Science

MAAAS

Supplementary Materials for

Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination

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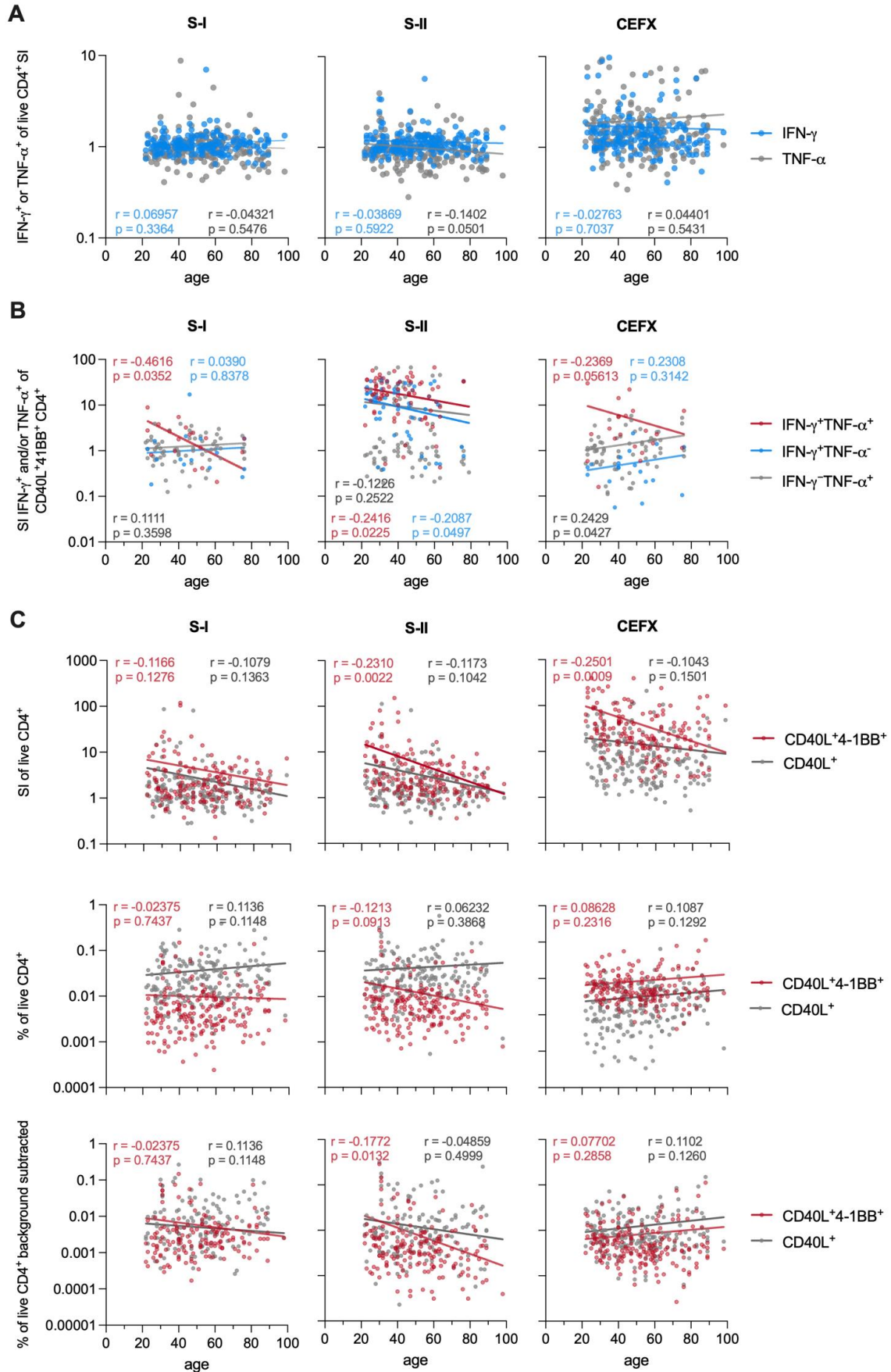
The PDF file includes:

