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Supplemental Information

Low-Avidity CD4$^+$ T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19

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Supplemental Figure 1

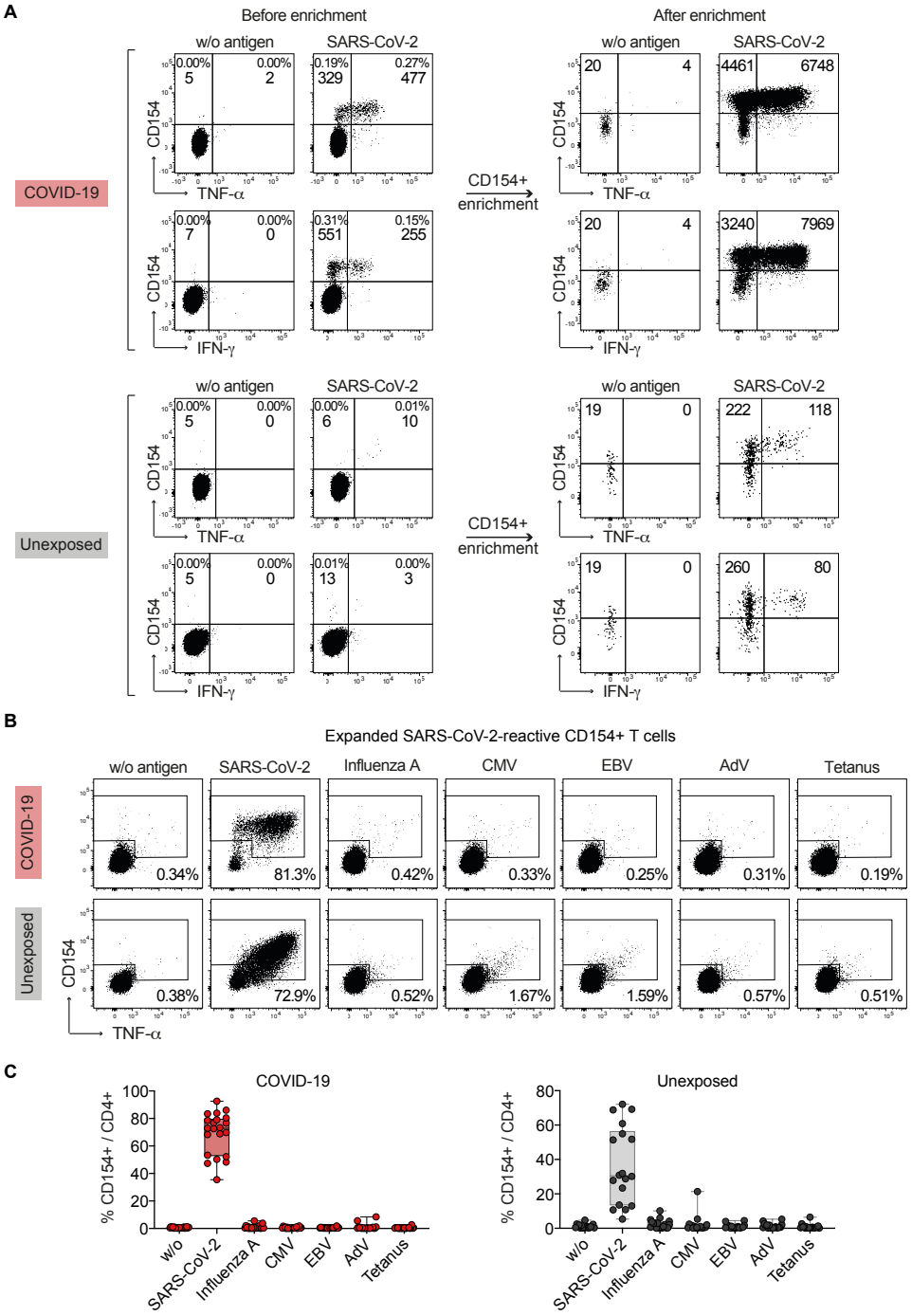

Figure S1. Detection of SARS-CoV-2 reactive CD4+ T cells by ARTE, Related to Figure 1.
(A) Ex vivo detection of SARS-CoV-2 pool-reactive CD4+ T cells by ARTE. Percentage within CD4+ T cells and absolute cell counts before and after magnetic CD154+ enrichment from 1×10e7 PBMCs of a COVID-19 patient and unexposed donor are indicated.
(B and C) Re-stimulation of FACS purified, expanded SARS-CoV-2 pool-reactive CD154+ T cells with the SARS-CoV-2 pool or control antigens. (B) Percentage of CD154+TNFα+ cells within CD4+ is indicated. (C) Statistical summary, each symbol represents one donor. Box-and-whisker plots display quartiles and range. Unexposed donors (n=18), COVID-19 patients (n=21).

