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Supplemental information

Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells

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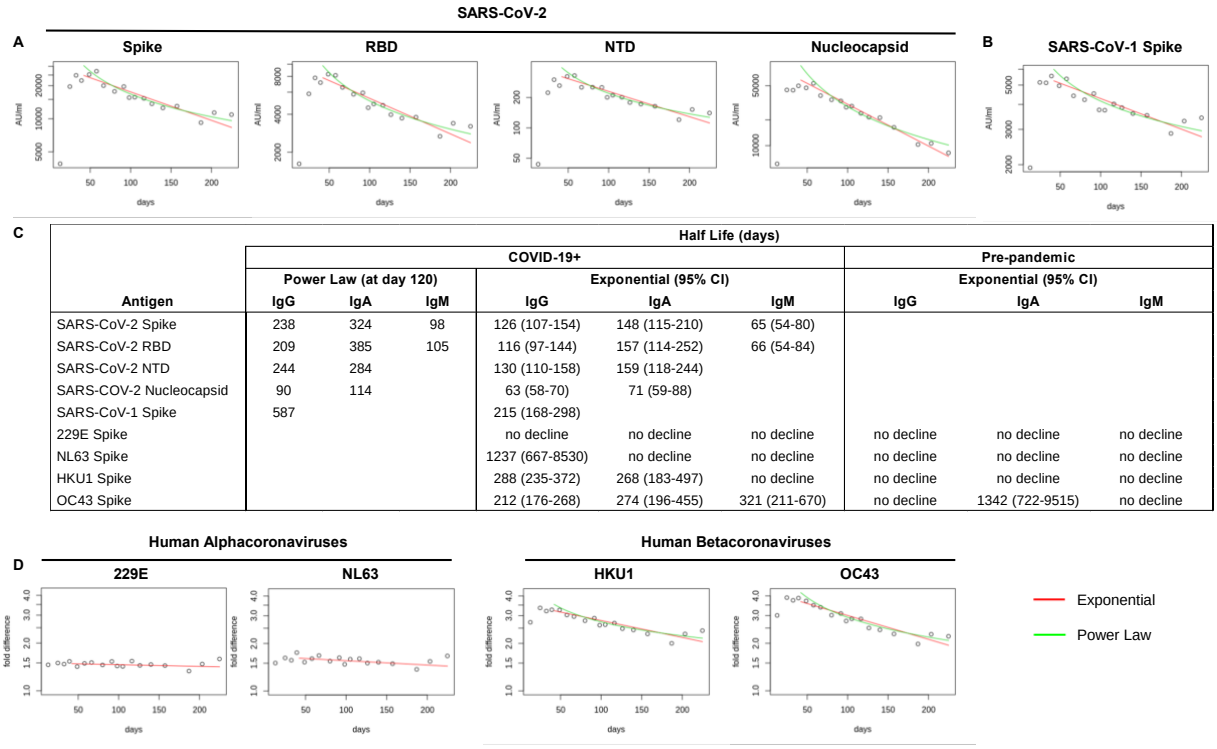
Table S1. Cohort Demographics and Baseline Characteristics (Related to STAR Methods Subject Details).

Characteristic	All (N=254)
Age, median (range)— years	48.5 (18-82)
Female sex at birth— no. (%)	141 (55.6)
Race or ethnic group— no. (%)	
White	226 (89.0)
Hispanic or Latino	21 (8.3)
Black or African American	15 (5.9)
Asian	11 (4.3)
Other[a]	7 (2.8)
Median time from symptom onset to enrollment (range)— days	53.5 (1-203)
Comorbid conditions— no. (%)	
Hypertension	46 (18.1)
Obesity	41 (16.1)
Chronic lung disease	23 (9.3)
HIV-1 and/or autoimmune disease	19 (7.7)
Type 2 diabetes mellitus	18 (7.3)
Heart disease	15 (6.0)
Cancer	10 (3.9)
Symptoms with initial illness— no. (%)	
Myalgia, fatigue	231 (90.9)
Headache	168 (66.1)
Fever	167 (65.7)
Cough	161 (63.4)
Loss of smell	146 (57.5)
Loss of taste	143 (56.3)
Shortness of breath	108 (42.5)
Diarrhea	102 (40.2)
Sputum production	43 (16.9)
None	9 (3.5)
Disease severity (WHO Score)—no. (%)	
Mild (1-2)	180[b] (70.9)
Moderate (3-4)	62 (24.4)
Severe (5-10)	12 (4.7)
Maximum number of visits—total	
1	9
2	103
3	62
4	51
5-7	29

