

Supplemental information

Generation of glucocorticoid-resistant

SARS-CoV-2 T cells for adoptive cell therapy

Rafet Basar, Nadima Uprety, Emily Ensley, May Daher, Kimberly Klein, Fernando Martinez, Fleur Aung, Mayra Shanley, Bingqian Hu, Elif Gokdemir, Ana Karen Nunez Cortes, Mayela Mendt, Francia Reyes Silva, Sunil Acharya, Tamara Laskowski, Luis Muniz-Feliciano, Pinaki P. Banerjee, Ye Li, Sufang Li, Luciana Melo Garcia, Paul Lin, Hila Shaim, Sean G. Yates, David Marin, Indreshpal Kaur, Sheetal Rao, Duncan Mak, Angelique Lin, Qi Miao, Jinzhuang Dou, Ken Chen, Richard E. Champlin, Elizabeth J. Shpall, and Katayoun Rezvani

Supplemental Information

Supplemental Figures

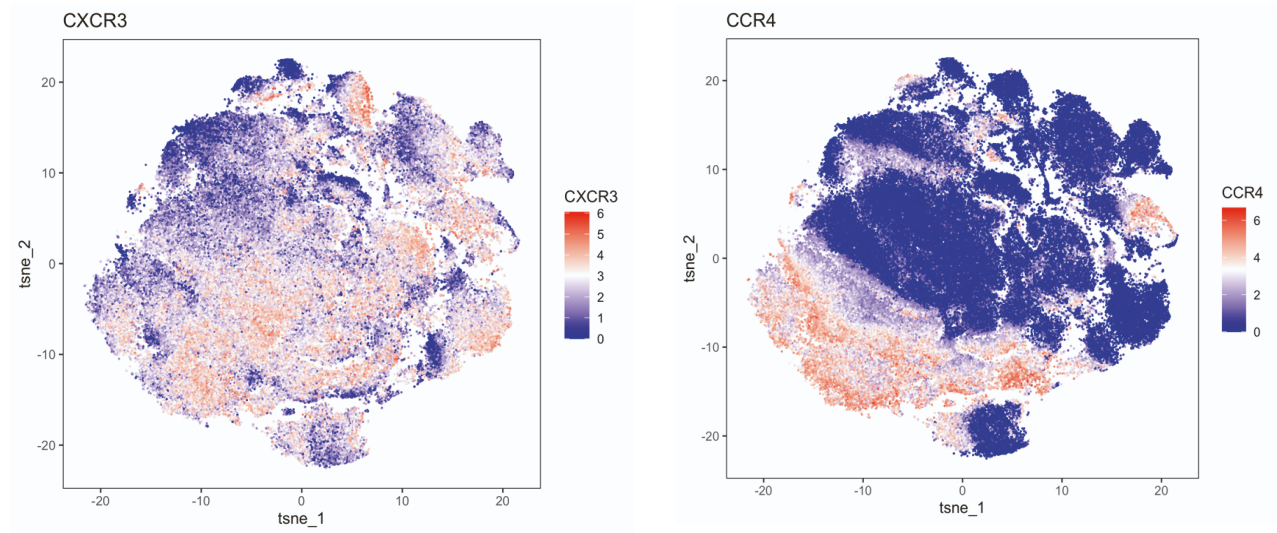


Figure S1. SARS-CoV-2 CTL's express chemokine receptors that support trafficking to the lungs, related to Figure 1. T-SNE plots showing expression of chemokine receptors that support trafficking to the lungs in SARS-CoV-2 CTL's, left panel showing CXCR3 and right panel showing CCR4.