Supplemental Figure 2

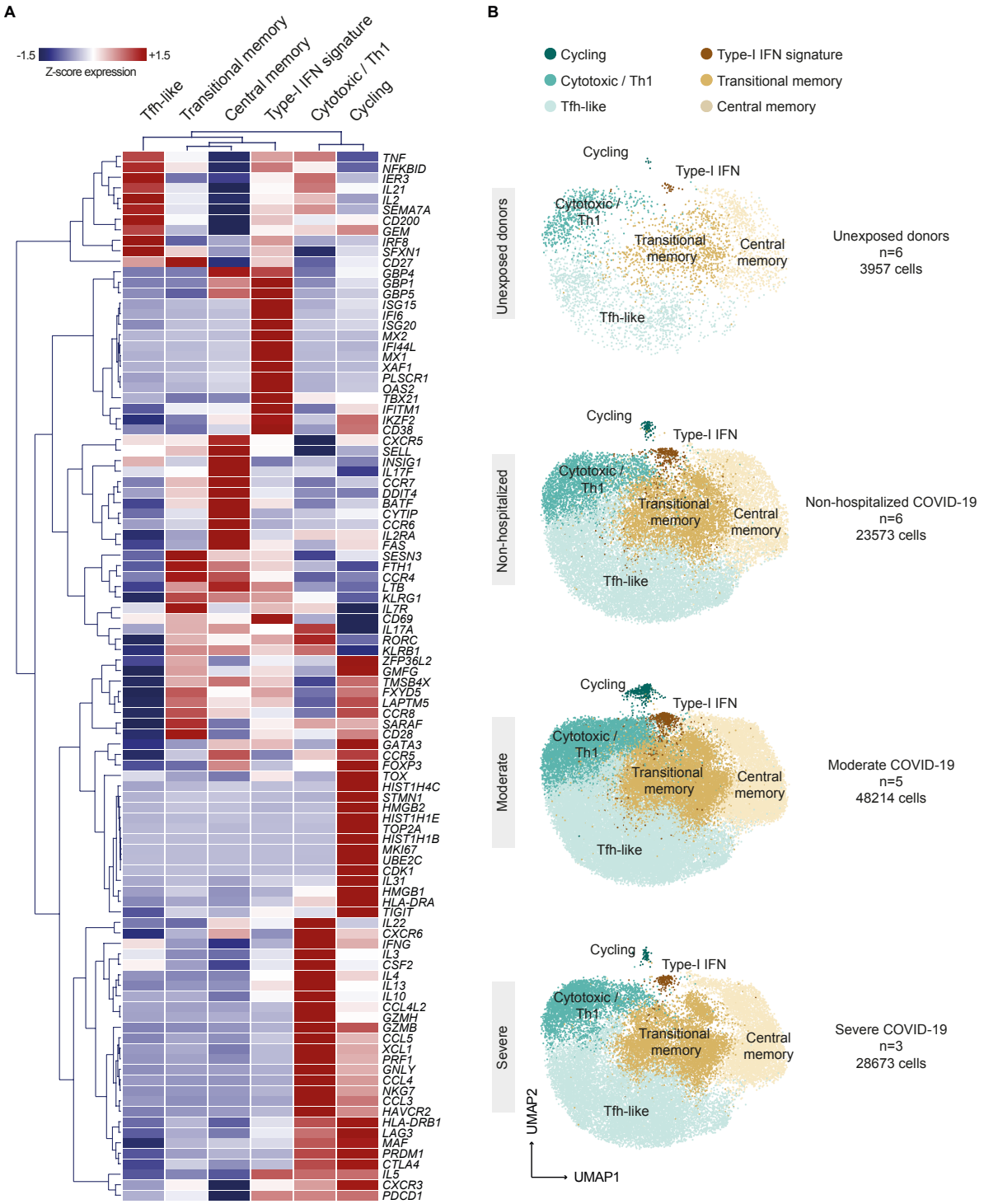

Figure S2. Gene expression of SARS-CoV-2 reactive CD4+ T cell clusters, Related to Figure 2.