[a]Individuals identifying as Other included: American Indian or Alaska Native; White (n=1); Asian, Black or African American (n=1); Asian; White (n=3); Native Hawaiian or other Pacific Islander; White (n=2); [b]6 participants had a positive Abbott SARS-CoV-2 Abbott SARS-CoV-2 IgG assay test but did not have a positive nasal SARS-CoV-2 PCR test.



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	Half Life (days)								
	COVID-19+						Pre-pandemic		
	Power Law (at day 120)			Exponential (95% CI)			Exponential (95% CI)		
Antigen	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
SARS-CoV-2 Spike	238	324	98	126 (107-154)	148 (115-210)	65 (54-80)			
SARS-CoV-2 RBD	209	385	105	116 (97-144)	157 (114-252)	66 (54-84)			
SARS-CoV-2 NTD	244	284		130 (110-158)	159 (118-244)				
SARS-COV-2 Nucleocapsid	90	114		63 (58-70)	71 (59-88)				
SARS-CoV-1 Spike	587			215 (168-298)					
229E Spike				no decline	no decline	no decline	no decline	no decline	no decline
NL63 Spike				1237 (667-8530)	no decline	no decline	no decline	no decline	no decline
HKU1 Spike				288 (235-372)	268 (183-497)	no decline	no decline	no decline	no decline
OC43 Spike				212 (176-268)	274 (196-455)	321 (211-670)	no decline	1342 (722-9515)	no decline

Figure S1. Modeling of antibody titer decline. Decline of IgG antibody titers was analyzed by an exponential decay model (red) and a power law model (green) for antibodies reactive to SARS-CoV-2 antigens (A) and SARS-CoV-1 spike (B). The half-lives estimated by the exponential and power law models (C). The half-lives estimated by the power law were calculated at day 120 after symptom onset. The fold difference in IgG antibody titers to endemic coronaviruses between COVID-19 patients and pre-pandemic controls plotted over days since symptom onset (D). Related to Figure 1 and 2.