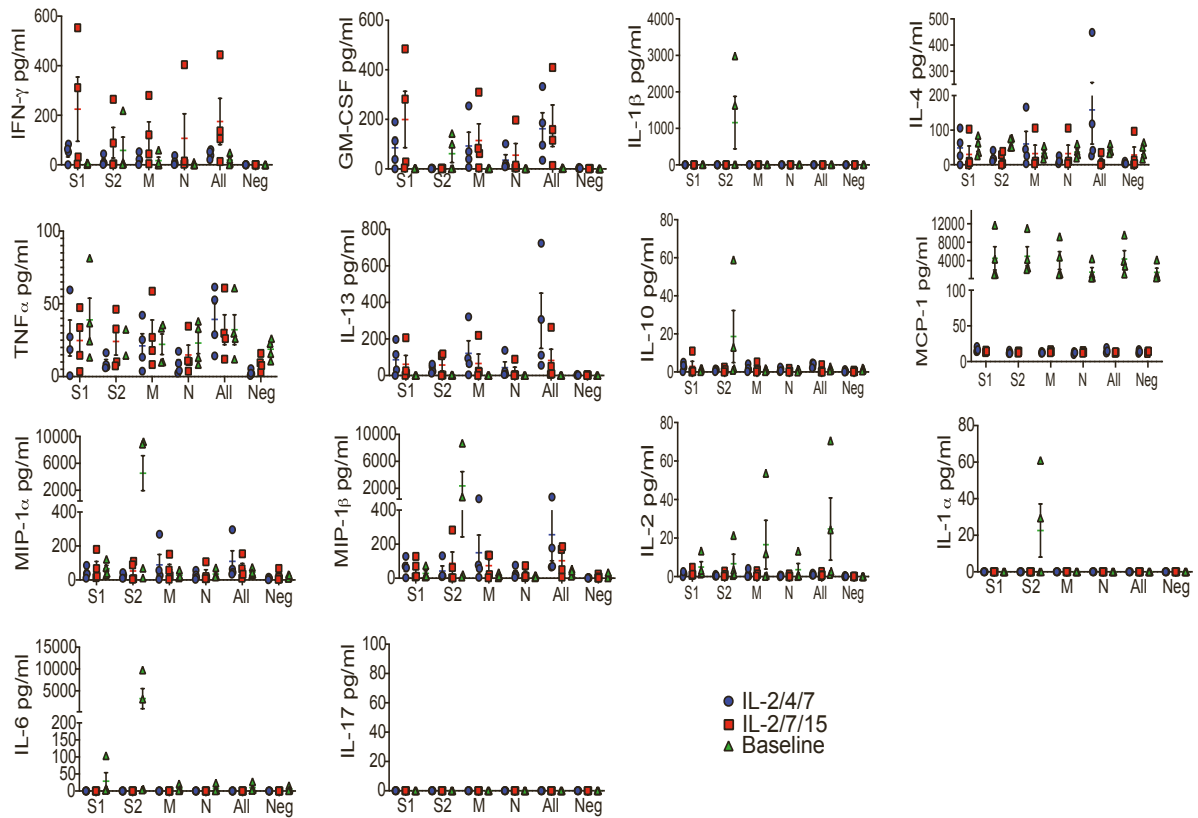


Figure S2. Cytokine profile from COVID-19 reactive T cells supports a functional profile without CRS, related to Figure 1. Multiplex cytokine analysis showing the concentration of different cytokines in pg/ml detected in supernatants from COVID-19 reactive T cells stimulated with the different peptide libraries (S1, S2, M and N, either separately or in combination) at baseline (green triangles) or after expansion with IL-2/4/7 (blue circles) or IL-2/7/15 (red squares). n=4 samples per group. Neg refers to negative control without peptide stimulation.

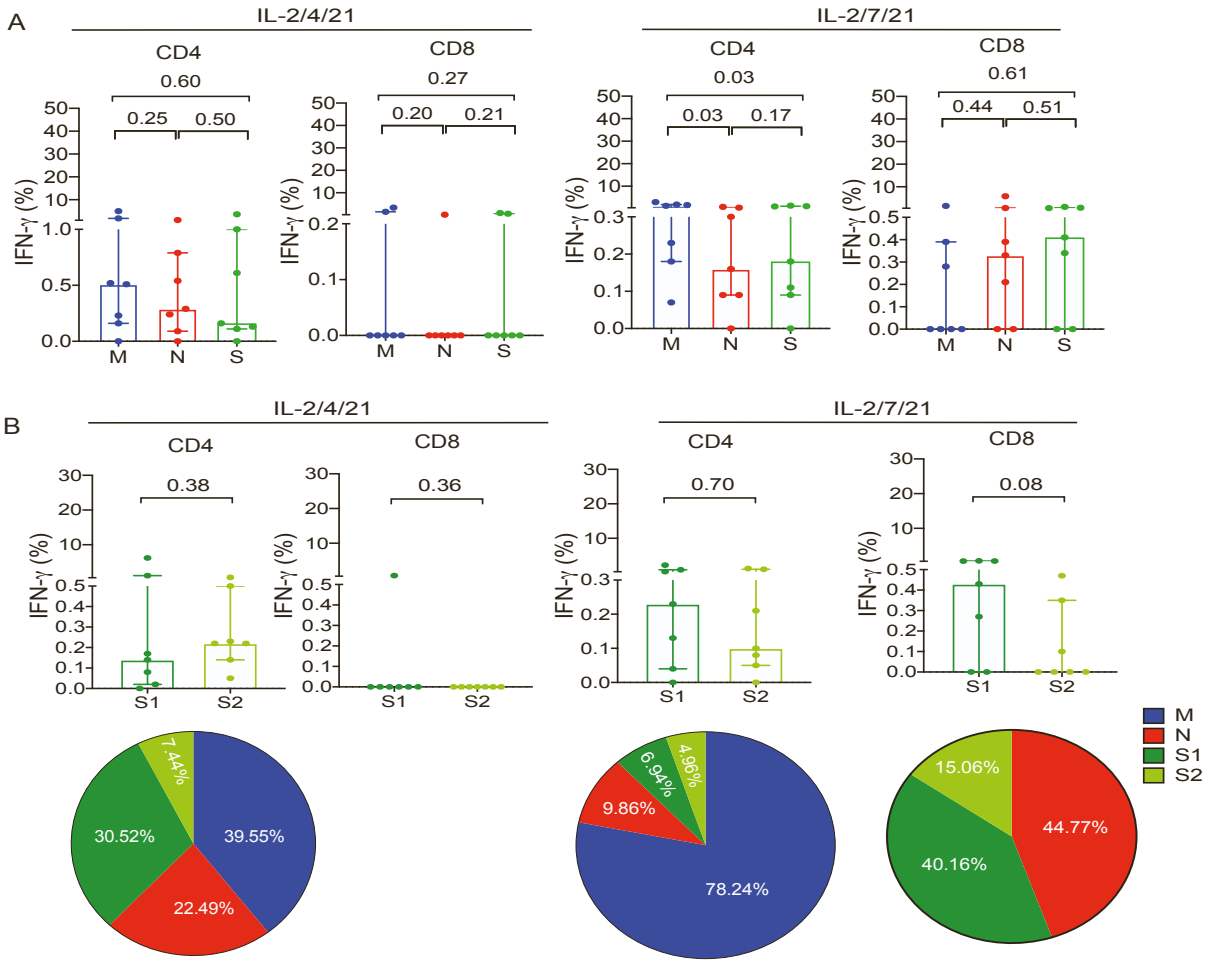


Figure S3. Expanded COVID-19 CTLs are directed against structural proteins, including both the C and N terminals of the S protein, related to Figure 3. **A**, Percentage of IFN- γ (+) COVID-19 reactive CD3⁺ T cells stimulated with the peptide libraries derived from the different structural proteins M (blue), N (red), S (green) cultured with different cytokine cocktails IL2/4/21 (left panel) or IL2/7/21 (right panel). **B**, Quantification of IFN- γ (+) COVID-19 reactive T cells subsets (CD4⁺ or CD8⁺) directed against N-terminus (S1, dark green) or the C-terminus (S2, light green) of the S protein in both IL-2/4/21 (left panels) and IL-2/7/21 (right panels) stimulation conditions (n=7 samples per group). Bars represent median values with interquartile range. p-values are indicated at the top of each graph. Corresponding pie charts showing the percent distribution of M (blue), N (red), S1 (dark green) and S2 (light green) reactive IFN- γ (+) T cells are depicted under each bar graph. Please note that a pie chart was not generated for the CD8⁺ T cells cultured with IL-2/4/21 due to the very low number of cells. Inset percentages (%) within each pie chart represent the fraction of IFN- γ (+) T cells that are reactive to specific peptides. Sum of all fractions is 100% representing the total IFN- γ (+) T cells. Statistical analysis by paired t-test (A, B).