Figs. S1 to S6
Tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist
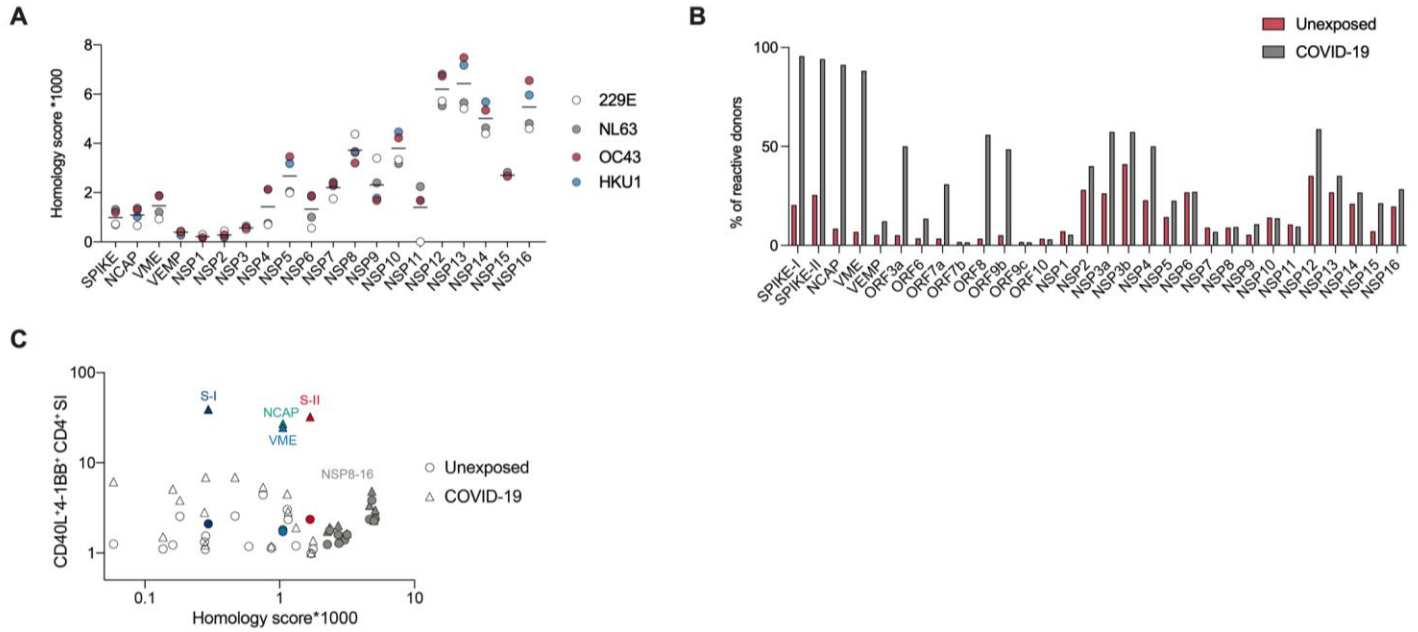
Fig. S1. HCoV homology scores and SARS-CoV-2 peptide pool reactivity
(A) Similarity between SARS-CoV-2 and the different endemic coronaviruses 229E, NL63, OC43, and HKU1 (isolate N1). Scores were calculated using a PAM30 substitution matrix for all 9-mers of the respective antigen against all 9-mers of the proteome of SARS-CoV-2. The homology score is the percentage of 9-mer pairs with a score above 30. **(B)** Frequency of donors with T cells reactive against the indicated peptide pools in unexposed and COVID-19 convalescents from Fig. 1A with an SI≥3. **(C)** To visualize the probability of homologous epitope recognition, a homology score for the comparison of a SARS-CoV-2 antigen with all four endemic coronaviruses 229E, NL63, OC43, and HKU1 (isolates N1, N2 N5) was plotted against the mean SI of 60 unexposed donors (circles) and 59 COVID-19 convalescents (triangles). For the calculation of the homology score all theoretical possible 9 aa fragments in each peptide of a respective PepMix were scored against all possible 9 aa fragments of the complete proteome of all other HCoVs using a PAM30 substitution matrix. The homology score is the percentage of individual comparisons with a score > 30.

1

A

S-I

S-II

CEFX

IFN-γ⁺ or TNF-α⁺ of live CD4⁺ SI

r = 0.06957 r = -0.04321
p = 0.3364   p = 0.5476

r = -0.03869 r = -0.1402
p = 0.5922   p = 0.0501

r = -0.02763 r = 0.04401
p = 0.7037   p = 0.5431

● IFN-γ
● TNF-α

B

S-I

S-II

CEFX

SI IFN-γ⁺ and/or TNF-α⁺ of CD40L⁺ 41BB⁺ CD4⁺

r = -0.4616 r = 0.0390
p = 0.0352   p = 0.8378

r = 0.1111
p = 0.3598

r = -0.1226
p = 0.2522

r = -0.2416 r = -0.2087
p = 0.0225   p = 0.0497

r = -0.2369 r = 0.2308
p = 0.05613 p = 0.3142

r = 0.2429
p = 0.0427

● IFN-γ⁺TNF-α⁺
● IFN-γ⁺TNF-α⁻
● IFN-γ⁻TNF-α⁺

C

S-I

S-II

CEFX

SI of live CD4⁺

r = -0.1166 r = -0.1079
p = 0.1276   p = 0.1363

r = -0.2310 r = -0.1173
p = 0.0022   p = 0.1042

r = -0.2501 r = -0.1043
p = 0.0009   p = 0.1501

● CD40L⁺4-1BB⁺
● CD40L⁺

% of live CD4⁺

r = -0.02375 r = 0.1136
p = 0.7437   p = 0.1148

r = -0.1213 r = 0.06232
p = 0.0913   p = 0.3868

r = 0.08628 r = 0.1087
p = 0.2316   p = 0.1292

● CD40L⁺4-1BB⁺
● CD40L⁺

% of live CD4⁺ background subtracted

r = -0.02375 r = 0.1136
p = 0.7437   p = 0.1148

r = -0.1772 r = -0.04859
p = 0.0132   p = 0.4999

r = 0.07702 r = 0.1102
p = 0.2858   p = 0.1260

● CD40L⁺4-1BB⁺
● CD40L⁺

age

Fig. S2. Cytokine characterization and activation molecule profiling of spike-specific CD4$^+$ T cells by participant age

(A, B) SI of IFN-γ^+ or TNF-α^+ of live CD4$^+$ T cells by age (A) and (B) SI of IFN-γ^+TNF-α^-, IFN-γ^-TNF-α^+ and IFN-γ^+TNF-α^+ CD40L$^+$41BB$^+$ CD4$^+$ T cells after stimulation of PBMCs with SARS-CoV-2 S-I, SARS-CoV-2 S-II, or CEFX in *n*=568 unexposed donors and *n*=174 COVID-19 convalescents. **(C)** SI of live CD4$^+$ T cells (upper row), frequencies of live CD4$^+$ T cells (middle row), and background (unstimulated control)-subtracted frequencies of live CD4$^+$ T cells (lower row) stimulated with S-I, S-II or CEFX and gated for CD40L$^+$4-1BB$^+$ or CD40L$^+$ expression.