Single cell transcriptomes of FACS purified ex vivo isolated CD154+ memory T cells following stimulation with pooled SARS-CoV-2 spike, membrane and nucleocapsid proteins from unexposed donors (n=6) and COVID-19 patients (n=14).

(A) Heatmap depicting Z-score normalized expression levels of the top 10 differential expressed marker genes of each cluster and other selected genes.

(B) UMAP visualization of the subset composition of SARS-CoV-2 reactive CD4+ T cells colored by functional gene expression clusters for unexposed donors (n=6) and non-hospitalized (n=6), moderate (WHO 4-5; n=5) and severe (WHO 6-7; n=3) COVID-19 patients.

Supplemental Figure 3

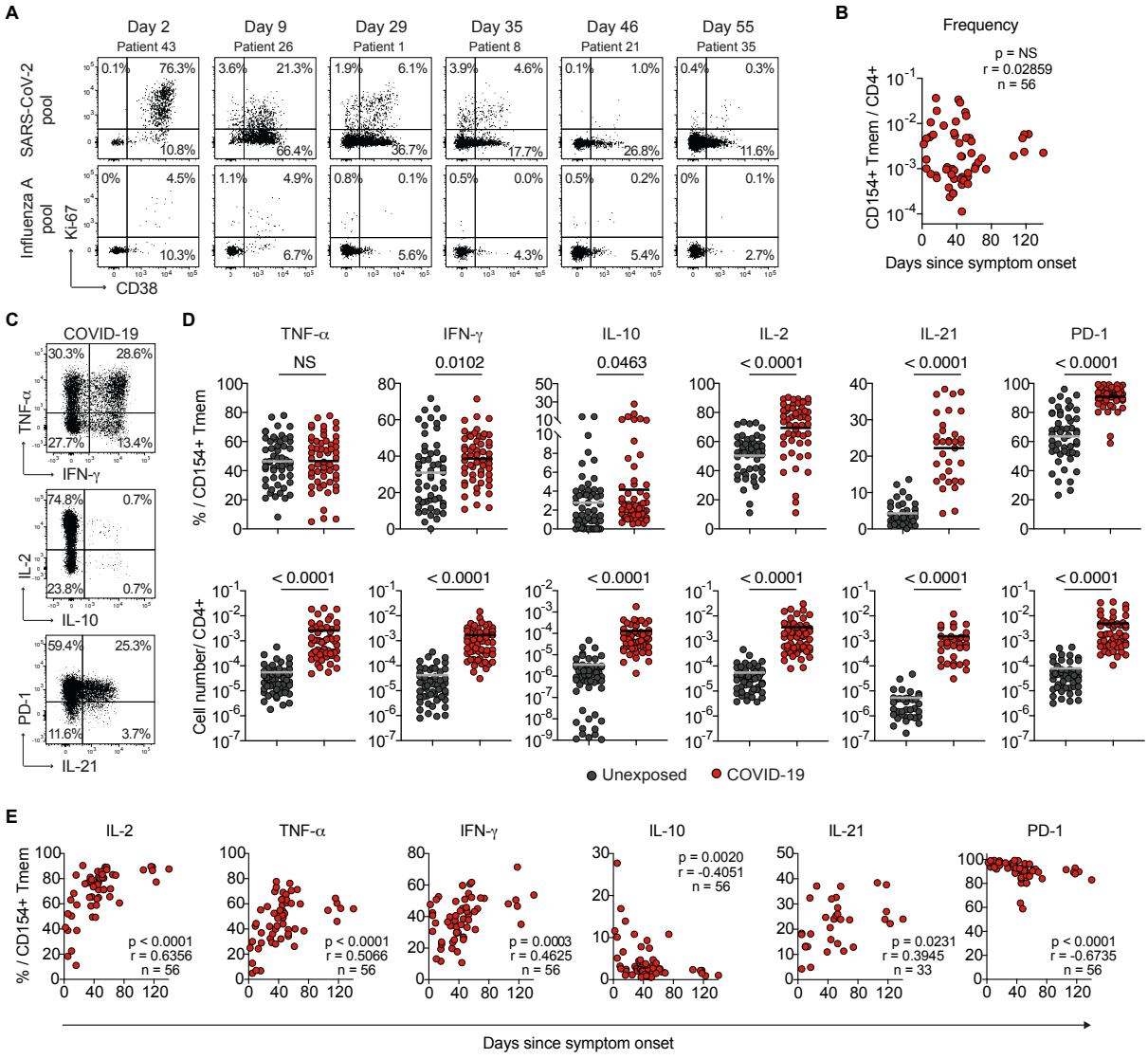

Figure S3. Inflammatory SARS-CoV-2-specific CD4+ T cell responses in COVID-19 patients, Related to Figure 2.

(A) Ex vivo Ki-67 and CD38 staining of SARS-CoV-2 pool- or Influenza A-reactive CD154+ Tmem from COVID-19 patients at different time points after disease onset. Percentage of Ki-67+ and/ or CD38+ cells within CD154+ Tmem are indicated.

(B) Spearman correlation of the frequencies of SARS-CoV-2 pool-reactive CD154+ Tmem and days since disease onset in COVID-19 patients (n=56).

(C) Ex vivo cytokine and phenotype staining of SARS-CoV-2 pool-reactive CD154+ Tmem from a COVID-19 patient. Percentage of marker positive cells within CD154+ Tmem are indicated.

(D) Ex vivo cytokine production and phenotype of SARS-CoV-2 pool-reactive cells. Upper row: relative frequencies within CD154+ Tmem and lower row: absolute frequencies within total CD4+ T cells. Unexposed donors (n=55; IL-21 n=36), COVID-19 patients (n=56; IL-21 n=33).

(E) Spearman correlation of cytokine and phenotypic marker expression of SARS-CoV-2 pool-reactive CD154+ Tmem and days since disease onset.

Each symbol in (B, D, E) represents one donor, horizontal lines indicate (D) mean. Statistical differences: (D) Two-tailed Mann-Whitney test.

