 2-month and 6-month timepoints as "2m" and "6m", respectively. "AIM $^{+}$ " in B and F denotes OX40 $^{+}$ CD40L $^{+}$ CD4 $^{+}$ T cells. 'P' values for A were calculated by Wilcoxon test, for C-E by Kruskal-Wallis test with Dunn's correction for comparisons within the pediatric and adult age groups and by Mann-Whitney test with Holm-Sidak correction for comparisons across the pediatric and adult age groups; and are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; non-significant 'p' values are not shown. 'r' in B and F-H indicates Spearman correlation coefficient.



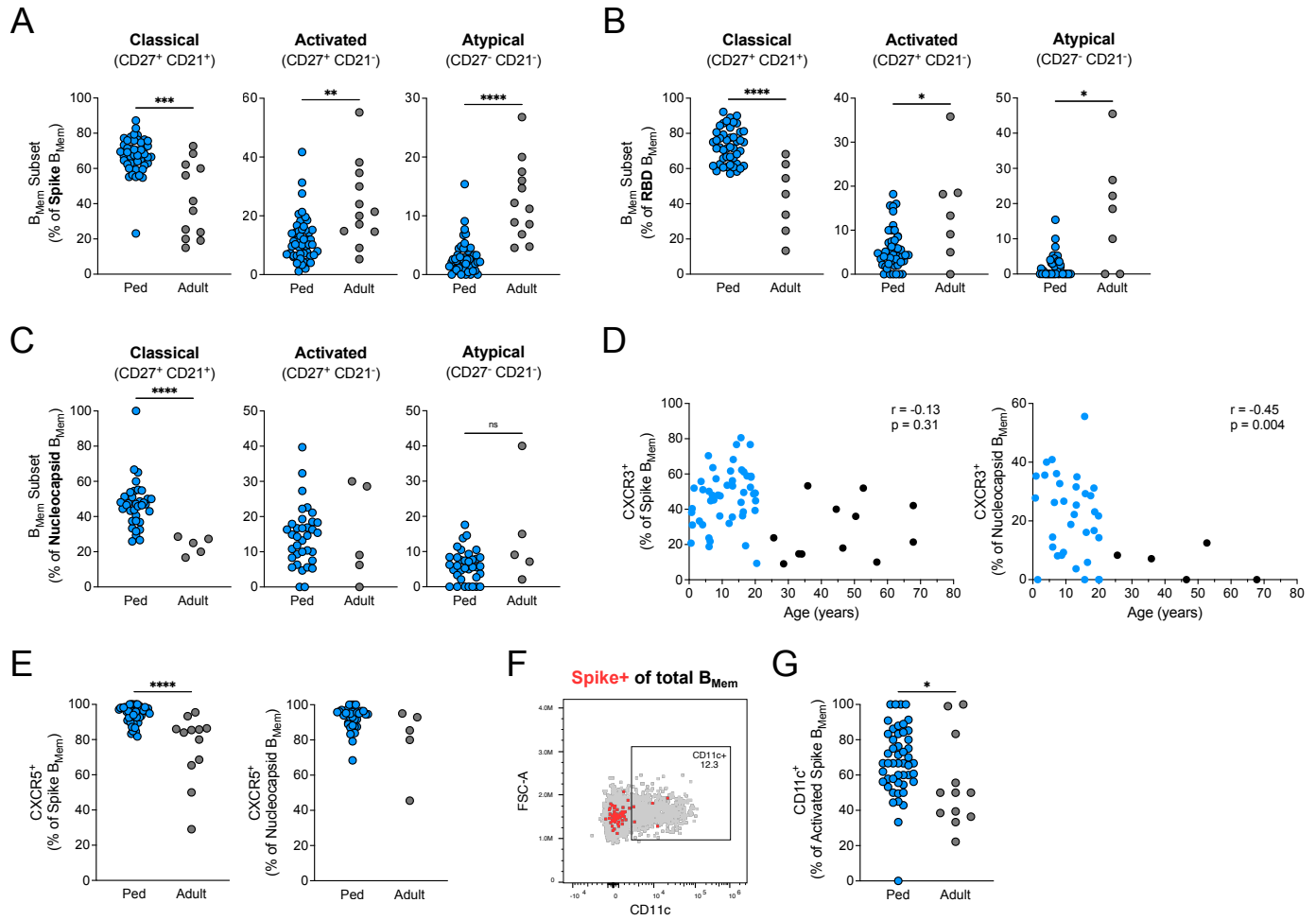
Supplemental Figure 5. cTFH response in SARS-CoV-2 infected children and adults