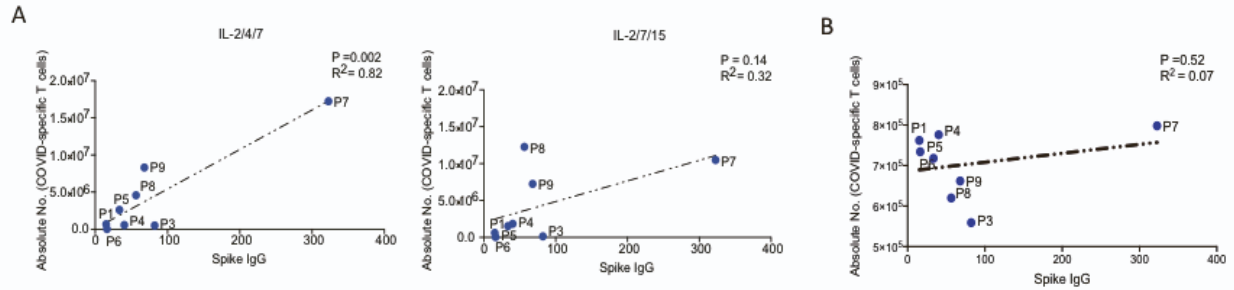


Figure S4. Absolute number of COVID-19 specific T cells cultured with IL-2/4/7 correlates with antibody titer of Spike protein IgG, related to Figure 3. A. Scatter plots showing correlation between absolute number of COVID-19 specific T cells (on Y axis) and antibody titers on the X axis (Spike IgG) of COVID-19 specific T cells expanded with IL-2/4/7 (left panel) or IL-2/7/15 (right panel). **B.** Scatter plot showing no correlation between absolute number of COVID-19 specific T cells (on Y axis) and antibody titers on the X axis (Spike IgG) of COVID-19 specific T cells prior to expansion. Statistical analysis by simple linear regression analysis (A, B).