Supplemental Figure 4

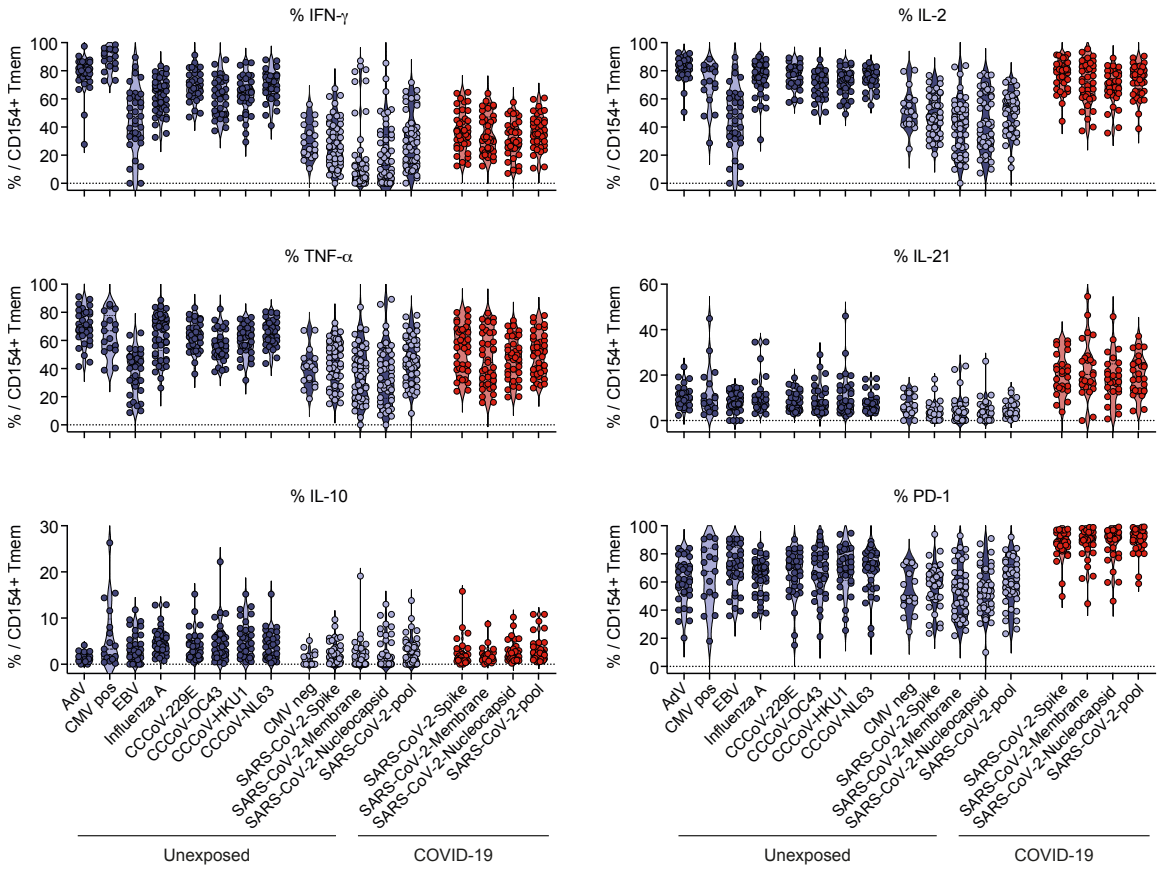

Figure S4. Pattern of SARS-CoV-2 reactive CD4+ T cells compared to other anti-viral responses, Related to Figure 2.
Ex vivo cytokine production and phenotype of SARS-CoV-2-reactive cells of re-convalescent COVID-19 patients in comparison to other anti-viral responses in SARS-CoV-2 unexposed donors (n=26-50). Each symbol represents one donor.