(A) Paired longitudinal spike- (left), non-spike- (right) specific cTFH frequencies at indicated timepoints post-infection from donors that provided samples for all three time points. (B) Representative flow cytometry plots from the indicated age-groups showing SARS-CoV-2 spike- (top) and non-spike- (bottom) specific AIM⁺ CD4⁺ T cells (blue) overlaid on total CD4⁺ T cells (grey) with the CXCR5⁺ gate representing cTFH cells. Numbers in blue indicate the frequency of SARS-CoV-2 specific cTFH amongst total CD4⁺ cells. (C) Frequencies of total cTFH cells among total non-naïve CD4⁺ T cells in children and adults at indicated time points. (D) Frequencies of PD1⁺ CXCR5⁺ cTFH cells among total non-naïve CD4⁺ T cells in children and adults at indicated time points. (E) Correlation of Spike-specific cTFH frequencies at the acute phase of infection plotted against neutralizing antibody measured during the convalescent phase using a pseudovirus (614G) assay shown as 50% inhibitory dilution. (F) Correlation of Spike-specific cTFH frequencies at the acute phase of infection with indicated antigen binding antibody levels measured 2 months post-infection using a Luminex based multiplex assay. (G) Correlation of neutralizing antibody titers at 50% inhibitory dilution measured 2 months post-infection with age. (H) Correlation of SARS-CoV-2 RBD-binding antibody levels measured 2 months post-infection with age. Centerlines and error bars in C and D represent the geometric mean and geometric standard deviation, respectively. Dotted line in A, E, and F indicates the limit of quantification of the assay determined by calculating the geomean of the background (DMSO treated); solid grey line indicates the lower limit of detection. Uninfected participants are indicated as "Un"; 2-month and 6-month timepoints as "2m" and "6m", respectively. "AIM⁺" denotes the frequencies of OX40⁺CD40L⁺ cells amongst total CD4⁺ T cells. 'P' values were calculated for A by Wilcoxon test, for (C-D) by Kruskal-Wallis test with Dunn's correction for comparisons within the pediatric and adult age groups and by Mann-Whitney test with Holm-Sidak correction for comparisons across the pediatric and adult age groups, and are indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; non-significant 'p' values are not shown. 'r' in (E-H) indicates Spearman correlation coefficient.