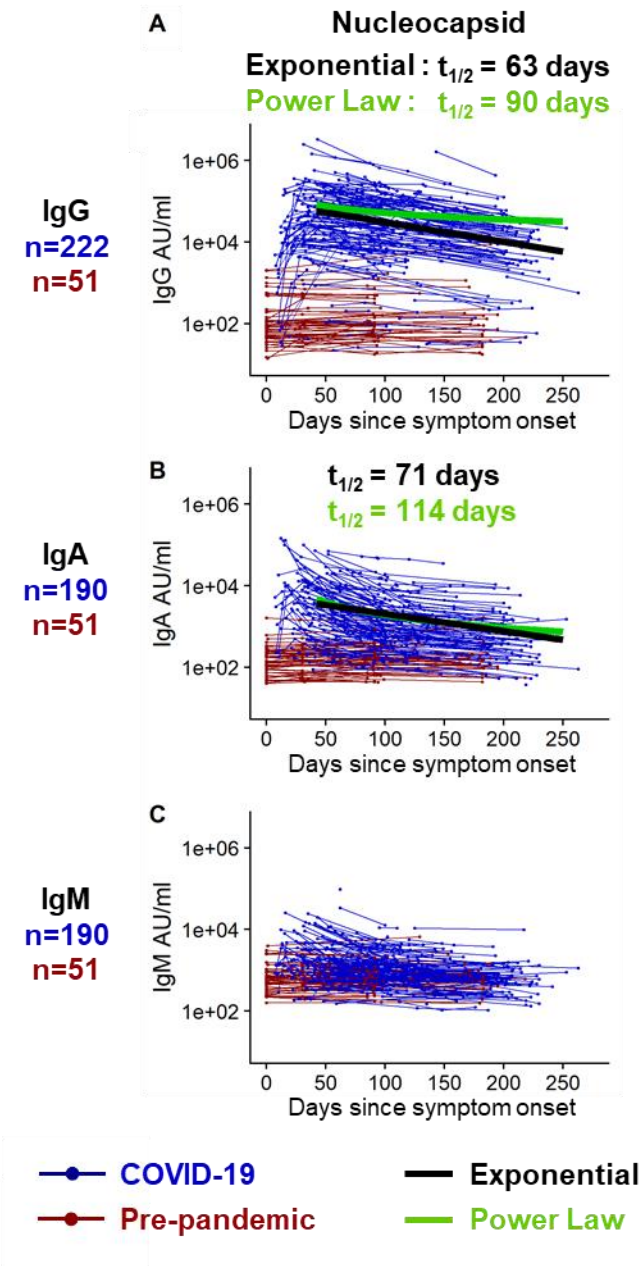

Figure S2. Longitudinal SARS-CoV-2 nucleocapsid binding antibody responses. IgG (A), IgA (B), and IgM (C) antibodies reactive to SARS-CoV-2 nucleocapsid were measured by an electrochemiluminescent multiplex immunoassay in triplicate and reported as arbitrary units per ml (AU/ml) as normalized by a standard curve. Longitudinal antibody titers of COVID-19 patients (in blue, n=222 COVID-19+ for IgG; n=190 COVID-19+ for IgA and for IgM) are plotted over days since symptom onset, whereas longitudinal pre-pandemic donor samples (in red, n=51 for IgG, IgA and IgM) were collected in the course of a non-SARS-CoV-2 vaccine study before 2019 and plotted over days since immunization. IgG decay curves and half-lives estimated by an exponential decay model are shown in black, whereas the decay curves and half-lives at day 120 post symptom onset estimated by a power law model are shown in green. Related to Figure 1.