A, B, C: Pearson correlation.
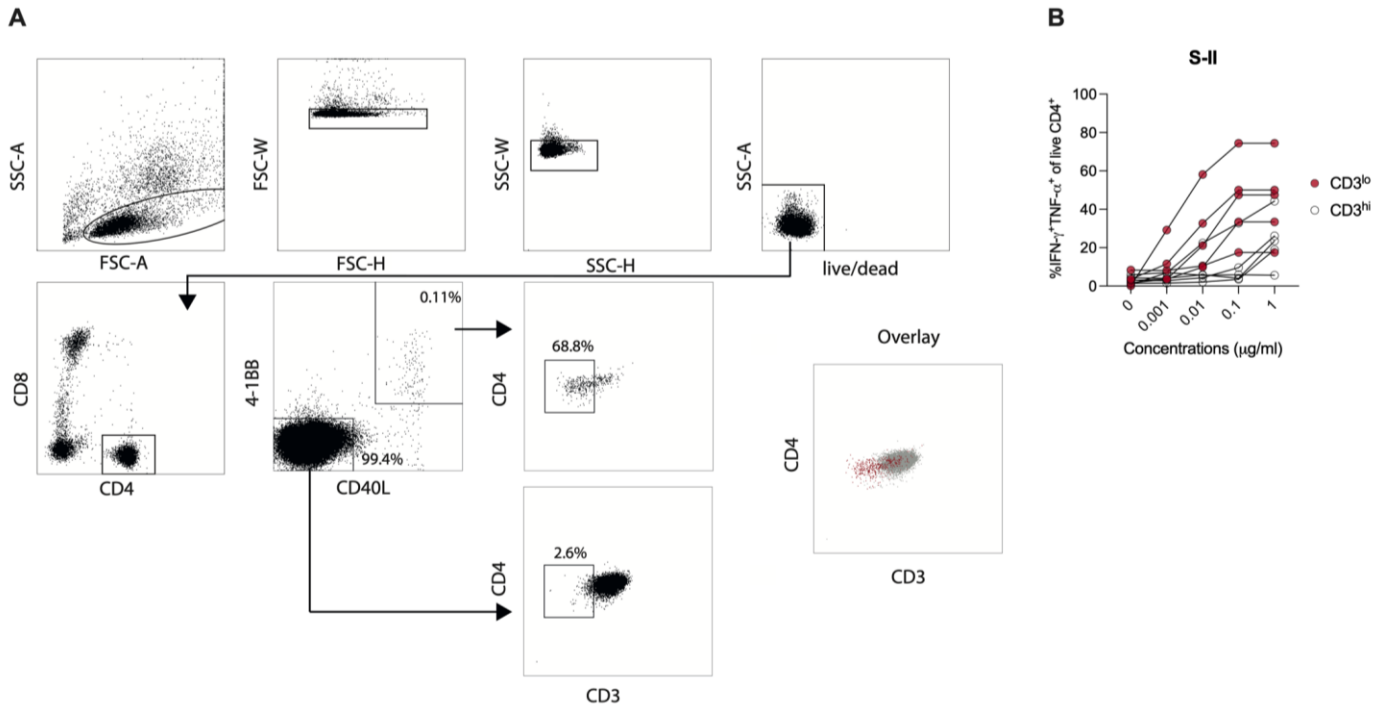
Fig. S3. CD3^{lo} gating in flow cytometry displays functional avidity.
(**A**) Representative gating strategy for CD3^{lo} cells within non-reactive CD4$^+$ T cells and S-II-reactive CD40L$^+$4-1BB$^+$ CD4$^+$ T cells. Cells were pre-gated on live, doublet-free CD4$^+$ lymphocytes. (**B**) S-II-reactive CD3^{lo} and CD3^{hi} CD40L$^+$41BB$^+$ CD4$^+$ T cells were sorted and expanded, respectively, and restimulated with indicated concentrations of S-II and the frequencies of IFN-γ^+TNF-α^+ CD4$^+$ T cells measured.

A

SARS-CoV-2 S-II pools

Y-axis: %CD40L+ 4-1BB+ CD4+ T cells (1 – 0.001)

Legend: Donor 1, Donor 2, Donor 3, Donor 4, Donor 5

X-axis: unstim, SARS2 S-I, SARS2 S-II, CMV, SEB, Pool27, Pool28, Pool29, Pool30, Pool31, Pool32, Pool33, Pool34, Pool35, Pool36, Pool37, Pool38, Pool39, Pool40, Pool41, Pool42, Pool43, Pool44, Pool45, Pool46, Pool47, Pool48, Pool49, Pool50, Pool51, Pool52

B

SARS-CoV-2 S-II peptides

Y-axis: %CD40L+ 4-1BB+ CD4+ T cells (100 – 0.1)

Legend: Donor 1, Donor 2, Donor 3, Donor 4, Donor 5

X-axis: No APC, Unstim, SARS2 S-I, SARS2 S-II, 229E S-I, 229E S-II, OC43 S-I, OC43 S-II, CMV, SEB, unspecific peptide 284, peptide 186, peptide 188, peptide 189, peptide 191, peptide 192, peptide 198, peptide 199, peptide 200, peptide 201, peptide 202, peptide 204, peptide 205, peptide 240, peptide 243, peptide 244, peptide 251, peptide 253, peptide 254, peptide 256, peptide 257