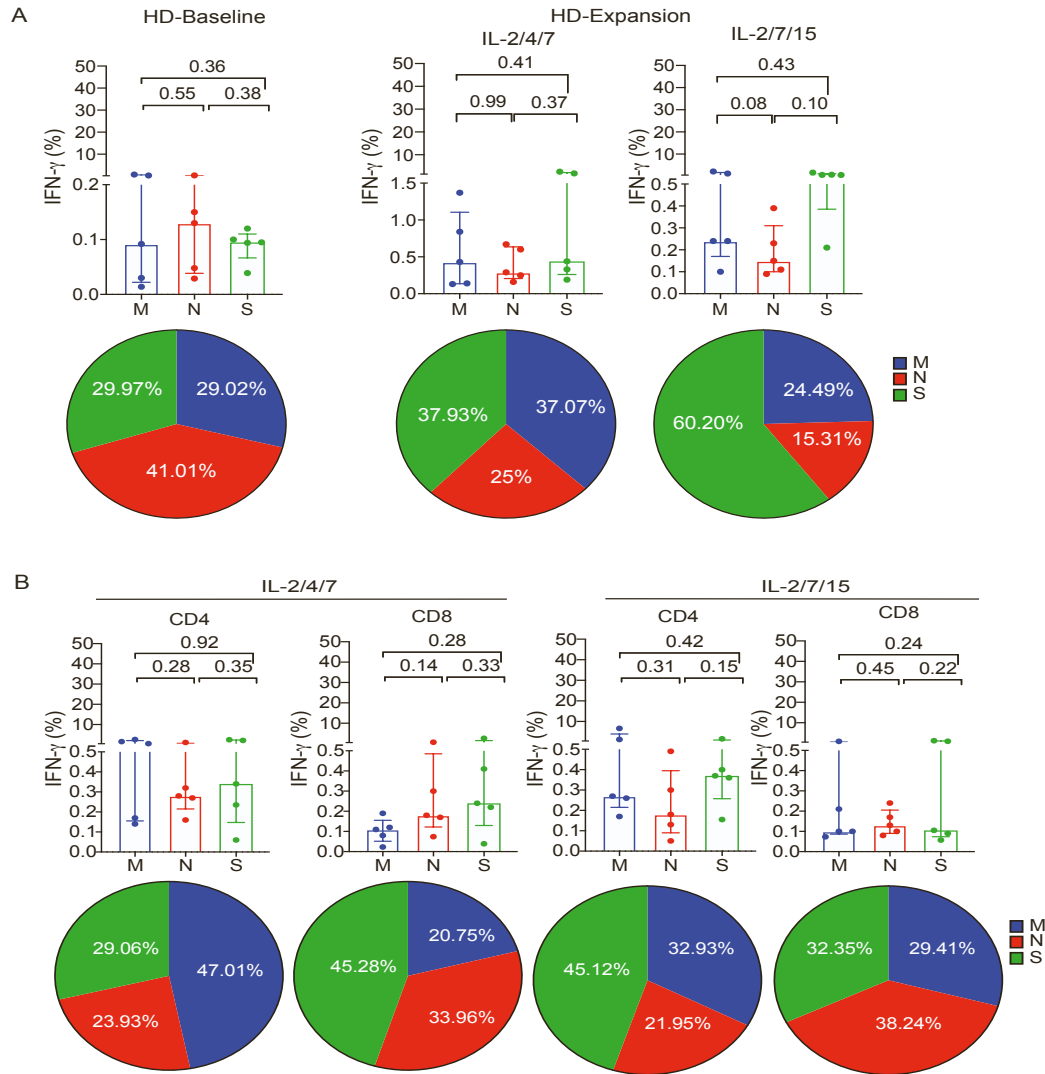


Figure S5. COVID-19 CTLs can be expanded from the PB of healthy donors but at lower frequencies compared to COVID-19-recovered donors, related to Figure 4. **A, B** Graphical analysis of IFN- γ (+) COVID-19 reactive CD3+ T cells from healthy donors stimulated with the peptide libraries derived from the different structural proteins M (blue), N (red), S (green) at **(A)** baseline or **(B)** cultured with IL-2/4/7 (left panel) or IL-2/7/15 (right panel). Corresponding pie charts show the distribution of M (blue), N (red) or S (green) reactive IFN- γ (+) T cells for each group (n=5 samples per group). Bars represent median values with interquartile range. p-values are indicated at the top of each graph. **C**, Percentage of IFN- γ (+) COVID-19 reactive CD4 and CD8 T cells generated from PB of healthy donors stimulated with the peptide libraries derived from the different structural proteins M (blue), N (red), S (green) after expansion with IL-2/4/7 (left panels) or IL-2/7/15 (right panels). Distribution of M (blue), N (red) or S (green) reactive IFN- γ (+) T cells for each group is shown in pie charts below corresponding graphs (n=5 samples per group). Bars represent median values with interquartile range. p-values are indicated at the top of each graph. Inset percentages (%) within each pie chart represent the fraction of IFN- γ (+) T cells that are reactive to specific peptides. Sum of all fractions is 100% representing the total IFN- γ (+) T cells. Statistical analysis by paired t-test (A, B).

Supplemental Tables

Table S1. Mass cytometry panel used for phenotyping of COVID-19 reactive T cells, related to Figure 1.

Tag	Antibody	Clone	Source
89Y	CD45	HI30	Fluidigm
141Pr	CCR6	G034E3	Fluidigm
142Nd	IL4	MP425D2	Fluidigm
143Nd	CD4	OKT4	Biolegend
144Nd	IL2	MQ1-17H12	BD Biosciences
145Nd	CD62L	DREG-56	Biolegend
146Nd	CD8	RPA-T8	Biolegend
147Sm	CD127	MB15-18C9	Miltenyi Biotec
148Nd	IL17a	BL168	Biolegend
149Sm	CD25	2A3	BD Biosciences
150Nd	CD28	CD28.2	BD Biosciences
151Eu	CD107a	H4A3	BD Biosciences
152Sm	CD95	DX2	BD Biosciences
153Eu	CCR4	205410	R&D Systems
154Sm	TIGIT	MSA43	Thermo Fisher
155Gd	CD27	M-T271	BD Biosciences
156Gd	CXCR4	12G5	Biolegend
158Gd	OX40	ACT35	Biolegend
159Tb	Perforin	delta G9	Miltenyi Biotec
160Gd	CD45RA	HI100	Biolegend
161Dy	TIM3	REA635	Miltenyi Biotec
162Dy	Ki-67	Ki-67	Biolegend
163Dy	CXCR3	G025H7	Biolegend
164Dy	CD45RO	UCHL1	Biolegend
165Ho	CTLA-4	L3D10	Biolegend
166Er	MIP1b	W15138A	Biolegend
167Er	CCR7	G043H7	Biolegend
168Er	IFNg	B27	Biolegend
169Tm	TNFa	MAb11	Biolegend
170Er	HLA-DR	L243	Biolegend
171Yb	CD161	191B8	Miltenyi
172Yb	KLRG1	13F12F2	Thermo Fisher
173Yb	Granzyme B	GB11	Fluidigm
174Yb	PD1	PD1.3.1.3	Miltenyi Biotec
175Lu	LAG3	11C3C65	Biolegend
176Yb	CD38	REA572	Miltenyi Biotec
209Bi	41BB	4B4-1	Biolegend
115In	CD57	HCD57	Biolegend
Pt194	CD3	UCTH1	Biolegend
Pt198	LD		

Table S2. Cytokine production and fold expansion of COVID-19 reactive T cells derived from the different recovered donors and expanded with IL-2/4/7 or IL-2/7/15 cytokine cocktails, related to Figure 1.