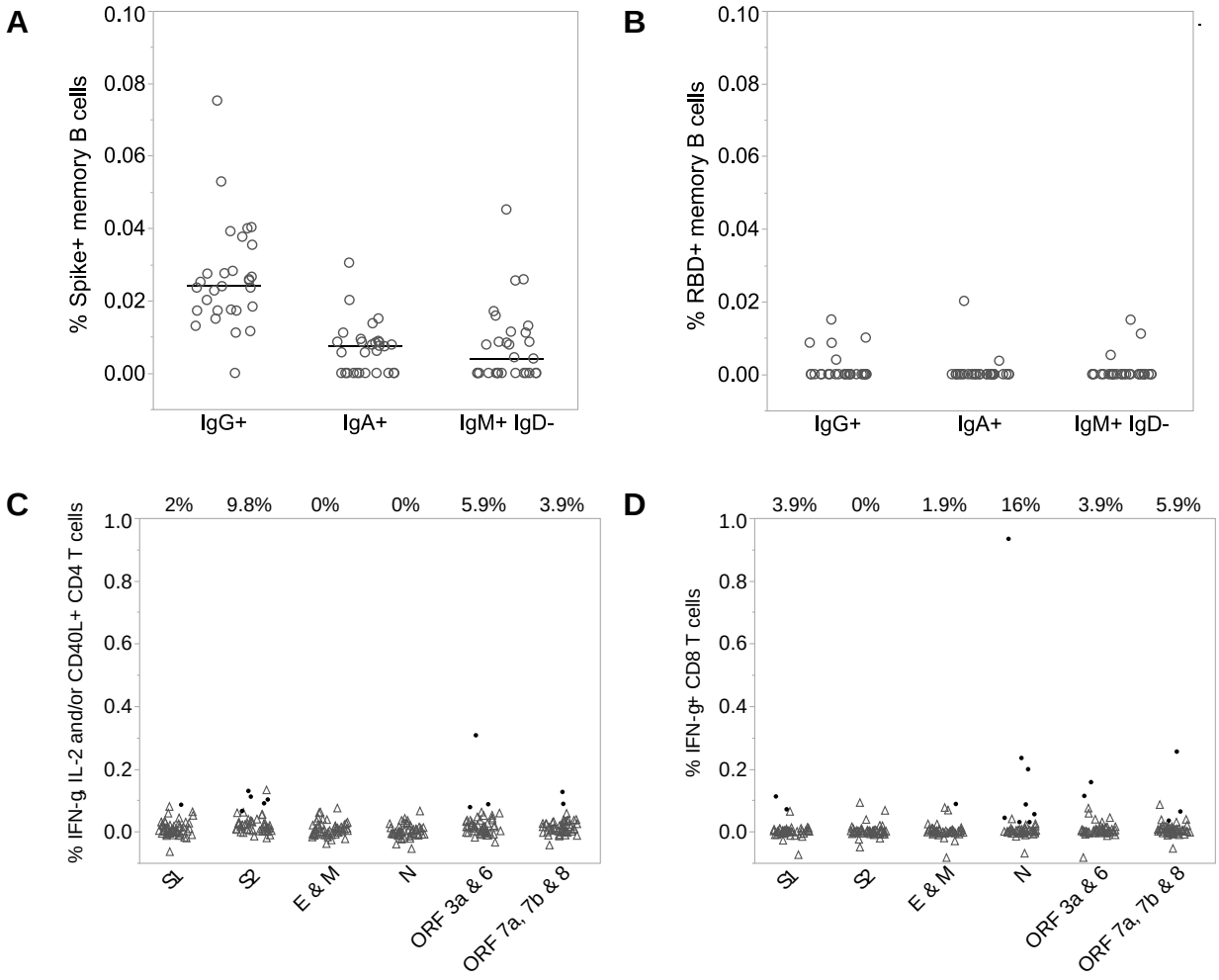

Figure S3. SARS-CoV-2 uninfected controls have few if any memory B and T cells recognizing SARS-CoV-2 antigens. Spike+ (A) and RBD+ (B) IgG+, IgA+ and IgM+ memory B cells in SARS-CoV-2 negative subjects are shown from PBMC collected before 2019 (n=29; tested in singlet). Line is at the median. Low frequencies of T cells recognizing SARS-COV-2 antigens are shown from donor samples not infected with SARS-CoV-2 (n=51). Background-subtracted CD4+ T cells expressing IFN-γ, IL-2 and/or CD40L (C), and IFN-γ+ CD8+ T cells (D) in response to stimulation with the SARS-CoV-2 antigens (on the x-axis) are shown. Positive T cell stimulations (as determined by MIMOSA) are indicated by a solid black circle, whereas samples that are negative are indicated by gray open triangles and the percent of positive responders are shown above the T cell graphs. Related to Figure 4, 5 and 6.

