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Supplemental Information

SARS-CoV-2-Specific T Cells Exhibit Phenotypic Features of Helper Function, Lack of Terminal Differentiation, and High Proliferation Potential

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SUPPLEMENTAL INFORMATION

Figure S1

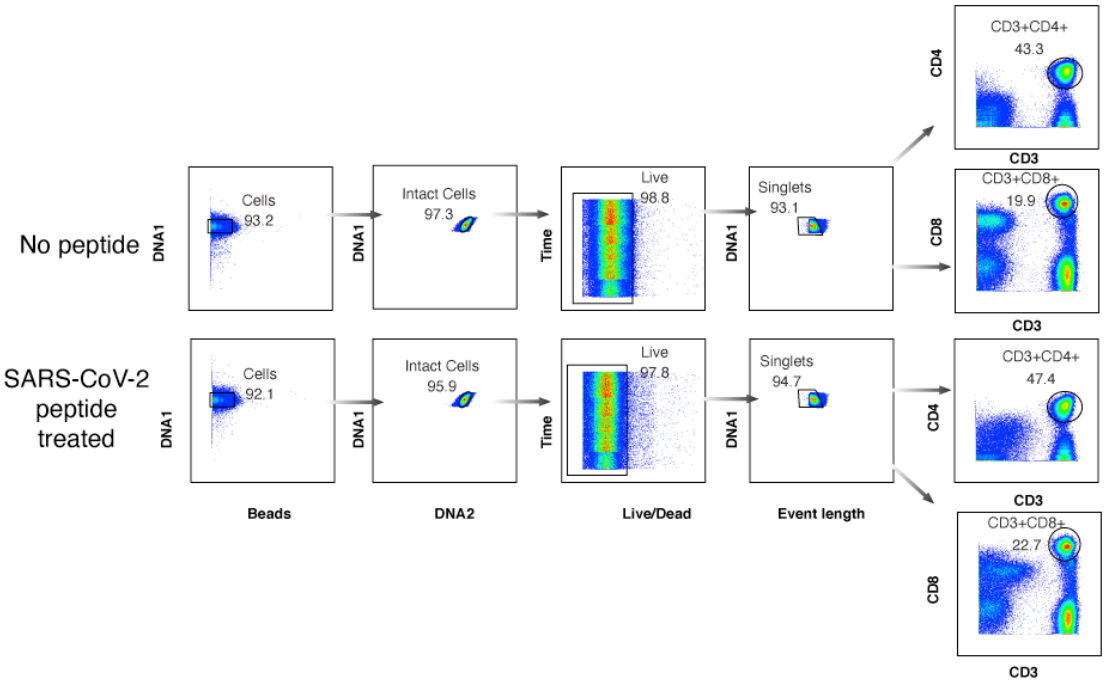

Figure S1. CyTOF gating strategy to identify CD4+ and CD8+ T cells from convalescent COVID-19 patients – Related to Figure 1. PBMCs were purified from freshly drawn blood specimens, treated as indicated in Fig. 1, and phenotyped by CyTOF. Shown is an example of the gating strategy leading to the identification of live, singlet CD4+ and CD8+ T cells.