			IL-2/4/7								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
Patient 1	177.37	IFN- γ	5.59	6.44	14.80	7.90	8.29	20.10	0.43	2.62	1.40
		IL-2	0.92	0.91	2.31	0.45	0.47	1.82	0.81	0.71	0.79
		TNF- α	3.18	2.67	6.52	3.14	3.10	7.12	1.45	1.13	2.03
Patient 3	506.42	IFN- γ	1.22	17.10	13.50	0.91	18.90	15.00	3.77	0.81	1.25
		IL-2	0.14	1.85	1.07	0.14	2.09	1.21	0.09	0.34	0.34
		TNF- α	0.67	7.50	3.99	0.57	8.41	4.59	0.66	0.00	0.11
Patient 4	540.69	IFN- γ	2.74	10.20	3.84	2.51	9.01	3.24	0.21	5.08	0.32
		IL-2	0.43	1.40	0.94	0.36	1.09	0.80	0.17	0.41	0.32
		TNF- α	1.66	5.35	3.23	1.63	5.06	3.26	1.05	3.01	2.42
Patient 5	897.59	IFN- γ	2.94	2.87	10.90	2.70	2.41	10.50	1.06	19.40	0.61
		IL-2	0.21	0.32	0.54	0.23	0.31	0.53	0.00	0.24	0.20
		TNF- α	1.23	1.18	4.42	1.30	1.26	4.64	0.53	3.07	0.61
Patient 6	7.16	IFN- γ	0.11	0.12	0.21	0.14	0.14	0.22	0.10	0.05	0.25
		IL-2	0.21	0.23	0.20	0.16	0.18	0.16	0.23	0.16	0.22
		TNF- α	0.19	0.22	0.25	0.05	0.04	0.07	0.10	0.12	0.32
Patient 7	45572.50	IFN- γ	33.60	9.81	10.30	33.40	9.31	10.80	42.00	19.40	3.99
		IL-2	1.41	1.10	0.97	1.44	1.11	0.99	0.61	0.43	0.22
		TNF- α	12.90	5.36	5.43	12.90	5.20	5.53	4.36	3.70	0.45
Patient 8	2645.58	IFN- γ	6.81	3.33	4.87	5.77	3.46	5.48	25.90	4.53	0.27
		IL-2	0.92	0.52	0.67	0.58	0.35	0.40	1.20	0.57	0.68
		TNF- α	2.80	0.87	1.71	2.64	0.88	1.81	5.65	0.97	0.68
Patient 9	3537.23	IFN- γ	18.90	3.51	12.50	17.50	2.77	12.70	19.00	14.00	1.02
		IL-2	3.80	1.16	1.34	3.51	0.93	1.32	1.13	1.12	0.18
		TNF- α	12.50	2.35	7.86	12.20	2.09	7.90	1.83	0.56	0.09
			IL-2/7/15								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
Patient 1	163.62	IFN- γ	1.65	3.92	1.96	2.12	4.65	2.24	0.24	1.63	2.65
		IL-2	1.08	1.56	1.64	0.49	0.81	0.92	0.38	0.23	1.75
		TNF- α	4.18	4.20	3.98	4.22	4.50	3.67	0.38	1.29	3.61
Patient 3	141.91	IFN- γ	0.37	1.40	2.83	0.44	1.51	3.10	0.35	0.26	0.12
		IL-2	0.15	0.47	0.45	0.20	0.53	0.51	1.27	0.90	1.19
		TNF- α	0.39	1.97	2.37	0.52	2.22	2.74	0.00	0.52	0.24
Patient 4	1746.55	IFN- γ	8.67	8.12	26.00	8.70	4.21	29.40	0.27	20.50	0.77
		IL-2	1.24	1.31	6.59	0.87	0.91	6.52	0.37	0.76	0.29
		TNF- α	3.93	3.60	10.10	3.99	2.41	11.20	0.69	7.27	0.76
Patient 5	530.27	IFN- γ	3.43	3.28	6.08	3.29	3.00	5.64	0.30	2.41	1.53
		IL-2	0.35	0.33	0.45	0.34	0.30	0.43	0.20	0.38	0.41
		TNF- α	1.40	1.40	2.31	1.52	1.46	2.47	0.91	1.25	0.82
Patient 6	15.97	IFN- γ	0.15	0.17	0.48	0.13	0.21	0.42	0.09	0.04	0.34
		IL-2	0.26	0.32	0.31	0.22	0.28	0.22	0.52	0.74	0.58
		TNF- α	0.24	0.48	0.78	0.10	0.60	0.33	0.09	0.10	0.28
Patient 7	27716.61	IFN- γ	20.80	5.36	5.51	34.20	7.19	9.23	7.35	3.84	1.58
		IL-2	0.98	0.59	0.65	1.64	0.85	0.95	0.26	0.26	0.27
		TNF- α	7.06	2.83	3.33	12.60	4.19	5.89	1.27	1.47	0.33
Patient 8	2912.07	IFN- γ	19.10	15.50	3.43	17.30	16.10	3.46	47.30	5.02	0.93
		IL-2	2.55	1.04	0.92	0.94	0.62	0.69	2.24	1.16	0.36
		TNF- α	4.90	1.70	1.91	3.90	1.43	1.82	18.50	1.45	1.14
Patient 9	3088.38	IFN- γ	17.80	0.90	8.86	18.00	0.78	9.89	5.45	6.26	1.36
		IL-2	2.12	0.40	0.93	2.08	0.36	0.91	0.33	0.49	0.45
		TNF- α	5.14	0.38	3.53	5.41	0.48	3.84	0.33	0.38	0.65

Percent IFN- γ , IL-2 and TNF- α production from total CD3+, and CD4+ and CD8+ subsets of COVID-19 reactive T cells derived from each recovered donor and stimulated with the peptide libraries derived from M, N and S structural proteins and fold expansion after culture with IL-2/4/7 or IL-2/7/15 cytokine cocktails.

Table S3. Cytokine production and fold expansion of COVID-19 reactive T cells derived from the different recovered donors and expanded with IL-2/4/21 or IL-2/7/21 cytokine cocktails, related to Figure 1.

			IL-2/4/21								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
Patient 3	0.57	IFN- γ	0.03	0.14	0.09	0.00	0.09	0.16	0.00	0.00	0.00
		IL-2	0.12	0.09	0.09	0.13	0.09	0.16	0.00	0.00	0.00
		TNF- α	0.10	0.14	0.14	0.06	0.00	0.12	0.00	0.00	0.00
Patient 4	0.53	IFN- γ	0.30	0.52	0.36	0.52	0.79	0.13	0.00	0.00	0.00
		IL-2	0.30	0.26	0.82	0.52	0.20	0.63	0.00	0.00	0.00
		TNF- α	0.40	0.17	0.21	0.26	0.00	0.13	0.00	7.69	0.00
Patient 5	82.96	IFN- γ	1.11	0.20	2.53	1.81	0.29	3.63	0.00	0.00	0.00
		IL-2	0.12	0.13	0.13	0.13	0.09	0.15	0.00	0.00	2.17
		TNF- α	0.66	0.09	1.66	0.87	0.13	2.36	0.00	0.00	0.00
Patient 6	0.08	IFN- γ	0.30	0.19	1.15	0.16	0.00	0.61	0.00	0.00	0.00
		IL-2	0.40	0.84	1.02	0.31	0.30	0.81	0.00	0.00	0.00
		TNF- α	0.90	0.47	0.00	0.47	0.45	0.00	3.70	0.00	0.00
Patient 7	996.18	IFN- γ	2.46	0.64	0.55	4.99	1.08	1.00	3.37	0.22	0.63
		IL-2	0.10	0.10	0.15	0.11	0.10	0.06	0.24	0.00	0.00
		TNF- α	0.93	0.34	0.28	1.95	0.50	0.42	0.72	0.22	0.00
Patient 8	0.71	IFN- γ	0.07	0.27	0.08	0.23	0.54	0.00	0.00	0.00	0.00
		IL-2	0.26	0.25	0.29	0.00	0.33	0.14	0.00	0.48	0.56
		TNF- α	0.19	0.13	0.12	0.23	0.33	0.14	0.00	0.00	0.00
Patient 9	6.77	IFN- γ	0.45	0.15	0.12	0.51	0.24	0.11	1.49	0.00	0.97
		IL-2	0.16	0.21	0.16	0.16	0.24	0.16	0.00	0.00	0.00
		TNF- α	0.24	0.15	0.14	0.34	0.20	0.24	2.99	0.00	0.00
			IL-2/7/21								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
Patient 3	0.85	IFN- γ	0.97	0.35	0.23	1.27	0.44	0.18	0.00	0.00	0.00
		IL-2	0.73	0.66	0.31	1.27	1.10	0.37	0.00	0.00	0.52
		TNF- α	1.70	0.31	0.17	2.55	0.88	0.18	0.00	0.72	0.00
Patient 4	2.72	IFN- γ	0.21	0.38	0.29	0.07	0.16	0.09	0.00	0.33	0.72
		IL-2	0.09	0.05	0.09	0.17	0.00	0.09	0.40	0.33	0.00
		TNF- α	0.13	0.19	0.16	0.17	0.22	0.18	0.40	0.00	0.00
Patient 5	109.68	IFN- γ	0.87	0.06	0.47	1.62	0.09	1.11	1.52	0.00	0.54
		IL-2	0.07	0.07	0.07	0.20	0.12	0.14	0.00	0.00	0.00
		TNF- α	0.18	0.07	0.20	0.44	0.20	0.59	0.76	0.57	0.54
Patient 6	1.82	IFN- γ	0.21	0.17	0.13	0.18	0.00	0.00	0.28	0.21	0.34
		IL-2	0.16	0.05	0.11	0.45	0.51	0.00	0.10	0.07	0.34
		TNF- α	0.19	0.22	0.14	0.45	1.01	2.58	0.94	0.35	0.93
Patient 7	415.98	IFN- γ	0.74	0.30	0.50	2.93	0.81	1.36	0.39	0.39	0.41
		IL-2	0.10	0.07	0.15	0.43	0.22	0.14	0.39	0.13	0.00
		TNF- α	0.44	0.18	0.25	2.22	0.81	0.61	0.39	0.26	0.10
Patient 8	2.03	IFN- γ	0.14	0.21	0.11	0.23	0.09	0.11	0.00	5.82	0.00
		IL-2	0.06	0.05	0.11	0.10	0.12	0.20	1.75	0.53	0.83
		TNF- α	0.13	0.25	0.26	0.25	0.33	0.44	0.00	0.53	0.42
Patient 9	78.48	IFN- γ	0.76	0.25	0.49	1.87	0.30	1.01	0.00	0.60	1.11
		IL-2	0.03	0.11	0.09	0.13	0.56	0.57	0.00	0.60	0.37
		TNF- α	0.19	0.14	0.25	0.52	0.19	0.64	0.00	0.60	0.00