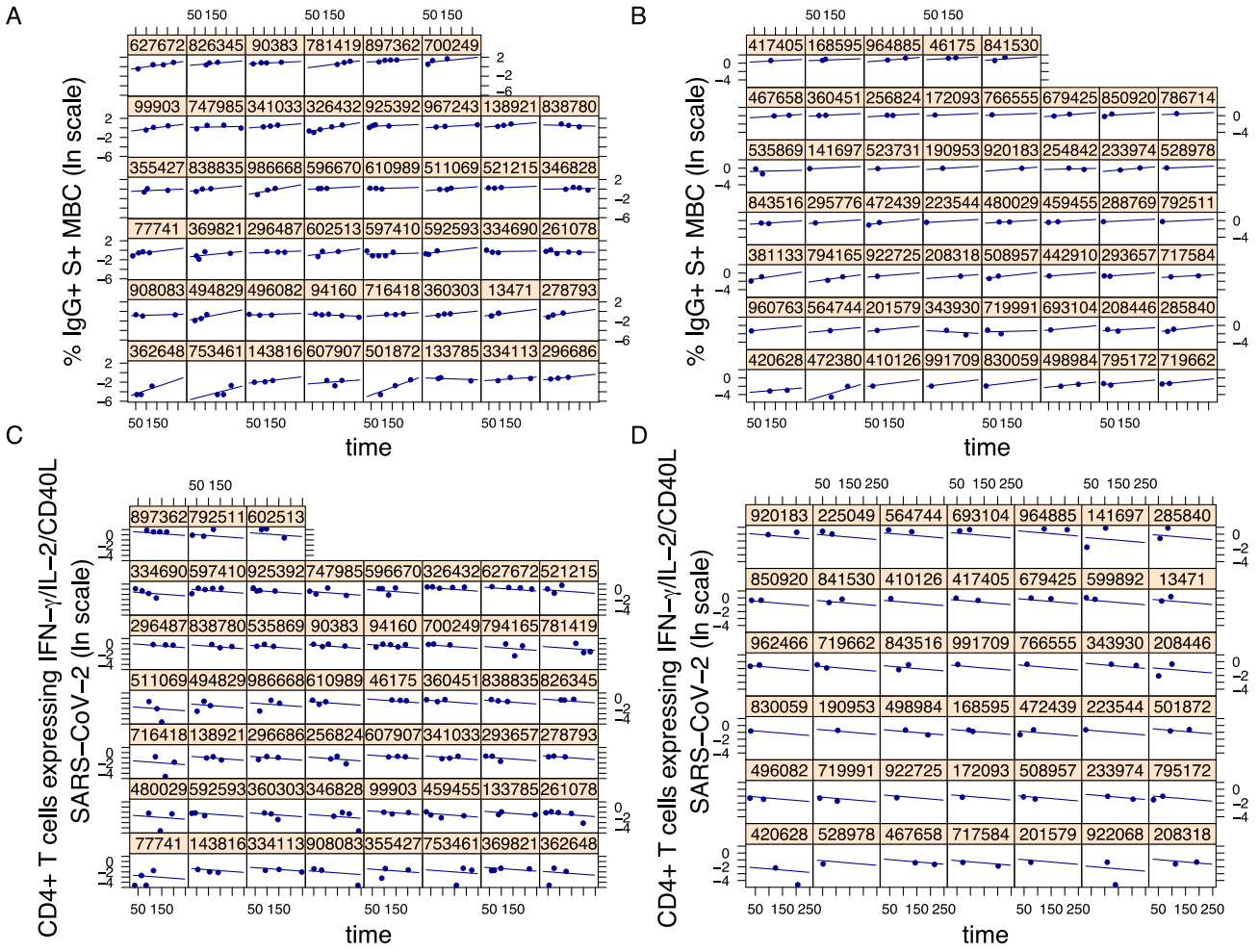

Figure S4. Representative individual-level estimates of SARS-CoV-2 B and T cell responses from 30 days post-symptom onset. Post-day 30 S+ IgG+ B cell responses ($\log_e$ scale) for individuals with data at 3 or more time points (A) and 1-2 time points (B) with fitted curves from a linear mixed effects model with random effects for the intercept and slope. Post-day 30 CD4+ T cell responses to SARS CoV-2 ($\log_e$ scale) for individuals with data at 3 or more time points (C) and 1-2 timepoints (D), with fitted curves from a nonlinear mixed effects model with random effects for the intercept and slope. The CD4+ T cell analyses only included individuals with a positive response to a least one SARS-CoV-2 antigen at one or more time points, where positive responses were determined by MIMOSA. Related to Figures 4 and 5.