Supplemental Figure 5

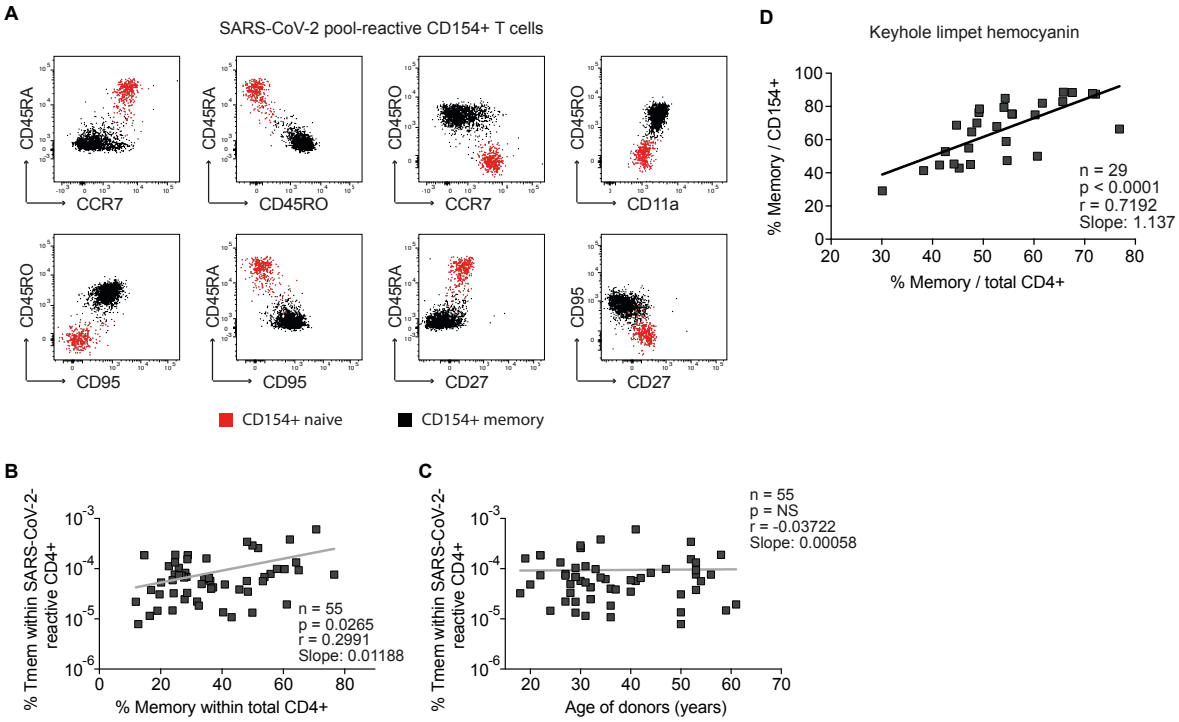

Figure S5. Proportion of neoantigen-specific memory T cells correlates with the proportion of memory cells in the total CD4+ pool, Related to Figure 3.
(A) Representative dot plot examples showing expression of phenotypic markers on SARS-CoV2-reactive CD154+ T cells from an unexposed donor. Naive antigen-specific T cells colored in red were defined as CD154+CD45RA+CCR7+ and are shown as overlay with the CD154+CD45RA- memory population (black).
(B) Spearman correlation between the frequencies of SARS-CoV-2 pool-reactive T cells in unexposed donors and the proportion of memory cells within the total CD4+ population.
(C) Spearman correlation between the frequencies of SARS-CoV-2 pool-reactive T cells in unexposed donors and the age of the donors.