Figure S2

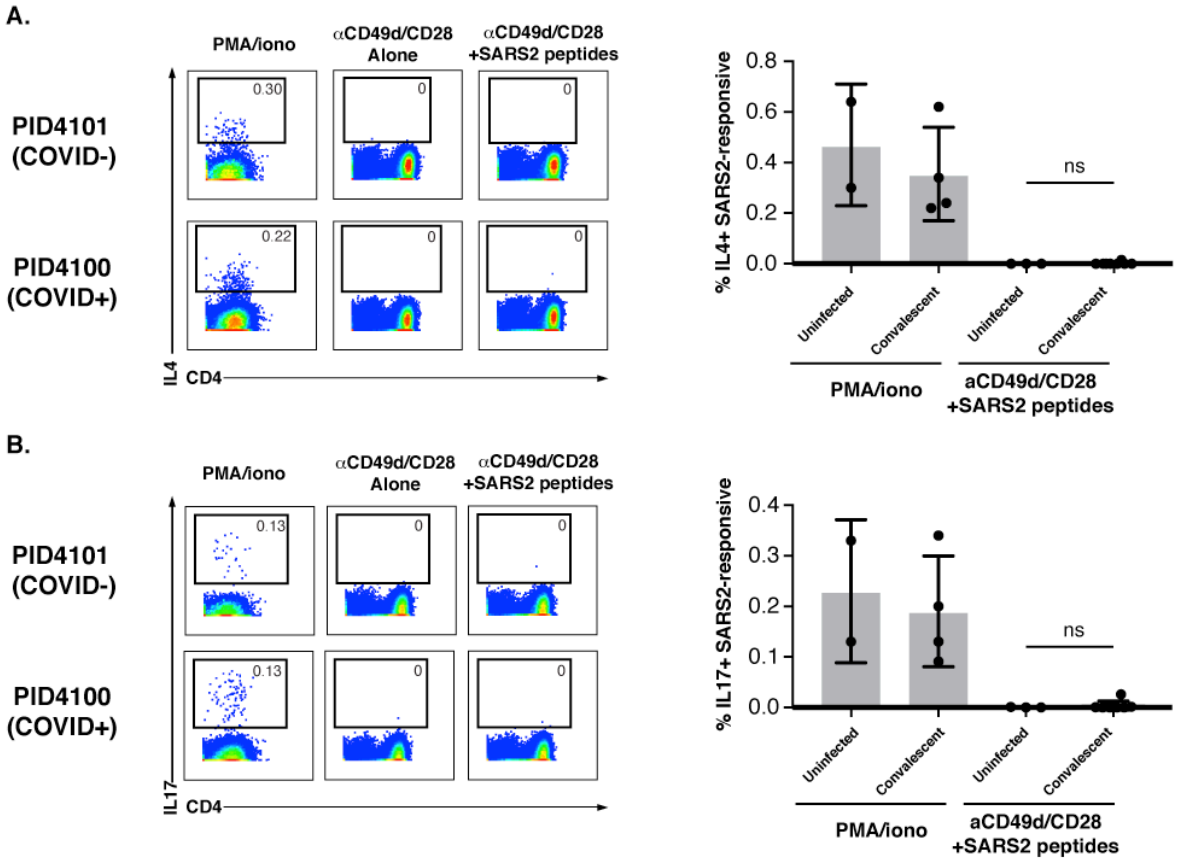

Figure S2. Blood from convalescent individuals lack SARS-CoV-2 spike-specific CD4+ T cells producing IL4 or IL17 – Related to Figure 1. Shown on the left are pseudocolor plots of CyTOF datasets reflecting the percentage of CD4+ T cells producing IL4 (**A**) or IL17 (**B**) in response to the indicated treatment condition, for one representative uninfected (COVID-) and one convalescent (COVID+) donor. Numbers correspond to the percentage of cells within the gates. PMA/ionomycin stimulation was used to demonstrate the presence of IL4- and IL17-producing T cells in these donors. Anti-CD49d/CD28 was used to provide co-stimulation during peptide treatment. Shown on the right are cumulative data of three uninfected individuals and nine convalescent individuals (Table S2). Results are gated on live, singlet CD4+ T cells. n.s. = non-significant as assessed using the Student's unpaired t test.