Percent IFN- γ , IL-2 and TNF- α production from total CD3+, and in CD4+ and CD8+ subsets of COVID-19 reactive T cells derived from each recovered donor and stimulated with the peptide libraries derived from M, N and S structural proteins and fold expansion after culture with IL-2/4/21 or IL-2/7/21 cytokine cocktails.

Table S4. Cytokine production of COVID-19 reactive T cells from recovered donors expanded with IL-2/4/7 or IL-2/7/15 against S1 and S2 (N and C terminals of the S protein), related to Figure 2.

		IL-2/4/7					
		CD3 %		CD4 %		CD8 %	
		S1	S2	S1	S2	S1	S2
Patient 1	IFN- γ	4.69	0.71	4.47	0.66	0.70	0.60
	IL-2	0.58	0.38	0.59	0.45	0.09	0.15
	TNF- α	7.19	1.11	5.97	0.75	0.26	0.45
Patient 3	IFN- γ	1.33	5.12	1.00	0.46	1.43	3.62
	IL-2	0.19	0.24	0.12	0.07	1.04	0.75
	TNF- α	2.62	0.92	1.86	0.51	7.04	1.09
Patient 4	IFN- γ	10.20	7.84	8.33	6.60	13.20	6.29
	IL-2	2.58	4.48	1.04	2.72	6.15	6.47
	TNF- α	2.85	1.52	2.30	0.88	3.71	1.15
Patient 5	IFN- γ	26.20	1.93	9.10	2.14	12.20	3.12
	IL-2	0.14	0.83	0.09	0.37	0.00	3.29
	TNF- α	4.62	2.63	3.62	1.60	9.38	2.44
Patient 6	IFN- γ	6.92	1.09	10.60	1.31	2.70	1.05
	IL-2	1.50	1.22	1.20	1.49	2.52	2.44
	TNF- α	9.47	0.72	12.80	0.86	0.00	0.00
Patient 7	IFN- γ	26.50	8.54	20.80	3.66	12.90	2.11
	IL-2	5.03	0.36	2.55	0.13	0.60	5.79
	TNF- α	2.42	1.30	1.67	0.61	8.70	2.63
Patient 8	IFN- γ	1.53	3.84	1.42	1.34	1.88	3.40
	IL-2	0.17	0.41	0.06	0.15	3.56	1.99
	TNF- α	3.62	1.67	2.81	1.08	27.30	13.70
Patient 9	IFN- γ	2.67	3.92	1.22	1.76	3.34	3.60
	IL-2	0.48	0.61	0.22	0.38	6.05	4.32
	TNF- α	0.45	1.63	0.33	1.00	3.97	14.70

		IL-2/7/15					
		CD3 %		CD4 %		CD8 %	
		S1	S2	S1	S2	S1	S2
Patient 1	IFN- γ	5.96	3.25	4.47	3.72	0.83	7.77
	IL-2	0.8	0.58	0.59	0.69	4.13	3.88
	TNF- α	7.59	1.17	5.97	0.93	5.79	0.97
Patient 3	IFN- γ	7.43	1.61	1.05	0.76	4.57	1.72
	IL-2	0.41	0.13	0.07	0.04	3.28	0.54
	TNF- α	5.06	2.48	3.70	1.45	26.20	18.40
Patient 4	IFN- γ	18.00	14.60	2.50	1.32	10.10	5.90
	IL-2	1.57	1.29	0.22	0.12	1.04	0.75
	TNF- α	1.94	1.93	1.53	1.31	8.33	8.86
Patient 5	IFN- γ	4.99	1.96	0.82	1.81	5.86	0.46
	IL-2	0.12	0.05	0.05	0.03	3.15	0.46
	TNF- α	1.63	4.14	0.84	3.05	12.20	22.60
Patient 6	IFN- γ	3.95	2.72	1.97	2.53	1.22	1.20
	IL-2	0.80	0.24	0.21	0.09	1.28	0.40
	TNF- α	2.46	2.29	1.51	1.41	18.70	15.30
Patient 7	IFN- γ	11.10	1.72	15.80	2.00	2.70	1.05
	IL-2	4.37	3.80	3.02	2.34	3.60	3.48
	TNF- α	10.50	0.95	13.90	1.03	0.36	0.00
Patient 8	IFN- γ	1.73	4.34	2.71	10.50	1.81	3.70
	IL-2	0.12	4.97	0.18	1.13	0.14	4.48
	TNF- α	1.57	2.10	1.38	2.25	16.10	13.90
Patient 9	IFN- γ	5.92	5.03	7.23	6.43	4.48	2.71
	IL-2	1.20	1.21	0.47	0.42	2.90	2.98
	TNF- α	1.59	1.41	4.48	0.38	0.40	0.27