(D) Pearson correlation between the proportion of memory cells within the antigen-specific T cells (y-axis) and the proportion of memory cells within the total CD4+ population is shown for the neoantigen keyhole limpet hemocyanin (KLH).
Each symbol in (B-D) represents one donor.

Supplemental Figure 6

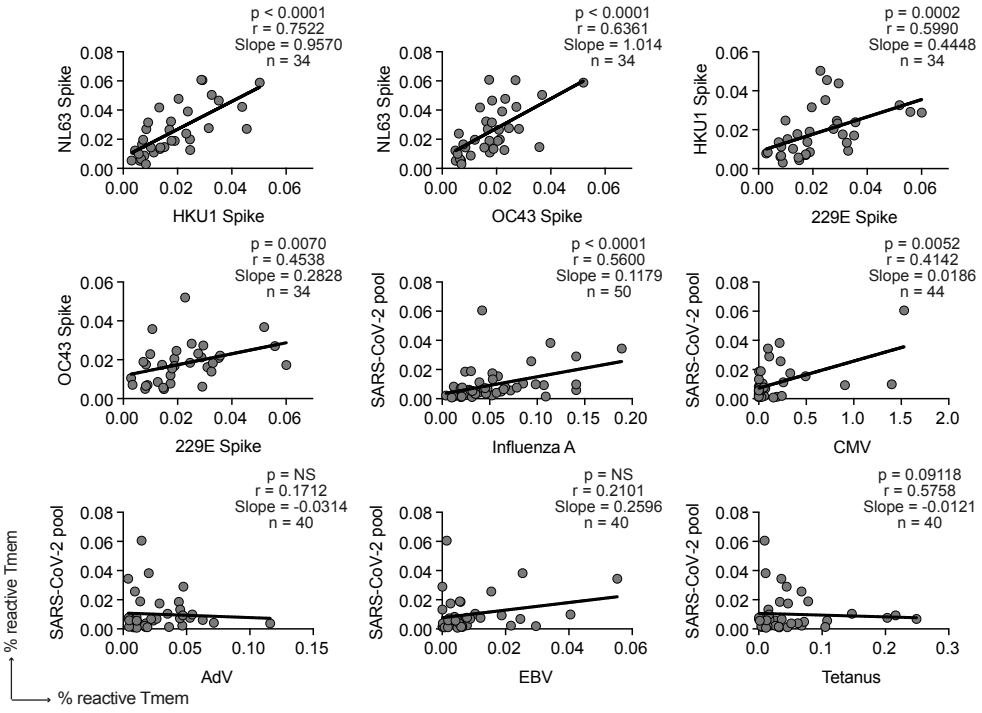

Figure S6. Correlations of SARS-CoV-2-reactive T cells of unexposed donors with the response against other common viruses, Related to Figure 5.
Spearman correlation between CD154+ Tmem frequencies reactive against different CCCoVs or SARS-CoV-2 and Influenza A (H1N1), Cytomegalovirus (CMV), Epstein–Barr Virus (EBV), Adenovirus (AdV) or tetanus in unexposed donors. Each symbol represents one donor.