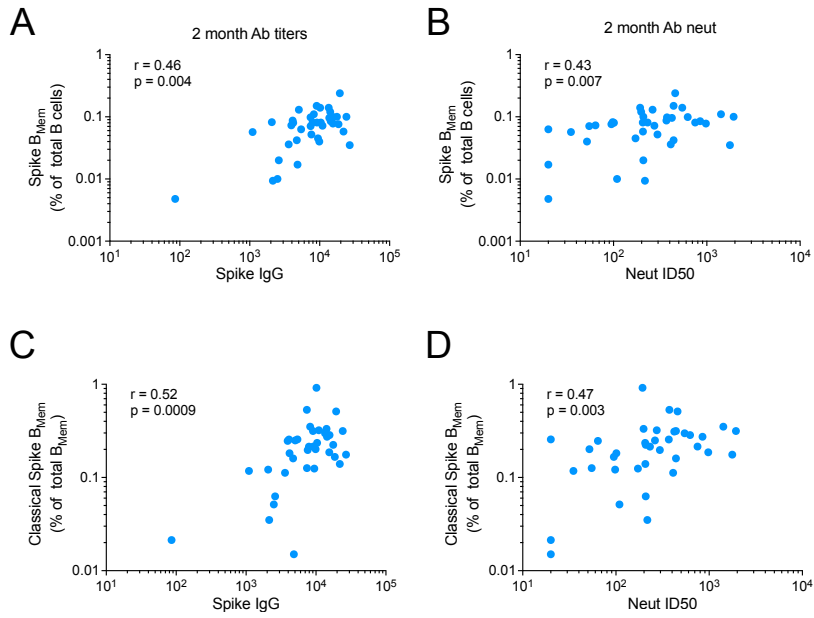
Supplemental Figure 6. Isotype distribution of SARS-CoV-2-specific memory B cells in children and adults

(A) Gating strategy for SARS-CoV-2-specific memory B cell analysis (additional representative gates are shown in their respective figures). (B) Representative flow cytometry plots showing Ig isotypes among total memory B cells (left) and spike-specific memory B cells (right). (C) Correlation of Nucleocapsid binding memory B cell frequencies with age in the pediatric group. (D) Correlations of IgM+ memory B cells as percent of Spike (left) and Nucleocapsid (right) binding memory B cells with age. (E) Frequencies of Spike Ig isotypes in asymptomatic ('Asym') or symptomatic ('Sym') pediatric or adult participants. $n=50$ in the pediatric group; $n=12$ in the adult group. 'P' values for E were calculated by by Kruskal-Wallis test with Dunn's correction. 'r' in C and D indicates Spearman correlation coefficient.



Supplemental Figure 7. SARS-CoV-2-specific memory B cell subsets in children and adults

(A-C) Frequencies of classical (left), activated (middle), and atypical (right) memory B cells indicated as percent of (A) Spike-binding memory B cells, (B) RBD-binding memory B cells, or (C) Nucleocapsid-binding memory B cells. (D) Correlations of CXCR3⁺ memory B cells as percent of Spike (left), and Nucleocapsid (right) binding memory B cells with age in the pediatric group. (E) Frequencies of CXCR5⁺ memory B cells indicated as percent of Spike or Nucleocapsid binding memory B cells. (F) Representative flow cytometry plot showing CD11c staining on SARS-CoV-2 specific memory B cells. (G) Frequency of CD11c⁺ activated memory B cells indicated as percent of Spike memory B cells. n=50 in the pediatric group; n=12 in the adult group. "Ped" in B-E represents the pediatric group. 'P' values for A-C, E, and G were calculated by Mann-Whitney test, and are indicated as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; non-significant 'p' values are not shown. 'r' in D indicates Spearman correlation coefficient.



Supplemental Figure 8. Correlation of pediatric Spike memory B cell frequencies with humoral responses

(A) Correlation of Spike IgG titers as determined by BAMA assay at 2-months post-infection with spike memory B cells indicated as percent of total B cells. (B) Correlation of Neut ID50 titers at 2-months post-infection with spike memory B cells indicated as percent of total B cells. (C) Correlation of Spike IgG titers as determined by BAMA assay at 2-months post-infection with classical memory B cells indicated as percent of total memory B cells. (D) Correlation of Neut ID50 titers at 2-months post-infection with classical memory B cells indicated as percent of total memory B cells. 'r' indicates the Spearman correlation coefficient.

Supplemental Table 1: Study Cohort Description.