Percent IFN- γ , IL-2 and TNF- α production from total CD3⁺, and CD4⁺ and CD8⁺ subsets of COVID-19 reactive T cells derived from each recovered donor and stimulated with the peptide libraries derived from S1 and S2 (N and C terminals of the S protein) after expansion with IL-2/4/7 or IL-2/7/15 cytokine cocktails.

Table S5. Cytokine production of COVID-19 reactive T cells from recovered donors expanded with IL-2/4/21 or IL-2/7/21 against S1 and S2 (N and C terminals of the S protein), related to Figure 2.

		IL-2/4/21					
		CD3 %		CD4 %		CD8 %	
		S1	S2	S1	S2	S1	S2
Patient 3	IFN- γ	0.04	0.08	0.02	0.05	0.00	0.00
	IL-2	0.12	0.16	0.13	0.16	0.00	0.00
	TNF- α	0.08	0.05	0.08	0.04	0.00	1.59
Patient 4	IFN- γ	0.17	0.19	0.14	0.23	0.00	0.00
	IL-2	0.40	0.57	0.68	0.23	0.00	0.00
	TNF- α	0.40	0.19	0.27	0.00	0.00	0.00
Patient 5	IFN- γ	4.15	0.14	6.21	0.22	0.00	0.00
	IL-2	0.17	0.08	0.24	0.09	0.00	0.00
	TNF- α	1.78	0.13	2.69	0.22	0.00	0.00
Patient 6	IFN- γ	0.33	0.14	0.17	0.22	0.00	0.00
	IL-2	0.00	0.57	0.00	0.44	0.00	0.00
	TNF- α	1.00	0.72	0.34	0.22	0.00	0.00
Patient 7	IFN- γ	0.68	0.21	1.12	0.60	0.67	0.00
	IL-2	0.21	0.18	0.31	0.22	0.22	0.33
	TNF- α	0.23	0.12	0.35	0.18	0.00	0.67
Patient 8	IFN- γ	0.08	0.09	0.00	0.14	0.00	0.00
	IL-2	0.35	0.16	0.14	0.00	0.66	0.61
	TNF- α	0.18	0.14	0.29	0.00	0.00	0.00
Patient 9	IFN- γ	0.03	0.26	0.08	0.50	0.00	0.00
	IL-2	0.10	0.13	0.11	0.12	0.00	0.00
	TNF- α	0.07	0.20	0.16	0.30	0.00	0.00
		IL-2/7/21					
		CD3 %		CD4 %		CD8 %	
		S1	S2	S1	S2	S1	S2
Patient 3	IFN- γ	0.05	0.10	0.00	0.00	0.58	0.00
	IL-2	0.11	0.21	0.09	0.43	0.00	0.69
	TNF- α	0.22	0.07	0.35	0.14	0.00	0.00
Patient 4	IFN- γ	0.20	0.13	0.23	0.10	0.00	0.00
	IL-2	0.07	0.21	0.10	0.24	0.00	0.87
	TNF- α	0.16	0.15	0.20	0.07	0.00	0.00
Patient 5	IFN- γ	0.89	0.05	2.22	0.08	0.00	0.00
	IL-2	0.08	0.14	0.17	0.11	0.00	0.83
	TNF- α	0.37	0.06	1.01	0.15	0.00	0.83
Patient 6	IFN- γ	0.20	0.13	0.34	1.00	0.43	0.10
	IL-2	0.06	0.08	0.34	1.50	0.22	0.00
	TNF- α	0.16	0.07	1.02	1.50	0.43	0.50
Patient 7	IFN- γ	0.31	0.12	0.86	0.21	0.63	0.47
	IL-2	0.06	0.04	0.11	0.39	0.00	0.00
	TNF- α	0.20	0.09	1.18	0.21	0.63	0.00
Patient 8	IFN- γ	0.10	0.09	0.13	0.05	0.56	0.00
	IL-2	0.08	0.07	0.18	0.18	0.00	0.00
	TNF- α	0.20	0.23	0.31	0.55	0.00	0.00
Patient 9	IFN- γ	0.05	0.63	0.04	1.24	0.27	0.35
	IL-2	0.08	0.07	0.37	0.41	0.54	0.35
	TNF- α	0.08	0.31	0.20	0.85	0.54	0.35

Percent IFN- γ , IL-2 and TNF- α production from total CD3+, and CD4+ and CD8+ subsets of COVID-19 reactive T cells derived from each recovered donor and stimulated with the peptide libraries derived from S1 and S2 (N and C terminals of the S protein) after expansion with IL-2/4/21 or IL-2/7/21 cytokine cocktails.

Table S6. Cytokine production of COVID-19 reactive T cells from healthy controls expanded with different cytokine cocktails against M, N and S structural proteins, related to Figure 4.