Supplemental Figure 7

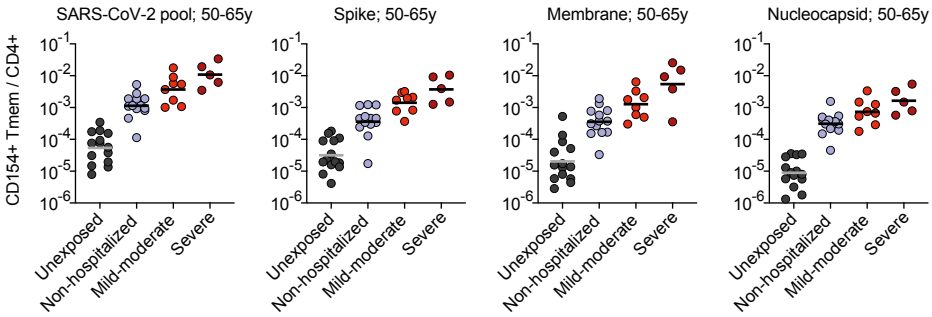

Figure S7. SARS-CoV-2-reactive T cell in age-selected donors, Related to Figure 6.
Frequencies of Tmem reactive against the indicated SARS-CoV-2 proteins in donors with an age range of 50-65. The COVID-19 severity level was assessed based on WHO criteria, whereby WHO groups 3-5 (moderate) and 6-7 (severe) were combined to increase statistical power (see table S1). Unexposed donors n=14, Non-hospitalized n=13 (WHO 1-2), moderate n=8 (WHO 3 n=1, WHO 4 n=4, WHO 5 n=3), severe n=5 (WHO 6 n=2, WHO 7 n=3). Each symbol in represents one donor, horizontal lines indicate mean.

Table S1. Cohort characteristics, Related to STAR Methods and Figures 1, 3 and 6.

	COVID-19 (n=56)	**Unexposed (n=64)**
Age mean	51 years (range 22-88)	37 years (range 18-61)
Gender		
Male	50% (28/56)	34% (22/64)
Female	50% (28/56)	66% (42/64)
Disease Severity[a]		
Non-hospitalized (WHO 1-2)	57% (32/56)	NA
Mild-moderate (WHO 3-5)	23% (13/56)	NA
Severe (WHO 6-7)	20% (11/56)	NA
SARS-CoV PCR positive		
Non-hospitalized (WHO 1-2)	84% (27/32)	NA
Mild-moderate (WHO 3-5)	100% (13/13)	NA
Severe (WHO 6-7)	100% (11/11)	NA
Total	91% (51/56)	NA
Antibody test positive[b, c]		
Non-hospitalized (WHO 1-2)	100% (32/32)	NA
Mild-moderate (WHO 3-5)	77% (10/13)	NA
Severe (WHO 6-7)	100% (11/11)	NA
Total	95% (53/56)	0% (0/64)

[a]WHO criteria
[b]Elecsys Anti-SARS-CoV-2, Roche Diagnostics GmbH
[c]Anti-SARS-CoV-2 ELISA, Euroimmun
NA=not applicable

Table S2. Patients characteristics, Related to STAR Methods and Figures 1, 3 and 6.