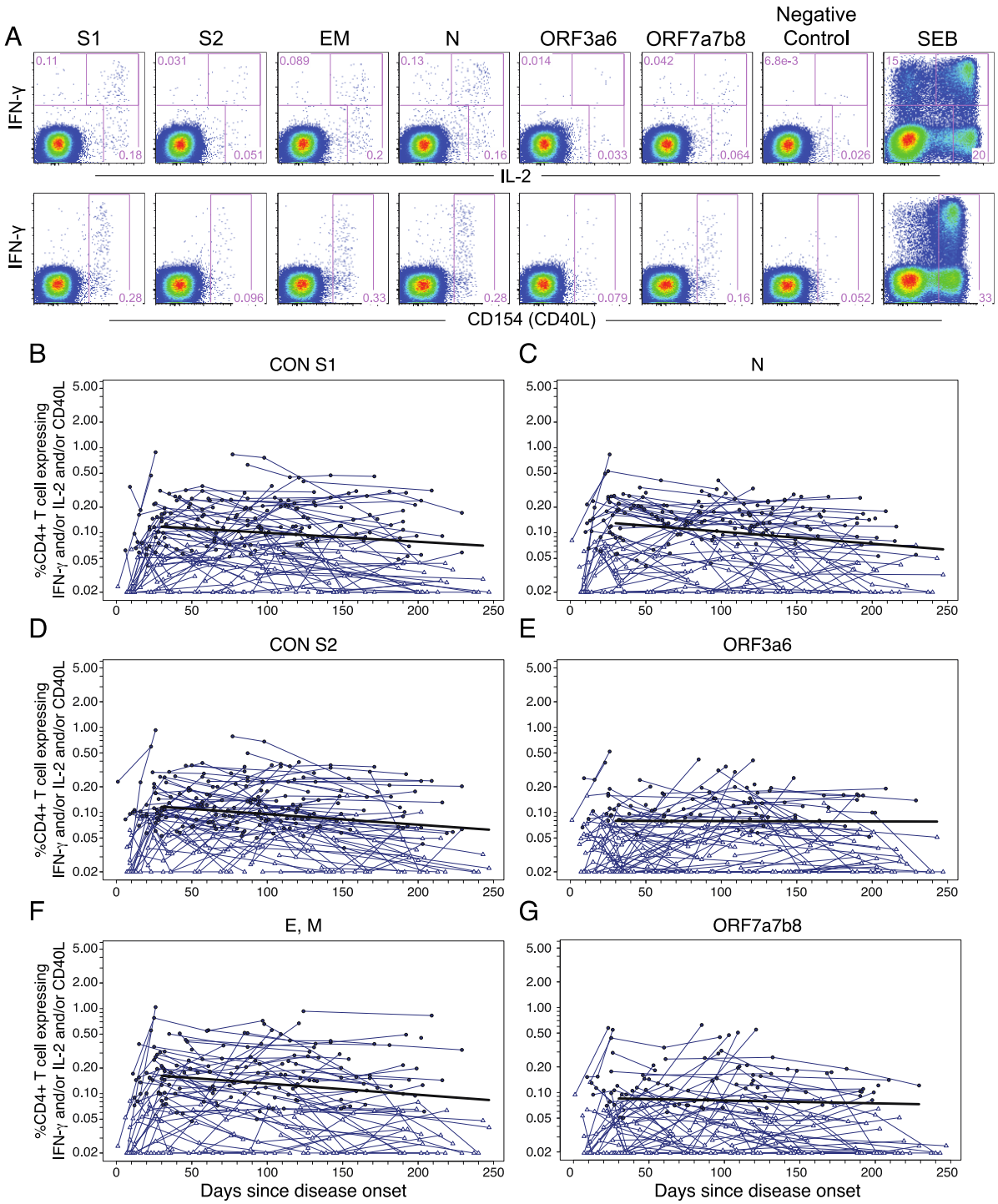

Figure S5. CD4+ T cell responses among SARS-CoV-2 convalescent subjects to individual SARS-CoV-2 peptide pools. (A) Representative SARS-CoV-2 specific CD4+ T cell responses to multiple SARS-CoV-2 antigens by intracellular cytokine staining (ICS) assay in PBMCs from a SARS-CoV-2 patient. Background-subtracted frequencies of IFN-γ+, IL-2+ and/or CD40L+ CD4+ T cells responding to: (B) S1, (C) S2, (D) envelope and membrane (EM), (E) N, (F) ORF3a and 6, (G) ORF7a, 7, and 8 (n=114; tested in single replicates). Positive responses as determined by MIMOSA are indicated by a solid circle and negative responses are indicated by open triangles. The bold black line represents the median fitted curve from a nonlinear mixed effects model of post-day 30 responses with random effects for the intercept and slope. The mixed effects models only include individuals with a positive response to the antigen(s) under consideration at one or more time points. Related to Figure 5.