Characteristics N (%)		0-3 years	4-11 years	12-15 years	16-20 years	Pediatric (0-20 years)	Adults (≥21 years)
		27	42	30	33	132	51
Age (years), median (IQR)		2.9 (1.2, 3.6)	7.5 (6.1, 9.3)	13.8 (13.1, 14.9)	17.9 (16.7, 19.5)	11.1 (5.7, 16.0)	42.4 (34.0, 56.4)
Sex							
Female		13 (48%)	15 (36%)	17 (57%)	16 (48%)	61 (46%)	25 (49%)
Male		14 (52%)	27 (64%)	13 (43%)	17 (52%)	71 (54%)	26 (51%)
Race/Ethnicity							
Non-Hispanic White		5 (19%)	3 (7%)	6 (20%)	6 (8%)	20 (15%)	32 (63%)
Non-Hispanic Black		3 (11%)	6 (14%)	4 (13%)	1 (14%)	14 (11%)	9 (18%)
Hispanic or Latino		17 (63%)	33 (79%)	20 (67%)	26 (78%)	96 (73%)	1 (2%)
Other race/ethnicity		2 (7%)	0	0	0	2 (2%)	9 (18%)
Comorbidities		7 (26%)	25 (40%)	18 (40%)	19 (42%)	50 (38%)	17 (33%)
	Asthma	1 (4%)	4 (10%)	1 (3%)	3 (9%)	9 (7%)	6 (12%)
	Obesity	4 (15%)	14 (33%)	10 (33%)	10 (30%)	38 (29%)	4 (8%)
	Other comorbidities	3 (11%)	2 (5%)	3 (10%)	4 (12%)	12 (9%)	12 (24%)
SARS-CoV-2 infection status							
Uninfected		4 (15%)	3 (7%)	3 (10%)	5 (15%)	15 (11%)	8 (16%)
Infected		23 (85%)	39 (93%)	27 (90%)	28 (85%)	117 (89%)	43 (84%)
	Viral load, log ₁₀ , median (IQR)	5.1 (2.4, 5.6)	4.0 (3.0, 5.7)	4.3 (3.2, 5.7)	4.4 (3.1, 5.2)	4.4 (3.0, 5.6)	4.6 (3.7, 6.1)
Infected and symptomatic		21 (91%)	28 (72%)	22 (81%)	23 (82%)	94 (80%)	42 (98%)
	Days of symptoms prior to sampling, median (IQR)	7 (5, 9)	8 (7, 9)	7 (5, 10)	10 (8, 11)	8 (6, 10)	8 (4, 20)
	Fever	10 (48%)	12 (43%)	15 (68%)	15 (65%)	52 (55%)	27 (64%)
	Cough	10 (48%)	15 (54%)	6 (27%)	17 (74%)	48 (51%)	31 (74%)
	Shortness of breath	1 (5%)	1 (4%)	3 (14%)	4 (17%)	9 (10%)	15 (36%)
	Sore throat	1 (5%)	5 (18%)	6 (27%)	9 (39%)	21 (22%)	17 (40%)
	Rhinorrhea	8 (38%)	6 (21%)	2 (9%)	6 (26%)	22 (23%)	21 (50%)
	Congestion	4 (19%)	7 (25%)	5 (23%)	4 (17%)	20 (21%)	23 (55%)
	Other symptoms	4 (19%)	4 (14%)	6 (27%)	5 (22%)	19 (20%)	40 (95%)
*Comorbidity data were unavailable for 14 of the adult participants.							

Supplemental Table 2: Sub-Cohort Characteristics by Analysis Type.

Analysis Cohort Characteristics	T cell analyses (n=175)	B cell analyses (n=62)
0-3 years, n (%)	25 (14%)	7 (11%)
4-11 years, n (%)	40 (23%)	16 (26%)
12-15 years, n (%)	30 (17%)	11 (18%)
16-20 years, n (%)	31 (18%)	16 (26%)
21+ years, n (%)	49 (28%)	12 (19%)
Infected, n (%)	152 (87%)	62 (100%)
Symptomatic infection, n (%)	135 (86%)	52 (84%)

Supplemental Table 3: T Cell Antibody Panel.