C

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                    1100       1120       1140       1160
                 *          *          *          *          *          *
NL63     : KQYTSACKTIEDALRLSAHLETNDVSSVLTFDSNAFSLAN----VTSFGD-YNLSSVIPQRNIHSSRIAGRSALEDLLFSKVVTSGLGT : 888
229E     : KQYTSACKTIEDALRNSARLESADVSEMLTFDKKAFTLAN----VSSFGD-YNLSSVIPSLPTSSGRVAGRSAIEDILFSKLVTSGLGT : 707
HKU1     : SEYGATGCDNHNSILNEVNDLDITQLCVANALMQGVTLSSNLNTNHSDVDNIDFKSLGCLGSQCGS-SSRSLLEDLLFNKVKLSDVGF   : 918
OC43     : VEYGSCGCDNHNAILTEVNELLDTTQLCVANSLMNGVTLSTKLKDGVNFNVDDINGLGCLGSCKASSRSAIEDLLFDKVKLSDVGF    : 921
MERS     : LQYGSFCTQLNRALTGIAAEQDRNTREVFAQVKQMYKTPT-----LKYFGGF-NFSQILP-DP---LKPTKRSFIEDLLFNKVTLADAGY : 905
SARS     : LQYGSFCTQLNRALSGIAAEQDRNTREVFAQVKQIYKTPP-----IKDFGGF-NFSQILP-DP---SKPSKRSFIEDLLFNKVTLADAGF : 815
SARS-CoV-2: LQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPP-----IKDFGGF-NFSQILP-DP---SKPSKRSFIEDLLFNKVTLADAGF : 833
           2Y  C  6  L              6         1         RS 6ED6LF K6      G
```

D

Y-axis: CD40L+ 4-1BB+ CD4+ SI (100 – 0.1)

Legend: Unexposed, COVID-19

Significance bars: ns, ns, ns, ns, *, *, **

X-axis: peptide 204, peptide 204_1, peptide 204_2, peptide 204_3, peptide 205, peptide 284, peptide 204, peptide 204_1, peptide 204_2, peptide 204_3, peptide 205, peptide 284

E

allele HLA-DPA1*01:03/	length	peptide	adjusted rank
DPB1*04:01	14	SFIEDLLFNKVTLA	0,8
DPB1*04:01	13	SFIEDLLFNKVTL	0,83
DPB1*04:01	11	SFIEDLLFNKV	0,97
DPB1*04:01	15	SFIEDLLFNKVTLAD	1,2
DPB1*04:02	14	SFIEDLLFNKVTLA	1,29
DPB1*04:01	13	SFIEDLLFNKVTL	1,37
DPB1*04:01	12	SFIEDLLFNKVT	1,6
DPB1*04:02	15	SFIEDLLFNKVTLAD	1,8
DPB1*04:02	11	SFIEDLLFNKV	1,83
DPB1*04:01	13	FIEDLLFNKVTLA	2,34
DPB1*04:02	12	SFIEDLLFNKVT	2,4
DPB1*04:01	14	FIEDLLFNKVTLAD	2,58
DPB1*04:01	12	FIEDLLFNKVTL	2,91
DPB1*04:01	14	FIEDLLFNKVTLAD	3,74
DPB1*04:02	14	FIEDLLFNKVTLAD	3,88
DPB1*04:01	11	FIEDLLFNKV	4,4
DPB1*04:02	12	FIEDLLFNKVTL	5,34
DPB1*04:01	11	FIEDLLFNKVT	7,43
DPB1*02:01	14	SFIEDLLFNKVTLA	8,18
DPB1*02:01	15	SFIEDLLFNKVTLAD	9,2
DPB1*02:01	13	SFIEDLLFNKVTL	9,82
DPB1*04:01	13	IEDLLFNKVTLA	11,54
DPB1*02:01	12	SFIEDLLFNKVT	14,53
DPB1*02:01	14	FIEDLLFNKVTLAD	15,08
DPB1*04:01	12	IEDLLFNKVTLA	15,12
DPB1*04:01	13	IEDLLFNKVTLAD	15,28
DPB1*02:01	11	SFIEDLLFNKV	16
DPB1*02:01	13	FIEDLLFNKVTLA	18,71
DPB1*02:01	13	FIEDLLFNKVTLAD	18,71
DPB1*04:02	12	IEDLLFNKVTLA	19,57

F

229E

allele DPA1*01:03/	length	peptide	adjusted rank
DPB1*04:02	14	SAIEDILFSKLVTS	2,58
DPB1*04:01	14	SAIEDILFSKLVTS	2,69
DPB1*04:02	15	SAIEDILFSKLVTSG	3,4
DPB1*04:01	13	SAIEDILFSKLVT	3,59