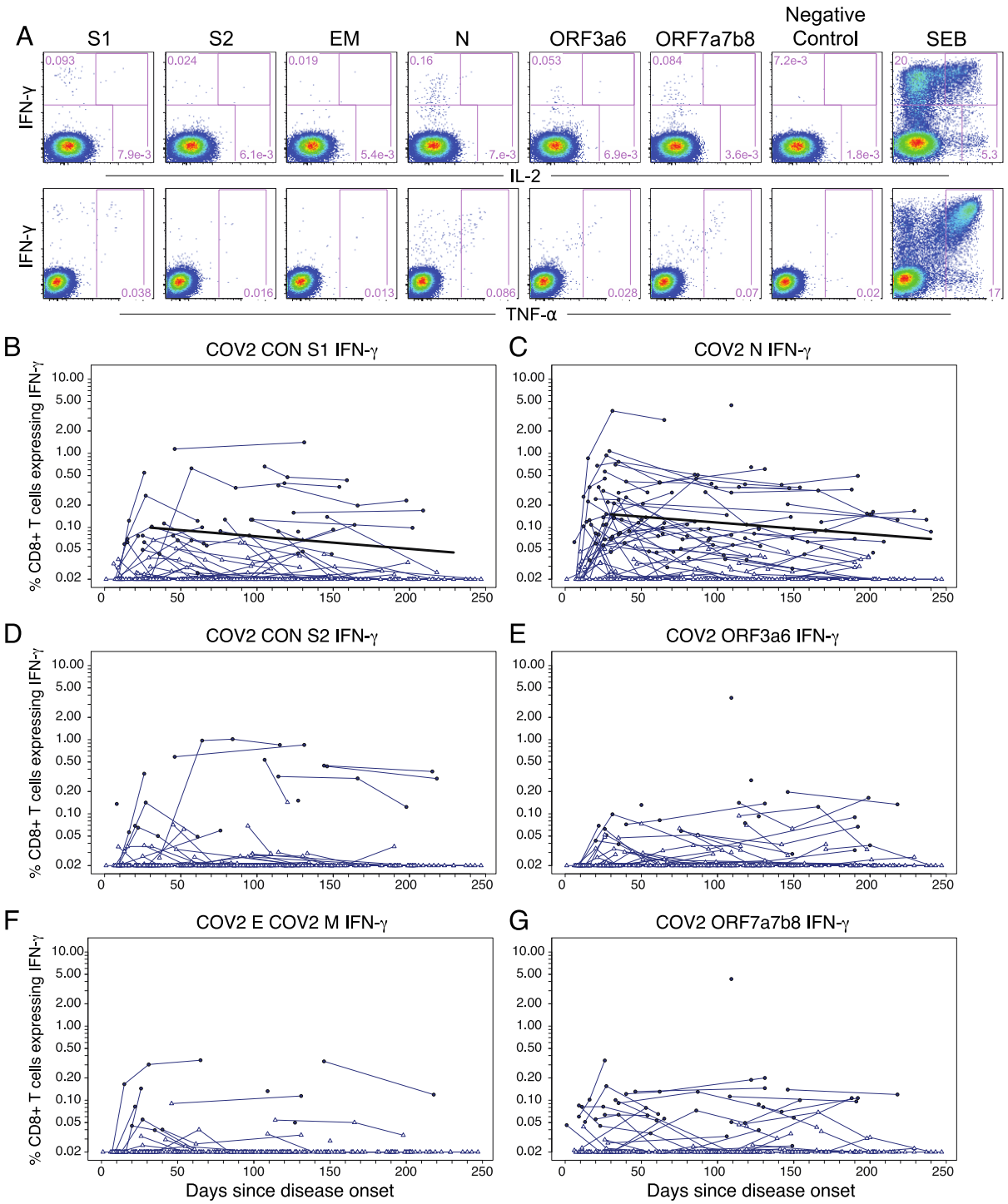

Figure S6. CD8 T+ cell responses among COVID-19 patients to individual SARS-CoV-2 peptide pools. (A) Representative SARS-CoV-2-specific CD8+ T cell responses to multiple SARS-CoV-2 antigens by intracellular cytokine staining (ICS) assay in PBMCs from a SARS-CoV-2 patient. Background-subtracted frequencies of IFN-γ+ CD8+ T cells responding to: (B) S1, (C) S2, (D) envelope and membrane (EM), (E) N, (F) ORF3a and 6, (G) ORF7a, 7, and 8 (n=114; tested in single replicates). Positive responses as determined by MIMOSA are indicated by a solid circle, and negative responses are indicated by open triangles. The bold black line represents the median fitted curve from a nonlinear mixed effects model of post-day 30 responses with random effects for the intercept and slope. The mixed effects models only included individuals with a positive response to the antigen(s) under consideration at one or more time points. Related to Figure 6.