Reagent	Clone	Source (Catalog #)	Dilution
CXCR5 AF700	J252D4	Biolegend (356915)	1:200
CXCR3 BV605	G025H7	Biolegend (353728)	1:200
CCR7 BV711	G043H7	Biolegend (353228)	1:100
CCR6 BUV496	11A9	BD Biosciences (612948)	1:200
ICOS BUV563	DX29	BD Biosciences (741421)	1:150
PD-1 BUV737	EH12.1	Biolegend (612791)	1:200
4-1BB BV421	4B4-1	Biolegend (309820)	1:200
CD69 FITC	FN50	Biolegend (310904)	1:250
CD40L PE-DAZZLE594	24-31	Biolegend (310840)	1:500
OX40 APC	Ber-ACT35	Biolegend (350008)	1:100
CD3 BUV395	UCHT1	BD Biosciences (563546)	1:200
CD38 BUV661	HIT2	BD Biosciences (612969)	1:100
CD8 BUV805	SK1	BD Biosciences (612889)	1:800
CD14 BV510	63D3	Biolegend (367124)	1:100
CD16 BV510	3G8	Biolegend (302048)	1:200
CD20 BV510	2H7	Biolegend (302340)	1:50
CD45RA BV570	HI100	Biolegend (304132)	1:400
CD27 BV785	O323	Biolegend (302832)	1:100
CD4 APC-FIRE810	SK3	Biolegend (344662)	1:50
TNFA EF450	Mab11	Thermo Fisher (48-7349-42)	1:50
IL-2 BV650	MQ1-17H12	Biolegend (500334)	1:50

Supplemental Table 4: Summary Statistics for Cell Counts Included in Spike-binding B_{Mem} Cell Analyses.

Pediatric Summary		Adult Summary	
Samples (N)	49	Samples (N)	12
Spike-specific Cell # (GeoMean)	125	Spike-specific Cell # (GeoMean)	61
Spike-specific Cell # (ArithMean)	170	Spike-specific Cell # (ArithMean)	109
Spike-specific Cell # (Median)	135	Spike-specific Cell # (Median)	43.5
Spike-specific Cell # (Range)	13-602	Spike-specific Cell # (Range)	20-525
Samples <20 spike B _{Mem} : n (%)	1 (2.04)	Samples <20 spike B _{Mem} : n (%)	0 (0)

Supplemental Table 5: Memory B Cell Antibody Panel.

Reagent	Clone	Source (Catalog #)	Dilution
IgG BUV395	G18-145	BD Biosciences (564229)	1:100
CXCR5 BUV496	RF8B2	BD Biosciences (741115)	1:200
CD19 BUV563	SJ25C1	BD Biosciences (612916)	1:200
CD79b BUV615	CB3-1	BD Biosciences (751380)	1:50
CD95 BUV737	DX2	BD Biosciences (612790)	1:200
CXCR3 BUV805	1C6	BD Biosciences (742048)	1:50
IgD Pacific Blue	IA6-2	Biolegend (348224)	1:50
CD20 BV510	2H7	Biolegend (302340)	1:100
IgM BV570	MHM-88	Biolegend (314517)	1:200
CD27 BB515	M-T271	BD Biosciences (564642)	1:200
IgA VioBright-FITC	IS11-8E10	Miltenyi Biotech (130-113-480)	1:400
CD3 PerCP	SK7	Biolegend (344814)	1:100
CD14 PerCP	63D3	Biolegend (367152)	1:200
CD16 PerCP	3G8	Biolegend (302030)	1:200
CD56 PerCP	HCD56	Biolegend (318342)	1:200
CD71 PE-Dazzle594	CY1G4	Biolegend (334120)	1:200
CD11c PE-Cy5	3.9	Biolegend (301610)	1:200
CD21 AF700	Bu32	Biolegend (354918)	1:50
CD38 APC-Fire810	HIT2	Biolegend (303550)	1:200