DPB1*04:01	15	SAIEDILFSKLVTSG	3,8
DPB1*04:01	13	SAIEDILFSKLVT	4,21
DPB1*04:01	13	AIEDILFSKLVTS	4,68
DPB1*04:01	14	AIEDILFSKLVTSG	4,74
DPB1*04:02	14	AIEDILFSKLVTSG	4,74
DPB1*04:02	12	SAIEDILFSKLV	4,74
DPB1*02:01	14	SAIEDILFSKLVTS	4,85
DPB1*02:01	13	SAIEDILFSKLVT	4,99
DPB1*02:01	15	SAIEDILFSKLVTSG	5,9
DPB1*04:01	12	SAIEDILFSKLVT	6,52
DPB1*04:02	12	SAIEDILFSKLV	6,52
DPB1*04:02	13	SAIEDILFSKLVT	6,55
DPB1*04:01	11	AIEDILFSKLV	8
DPB1*04:02	12	SAIEDILFSKLVT	8,9
DPB1*02:01	14	AIEDILFSKLVTSG	9,05
DPB1*02:01	11	SAIEDILFSKL	9,14
DPB1*02:01	12	SAIEDILFSKLV	9,19
DPB1*04:01	13	IEDILFSKLVTSG	9,51
DPB1*02:01	13	AIEDILFSKLVTS	9,67
DPB1*04:01	13	AIEDILFSKLVTS	9,98
DPB1*04:01	12	IEDILFSKLVTS	10,97
DPB1*02:01	13	SAIEDILFSKLVTSG	11,7
DPB1*04:02	12	IEDILFSKLVTS	11,86
DPB1*04:02	11	AIEDILFSKLV	12,57
DPB1*02:01	11	SAIEDILFSKL	14,28
DPB1*04:02	11	SAIEDILFSKL	14,28
DPB1*02:01	12	IEDILFSKLVTS	14,83
DPB1*04:01	11	IEDILFSKLVT	16,57

NL63

allele DPA1*01:03/	length	peptide	adjusted rank
DPB1*04:01	14	SALEDLLFSKVVTS	3,77
DPB1*04:02	14	SALEDLLFSKVVTS	4,2
DPB1*04:01	13	SALEDLLFSKVVT	4,21
DPB1*04:01	15	SALEDLLFSKVVTSG	5,1
DPB1*04:02	14	SALEDLLFSKVVTSG	5,4
DPB1*04:01	13	SALEDLLFSKVVT	5,46
DPB1*04:01	12	SALEDLLFSKVV	5,63
DPB1*04:01	13	ALEDLLFSKVVTSG	7,02
DPB1*04:01	14	SALEDLLFSKVVTSG	7,11
DPB1*04:01	12	SALEDLLFSKVV	7,41
DPB1*04:02	14	ALEDLLFSKVVTSG	7,86
DPB1*04:01	13	ALEDLLFSKVVTS	8,11
DPB1*02:01	14	SALEDLLFSKVVTS	8,83
DPB1*04:02	12	ALEDLLFSKVV	9,49
DPB1*02:01	13	SALEDLLFSKVVT	10,14
DPB1*04:01	11	ALEDLLFSKV	10,28
DPB1*04:01	14	SALEDLLFSKVV	10,28
DPB1*02:01	15	ALEDLLFSKVVT	11
DPB1*04:02	12	ALEDLLFSKVVT	13,94
DPB1*02:01	12	SALEDLLFSKVV	14,23
DPB1*02:01	14	ALEDLLFSKVVTSG	15,08
DPB1*04:01	11	ALEDLLFSKVV	17,14
DPB1*02:01	13	ALEDLLFSKVVTS	17,15
DPB1*04:01	13	LEDLLFSKVVTSG	17,15
DPB1*04:02	11	SALEDLLFSKV	17,71
DPB1*04:02	13	LEDLLFSKVVTSG	18,71
DPB1*04:01	12	LEDLLFSKVVTS	19,57

OC43

allele DPA1*01:03/	length	peptide	adjusted rank
DPB1*04:01	14	SAIEDLLFDKVKLS	6,25
DPB1*04:01	13	SAIEDLLFDKVKL	7,8
DPB1*04:01	15	SAIEDLLFDKVKLSD	8,7
DPB1*04:01	14	AIEDLLFDKVKLSD	9,51
DPB1*04:01	14	SAIEDLLFDKVKLS	9,58
DPB1*04:02	14	SAIEDLLFDKVKLS	10,01
DPB1*04:02	13	SAIEDLLFDKVKL	12,79
DPB1*04:02	14	AIEDLLFDKVKLSD	14
DPB1*04:01	13	AIEDLLFDKVKLS	14
DPB1*04:01	14	AIEDLLFDKVKLSD	14,66
DPB1*02:01	15	SAIEDLLFDKVKLSD	15
DPB1*04:01	15	SAIEDLLFDKVKLSD	15,08
DPB1*04:01	12	AIEDLLFDKVKL	15,72
DPB1*04:01	13	IEDLLFDKVKLS	17,15
DPB1*04:01	13	SAIEDLLFDKVK	18,68
DPB1*02:01	13	SAIEDLLFDKVKL	18,71

HKU1

allele DPA1*01:03/	length	peptide	adjusted rank
DPB1*04:01	13	SLLEDLLFNKVKL	2,03