		CD3 %			CD4 %			CD8 %		
		M	N	S	M	N	S	M	N	S
IL-2/4/7	IFN- γ median	0.43	0.29	0.44	0.55	0.28	0.34	0.11	0.18	0.24
	IFN- γ min	0.13	0.16	0.19	0.14	0.16	0.06	0.02	0.07	0.04
	IFN- γ max	1.37	0.67	2.57	2.59	1.12	2.50	0.19	0.67	2.59
	IL-2 median	0.14	0.50	0.16	0.10	0.16	0.10	0.24	0.18	0.24
	IL-2 min	0.03	0.12	0.04	0.03	0.14	0.04	0.00	0.09	0.02
	IL-2 max	0.31	0.74	0.63	0.23	1.27	0.17	0.41	0.63	0.29
	TNF- α median	0.25	0.23	0.16	0.26	0.57	0.27	0.04	0.22	0.19
	TNF- α min	0.15	0.17	0.06	0.11	0.07	0.07	0.02	0.12	0.02
	TNF- α max	1.11	2.34	1.59	2.19	5.17	3.55	0.18	0.28	0.67
IL-2/7/15	IFN- γ median	0.24	0.15	0.59	0.27	0.18	0.37	0.10	0.13	0.11
	IFN- γ min	0.10	0.09	0.21	0.17	0.05	0.16	0.07	0.08	0.06
	IFN- γ max	2.13	0.39	1.64	6.63	0.49	1.58	0.91	0.24	1.22
	IL-2 median	0.17	0.12	0.18	0.10	0.12	0.11	0.11	0.08	0.15
	IL-2 min	0.05	0.00	0.07	0.09	0.00	0.07	0.02	0.00	0.07
	IL-2 max	0.25	0.25	0.29	0.25	0.14	0.31	0.22	0.21	0.27
	TNF- α median	0.12	0.12	0.18	0.24	0.21	0.31	0.10	0.10	0.14
	TNF- α min	0.09	0.02	0.09	0.10	0.00	0.14	0.06	0.01	0.08
	TNF- α max	1.44	0.14	0.38	4.88	0.23	1.14	0.24	0.18	0.22

Percent IFN- γ , IL-2 and TNF- α production (median, minimum and maximum values) from total CD3+, and CD4+ and CD8+ subsets of COVID-19 reactive T cells stimulated with the peptide libraries derived from M, N and S structural proteins after expansion with the two different cytokine cocktails IL-2/4/7 or IL-2/7/15.

Table S7. Cytokine production and fold expansion of COVID-19 reactive T cells derived from the different healthy controls and expanded with IL-2/4/7 or IL-2/7/15 cytokine cocktails, related to Figure 4.

			IL-2/4/7								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
HD 1	21.31	IFN- γ	0.14	0.29	2.57	0.17	0.32	2.16	0.02	0.07	0.04
		IL-2	0.31	0.74	0.63	0.11	0.14	0.07	0.26	0.63	0.29
		TNF- α	0.15	0.23	0.06	0.14	0.27	0.58	0.02	0.22	0.11
HD 2	8.13	IFN- γ	0.13	0.25	0.44	0.14	0.28	0.24	0.19	0.30	0.22
		IL-2	0.25	0.50	0.27	0.10	0.16	0.10	0.41	0.30	0.27
		TNF- α	0.16	0.21	0.16	0.26	0.57	0.27	0.18	0.27	0.19
HD 3	2.85	IFN- γ	0.43	0.16	0.19	0.55	0.16	0.06	0.11	0.18	0.24
		IL-2	0.14	0.12	0.13	0.23	0.15	0.10	0.24	0.18	0.06
		TNF- α	0.25	0.17	0.12	0.11	0.07	0.07	0.17	0.12	0.38
HD4	27.74	IFN- γ	1.37	0.67	0.33	1.57	1.12	0.34	0.08	0.17	0.41
		IL-2	0.04	0.65	0.04	0.04	1.27	0.04	0.00	0.15	0.02
		TNF- α	1.11	2.34	0.22	1.38	5.17	0.27	0.03	0.28	0.02
HD5	41.84	IFN- γ	0.84	0.60	1.83	2.59	0.27	2.50	0.12	0.67	2.59
		IL-2	0.03	0.15	0.16	0.03	0.22	0.17	0.03	0.09	0.24
		TNF- α	0.63	0.62	1.59	2.19	0.96	3.55	0.04	0.22	0.67

			IL-2/7/15								
			CD3 %			CD4 %			CD8 %		
	Fold Expansion		M	N	S	M	N	S	M	N	S
HD 1	15.73	IFN- γ	0.24	0.23	0.56	0.26	0.30	0.36	0.10	0.10	0.11
		IL-2	0.25	0.25	0.29	0.09	0.12	0.09	0.22	0.18	0.18
		TNF- α	0.09	0.12	0.09	0.13	0.21	0.23	0.07	0.10	0.08
HD 2	9.01	IFN- γ	0.10	0.11	0.21	0.17	0.13	0.16	0.07	0.17	0.06
		IL-2	0.17	0.13	0.18	0.10	0.12	0.11	0.15	0.21	0.27
		TNF- α	0.12	0.14	0.15	0.24	0.22	0.34	0.10	0.13	0.11
HD 3	4.00	IFN- γ	0.24	0.15	0.59	0.27	0.18	0.37	0.10	0.08	0.09
		IL-2	0.13	0.12	0.14	0.13	0.14	0.18	0.10	0.08	0.13
		TNF- α	0.12	0.13	0.18	0.10	0.11	0.14	0.22	0.18	0.15
HD4	53.95	IFN- γ	2.13	0.09	0.67	6.63	0.05	0.40	0.21	0.24	0.96
		IL-2	0.05	0.00	0.07	0.10	0.00	0.07	0.02	0.00	0.07
		TNF- α	1.44	0.02	0.33	4.88	0.00	0.31	0.06	0.07	0.22
HD5	24.77	IFN- γ	1.23	0.39	1.64	1.24	0.49	1.58	0.91	0.13	1.22
		IL-2	0.17	0.00	0.18	0.25	0.01	0.31	0.11	0.01	0.15
		TNF- α	0.47	0.07	0.38	1.06	0.23	1.14	0.24	0.01	0.14

Percent IFN- γ , IL-2 and TNF- α production from total CD3+, and CD4+ and CD8+ subsets of COVID-19 reactive T cells derived from each healthy donor and stimulated with the peptide libraries derived from M, N and S structural proteins and fold expansion after culture with IL-2/4/7 or IL-2/7/15 cytokine cocktails.