ID	Age	Gender	Classification	WHO class	Days since symptom onset	Status	SARS-CoV-2 RNA Test	SARS-CoV-2 antibodies	SARS-CoV-2 antibodies[a]
1	33	male	Non-hospitalized	WHO 1/2	29	convalescent	positive	positive	53.48
2	33	female	Non-hospitalized	WHO 1/2	31	convalescent	positive	positive	7.06
8	47	female	Non-hospitalized	WHO 1/2	35	convalescent	positive	positive	77.66
9	51	male	Non-hospitalized	WHO 1/2	46	convalescent	positive	positive	39.93
10	34	male	Non-hospitalized	WHO 1/2	32	convalescent	not done	positive	3.14
12	37	female	Non-hospitalized	WHO 1/2	37	convalescent	positive	positive	72.28
13	62	female	Non-hospitalized	WHO 1/2	37	convalescent	positive	positive	35.77
14	84	female	Non-hospitalized	WHO 1/2	34	convalescent	positive	positive	4.11
15	34	male	Non-hospitalized	WHO 1/2	28	convalescent	negative	positive	12.24
16	27	female	Non-hospitalized	WHO 1/2	36	convalescent	negative	positive	1.27
17	27	male	Non-hospitalized	WHO 1/2	36	convalescent	positive	positive	21.62
21	22	male	Non-hospitalized	WHO 1/2	46	convalescent	positive	positive	23.99
27	59	female	Non-hospitalized	WHO 1/2	62	convalescent	not done	positive	10.42
28	27	female	Non-hospitalized	WHO 1/2	46	convalescent	negative	positive	39.89
35	40	male	Non-hospitalized	WHO 1/2	55	convalescent	positive	positive	72.44
36	59	male	Non-hospitalized	WHO 1/2	46	convalescent	positive	positive	77.5
37	28	female	Non-hospitalized	WHO 1/2	45	convalescent	positive	positive	3.26
38	46	male	Non-hospitalized	WHO 1/2	65	convalescent	positive	positive	25.7
61	29	female	Non-hospitalized	WHO 1/2	53	convalescent	positive	positive	1.92
63	27	female	Non-hospitalized	WHO 1/2	61	convalescent	positive	positive	63.71
81	56	male	Non-hospitalized	WHO 1/2	53	convalescent	positive	positive	33.9
82	33	female	Non-hospitalized	WHO 1/2	48	convalescent	positive	positive	4.05
83	47	female	Non-hospitalized	WHO 1/2	51	convalescent	positive	positive	100.5
84	52	male	Non-hospitalized	WHO 1/2	53	convalescent	positive	positive	38.42
86	52	female	Non-hospitalized	WHO 1/2	45	convalescent	positive	positive	96.99
89	29	male	Non-hospitalized	WHO 1/2	74	convalescent	positive	positive	14.3
126	61	male	Non-hospitalized	WHO 1/2	106	convalescent	positive	positive	7.61[b]
142	58	female	Non-hospitalized	WHO 1/2	118	convalescent	positive	positive	4.03[b]
143	61	female	Non-hospitalized	WHO 1/2	116	convalescent	positive	positive	5.24[b]
144	57	female	Non-hospitalized	WHO 1/2	118	convalescent	positive	positive	3.18[b]
151	51	female	Non-hospitalized	WHO 1/2	57	convalescent	positive	positive	1.5[b]
154	61	female	Non-hospitalized	WHO 1/2	57	convalescent	positive	positive	6.95[b]
85	56	male	mild-moderate	WHO 3	43	convalescent	positive	positive	11.02
80	38	male	mild-moderate	WHO 3	5	active	positive	negative	0.06
26	68	female	mild-moderate	WHO 4	9	convalescent	positive	positive	10.45
49	65	male	mild-moderate	WHO 4	25	convalescent	positive	positive	54.46
65	57	female	mild-moderate	WHO 4	13	active	positive	negative	0.883
77	54	female	mild-moderate	WHO 4	50	convalescent	positive	positive	81.96
22	88	female	mild-moderate	WHO 4	16	active	positive	negative	0.122
79	33	female	mild-moderate	WHO 4	5	active	positive	negative	0.159
129	58	male	mild-moderate	WHO 4	140	convalescent	positive	positive	6.62[b]
78	53	male	mild-moderate	WHO 5	69	convalescent	positive	positive	3.85
110	69	male	mild-moderate	WHO 5	44	convalescent	positive	positive	20.67
111	60	male	mild-moderate	WHO 5	5	active	positive	positive	11.4
24	51	female	mild-moderate	WHO 5	38	active	positive	positive	18.84
66	70	male	severe	WHO 6	17	convalescent	positive	positive	4.54
76	76	male	severe	WHO 6	51	convalescent	positive	positive	75.97
43	50	female	severe	WHO 6	2	active	positive	positive	14.05
46	68	male	severe	WHO 6	39	convalescent	positive	positive	5.07
68	63	male	severe	WHO 6	10	active	positive	positive	16.15
45	52	male	severe	WHO 7	17	convalescent	positive	positive	45.68
88	62	female	severe	WHO 7	42	convalescent	positive	positive	63.12
115	67	female	severe	WHO 7	123	convalescent	positive	positive	19.7
44	78	male	severe	WHO 7	16	convalescent	positive	positive	11.36
47	61	male	severe	WHO 7	37	convalescent	positive	positive	62.06
48	68	male	severe	WHO 7	10	active	positive	positive	9.99

[a]Elecsys Anti-SARS-CoV-2 IgG/IgM. Roche Diagnostics GmbH; positive >1.0
[b]Anti-SARS-CoV-2 IgG ELISA, Euroimmun, Lübeck, Germany; positive >1.0