DPB1*04:01	14	SLLEDLLFNKVKLS	2,05
DPB1*04:02	14	SLLEDLLFNKVKLS	2,91
DPB1*04:01	15	SLLEDLLFNKVKLSD	3
DPB1*04:02	13	SLLEDLLFNKVKL	3,12
DPB1*04:02	15	SLLEDLLFNKVKLSD	4
DPB1*04:01	13	LLEDLLFNKVKLS	4,05
DPB1*04:01	12	SLLEDLLFNKVKL	4,15
DPB1*04:01	14	LLEDLLFNKVKLSD	4,2
DPB1*04:01	11	SLLEDLLFNKV	4,23
DPB1*04:01	12	LLEDLLFNKVKL	5,04
DPB1*04:01	14	LLEDLLFNKVKLSD	5,93
DPB1*04:02	14	LLEDLLFNKVKLSD	6,14
DPB1*04:02	13	LLEDLLFNKVKL	6,23
DPB1*04:02	11	SLLEDLLFNKV	7,43
DPB1*02:01	14	SLLEDLLFNKVKLS	7,65
DPB1*04:02	12	LLEDLLFNKVKL	7,71
DPB1*02:01	13	SLLEDLLFNKVKL	8,1
DPB1*02:01	15	SLLEDLLFNKVKLSD	8,58
DPB1*04:01	11	LLEDLLFNKVK	9,14
DPB1*04:01	14	LEDLLFNKVKLSD	11,38
DPB1*04:01	15	LLEDLLFNKVKLSD	14
DPB1*04:01	12	LEDLLFNKVKLS	14,53
DPB1*04:02	11	LLEDLLFNKVK	14,85
DPB1*04:02	13	LLEDLLFNKVKLSD	17,15
DPB1*04:02	13	LLEDLLFNKVKLSD	17,15
DPB1*02:01	13	LLEDLLFNKVKLS	18,71

Fig. S4. Identification of S816-830 by narrowing down the sequence regions mapped in SARS-CoV-2 peptide pools that exhibited highest T cell cross-reactivity.

(A) Expanded SARS-CoV-2 S-I- or S-II specific CD40L$^+$4-1BB$^+$CD4$^+$ short-term-culture T cells were restimulated with different matrix pools in the presence autologous feeder cells and the frequency of CD40L$^+$4-1BB$^+$ reactive cells determined. The cut-off is set at 0.01 based on unstimulated controls. **(B)** Expanded SARS-CoV-2 S-I- or S-II reactive CD40L$^+$4-1BB$^+$CD4$^+$ T cells were restimulated with single peptide candidates determined from matrix stimulation (shown in A) in the presence of autologous feeder cells and the frequency CD40L$^+$4-1BB$^+$ reactive CD4$^+$ T cells was determined. The cut-off is set at 0.5 based on unstimulated controls. **(C)** Position of single peptides 204 and 205 (yellow) in sequence overlay of the known 7 human coronaviruses. Color code indicates homology: black: identical amino acid; gray: conservative amino acid replacement. **(D)** Identification of the dominant peptide at the 204 to 205 sequence intersection. The number behind the underline character indicates the number of amino acids by which the 15-mer was shifted towards the C-terminus beginning with the sequence of 204 and ending with the sequence of 205. A representative selection of donors who displayed an SI≥3 in one or more stimulations is shown. **(E, F)** MHC-II HLA binding prediction of HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01:03/DPB1*04:01 and HLA-DPA1*01:03/DPB1*04:02 with S816-830 and derived fragments **(E)** or HCoV-derived homologous peptides and fragments thereof **(F)**.

A

B

229E NL63 OC43 HKU1

C

229E NL63 OC43 HKU1
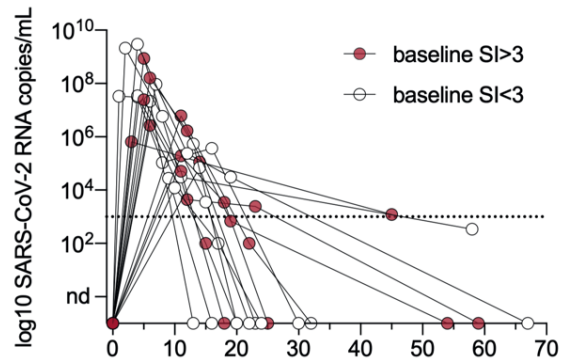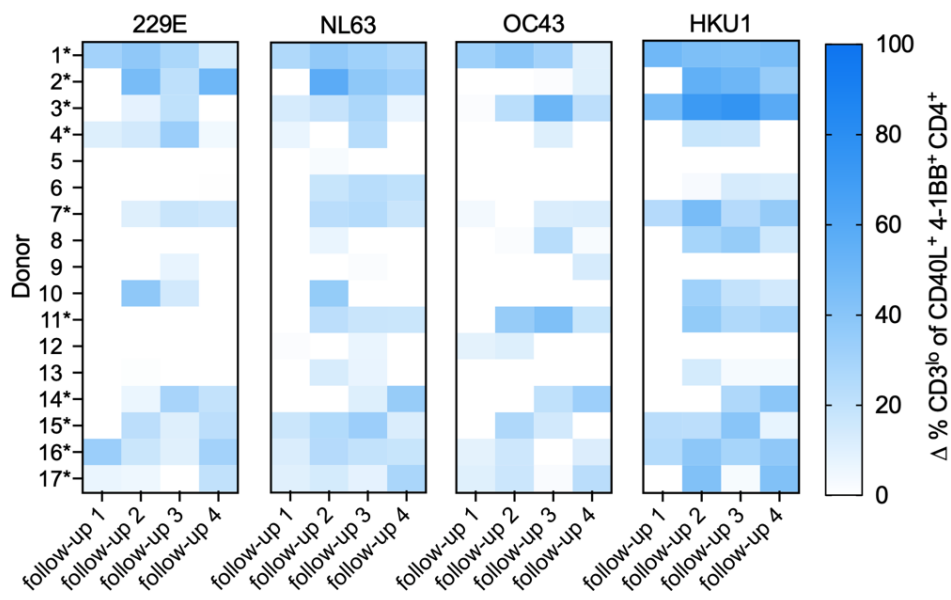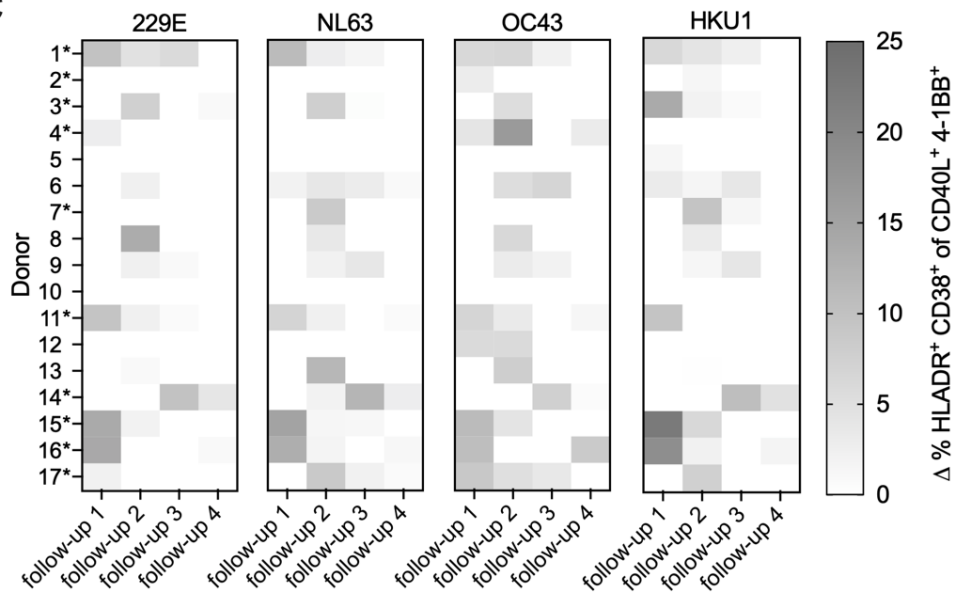
Fig. S5. SARS-CoV-2 RNA concentration kinetics and HCoV-reactive T cell characteristics upon SARS-CoV- infection.

(A) Viral RNA copies per mL of diluted swab material at indicated time points. The dotted line indicates the limit of quantification (nd = not detected). Donors with an SI≥3 for SARS-CoV-2 S-II at baseline are represented as red circles, individuals with an SI<3 are shown as white circles. **(B, C)** Heatmaps show the delta (Δ) frequency of CD3^{lo} **(B)** and the Δ frequency of HLADR$^+$CD38$^+$ cells **(C)** among CD40L$^+$4-1BB$^+$ CD4$^+$ T cells after stimulation with S-II pools of the indicated HCoVs. Δ represents the change of the parameter at the given time point relative to baseline (i.e., white depicts no increase). "*" identifies S816-830 peptide responders.
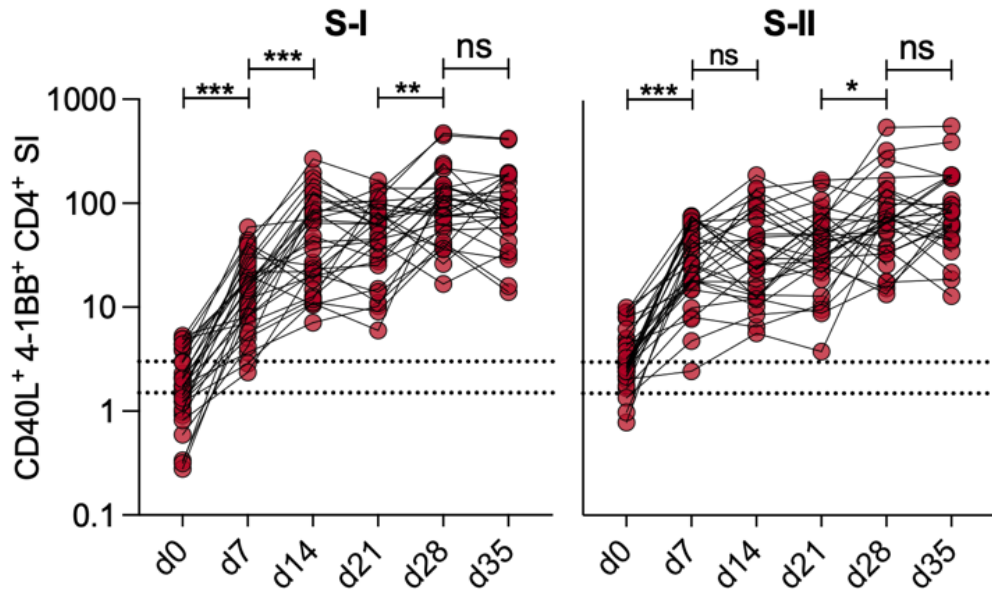
Fig. S6. Individual T cell responses to vaccination.

Response kinetics of CD40L$^+$4-1BB$^+$ CD4$^+$ T cells in individual vaccinees upon stimulation with SARS-CoV-2 S-I or S-II, as shown in Fig. 6B. *$P<0.05$, **$P<0.01$, ***$P<0.001$, and ns for $P>0.05$ (paired Student's *t* test). Secondary vaccination occurred on day 21.

Table S1. Donor characteristics.

Cohort	Subcohort	Number donors	Age mean (St.d.)	Age range	Gender (f/m)	ICU	Hospitalization	Mild/ asymptomatic	Measurement day p.s.o. mean (St.d.)	Measurement day p.s.o. range	Figure(s)
Orfeome											Fig. 1, S1
	COVID-19 convalescents	59	50.6 (15.0)	21-79	24/35	20	17	22	152 (57)	17-262	
	Unexposed	60	45.7 (16.1)	25-76	36/24	-	-	-	-	-	
Basic CCC											Fig. 2, 3, S3
	COVID-19 WHO 1-2	133	43.8 (13.6)	18-78	85/48	-	-	133	128 (74)	21-304	
	COVID-19 WHO 3-4	19	53.1 (12.8)	31-75	7/12	-	19	-	137 (41)	50-214	
	COVID-19 WHO 5-7	22	59.2 (13.3)	27-79	6/16	22	-	-	143 (59)	61-229	
	Unexposed	568	47.5 (11.8)	18-98	439/129	-	-	-	-	-	
Other pathogen pools											Fig. 2
	Young (<30)	20	23.5 (2.4)	20-29	11/9	-	-	-	-	-	
	Old (>65)	19	70.4 (3.8)	65-76	11/8	-	-	-	-	-	
Follow-up											Fig. 5
	COVID-19 convalescents	17	39.2 (10.8)	22-58	11/6			17	see Table 2	see Table 2	
Vaccination											
	Volunteers	31	40.9 (11.5)	20-67	12/17	-	-	-	-	-	Fig. 6, S5

p.s.o.=post symptom onset

Table S2. Characteristics of the follow-up donors.

Donor	Gender	Age	BMI (kg/m²)	Symptoms	Comorbidities	BL %CD4+	BL S-I SI	BL S-II SI	day BL	day FU1	day FU2	day FU3	day FU4
A	m	30	22.9	Fatigue. headache. cough. fever. nausea	Allergies. asthma	48.0	1.1	5.0	-76	6	12	19	54
B	f	47	33.2	Cough. loss of taste and smell. body ace	Allergies	53.8	0.5	1.6	-110	-	15	22	29
C	m	31	25.5	Fatigue. headache. cough. fever. loss of taste and smell. dermatological symptoms. body ace	-	47.8	3.1	1.9	-32	8	15	22	35
D	f	31	34.8	Fatigue. headache. cough. fever. loss of taste and smell. nausea. diarrhea. body ache	Allergies. migraine. bronchial hyperreactivity	55.6	2.0	2.1	-39	4	12	19	67
E	f	40	32	Fatigue. headache. cough. fever. loss of taste and smell. nausea. diarrhea. dermatological symptoms	Allergies. asthma. migraine. Hashimoto-Thyroiditis	61.1	1.6	1.3	-64	7	13	20	48
F	f	47	33.1	Cough. fever. nausea. diarrhea	-	73.4	3.5	6.4	-73	5	11	18	54
G	f	31	31.1	Fatigue. headache. cough. fever. body ace	Acne vulgaris. pseudotumor cerebri	66.0	1.1	1.3	-82	4	11	18	58
H	f	31	27.1	Fatigue. headache. loss of taste and smell. body ace	Thyroidectomy	67.0	2.9	0.8	-38	-	10	24	53
I	f	22	28.5	Fatigue. headache. cough. fever. loss of taste and smell. nausea. diarrhea. dermatological symptoms. body ace	Gastritis	60.3	2.5	5.9	-37	-	11	25	54
J	f	37	20.3	Fatigue. headache. cough. loss of taste and smell. nausea	Allergies	56.4	1.0	1.4	-19	-	14	23	71
K	m	52	29.2	Fatigue. headache. cough. fever. body ace	Allergies	66.8	4.0	5.0	-39	-	11	22	58
L	f	55	37.7	Fatigue. headache. cough. fever. loss of taste and smell. nausea. dermatological symptoms. body ace	Allergies. asthma. hypertonia	78.8	2.3	2.7	-13	9	16	23	57
M	m	58	24.2	Headache. fever. loss of taste and smell	Cardiac arrhythmia	56.9	0.3	3.0	-82	5	12	23	56
N	m	53	23.5	Fatigue. headache. cough. loss of taste and smell. body ace	-	66.4	4.3	5.8	-56	3	14	-	45
O	f	40	22.1	Fatigue. headache. fever. diarrhea. dermatological symptoms. body ace	-	59.7	1.2	2.3	-84	6	13	20	42
P	f	31	21.1	Fatigue. headache. loss of smell. dermatological symptoms. body ace	-	61.4	3.3	3.8	-111	7	15	20	42
Q	m	31	24.2	Loss of taste and smell	Arterial hypertonia	53.0	1.0	2.0	-68	8	16	30	66

BL=baseline, FU=follow-up. None of the donors required hospitalization.

Table S3. List of antibodies used for flow cytometric analyses.

Marker	Fluorochrome	Clone	Manufacturer	RRID	Titration
CD3	FITC	REA613	Miltenyi	AB_2725966	1:50
CD4	VioGreen	REA623	Miltenyi	AB_2726041	1:50
CD8	VioBlue	REA734	Miltenyi	AB_2659239	1:50
HLA-DR	PerCpVio700	REA805	Miltenyi	AB_2652172	1:50
CD38	APC	REA671	Miltenyi	AB_2726164	1:100
CD40L	PEVio770	REA238	Miltenyi	AB_2751209	1:50
4-1BB	PE	REA765	Miltenyi	AB_2654986	1:50
IFN-g	A700	B27	Biolegend	AB_961351	1:100
TNF-a	BV605	MAb11	Biolegend	AB_11203719	1:100