Figure S3

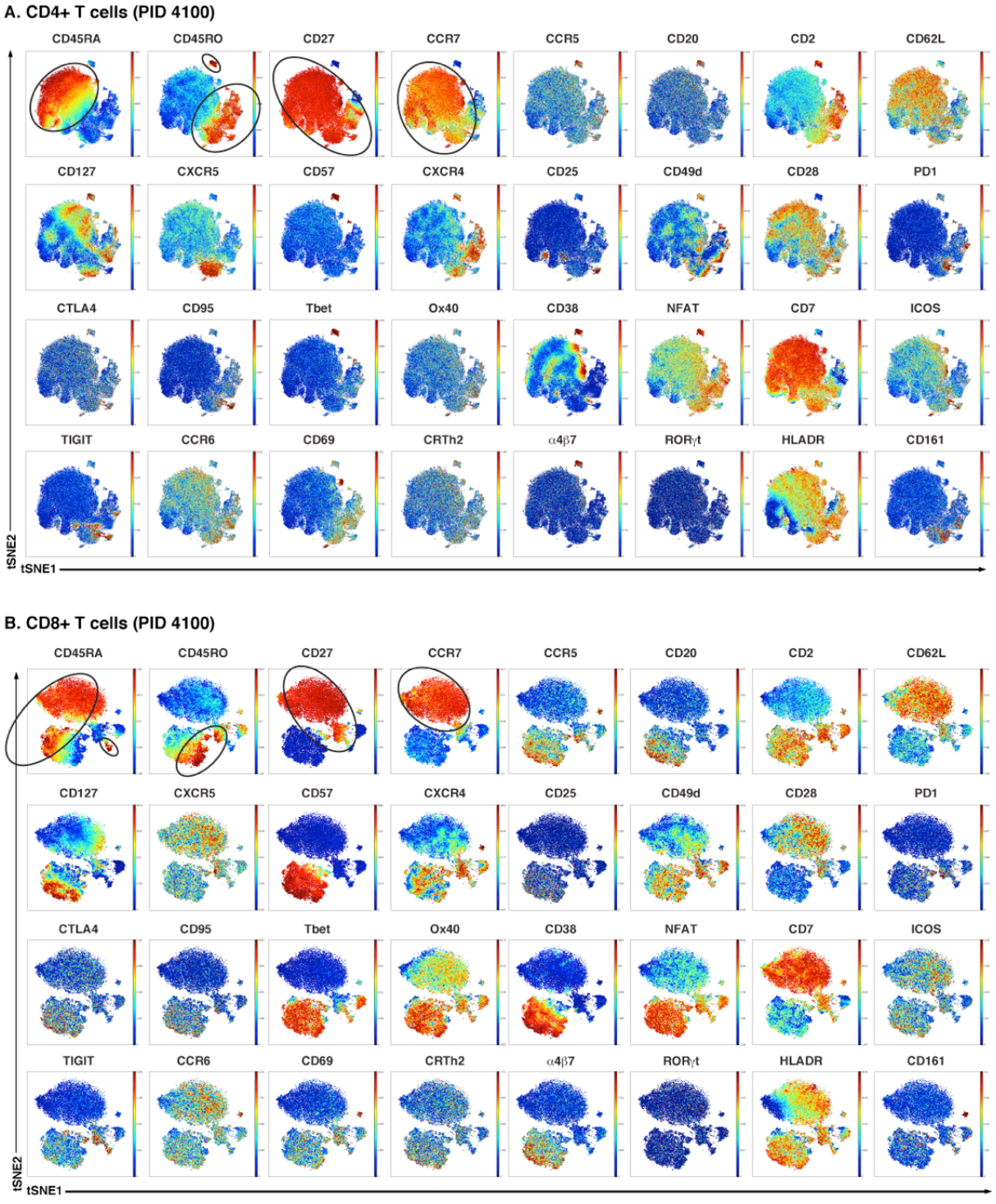

Figure S3. Heatmaps of antigen expression in T cells from representative donor – Related to Figure 2. Shown are t-SNE plots of CyTOF datasets reflecting CD4+ (A) and CD8+ (B) T cells from representative convalescent donor PID4100. Regions of the t-SNE harboring SARS-CoV-2- and CMV-specific T cells are shown in Fig. 2.

Circled in the first four plots are regions with high expression levels of CD45RA (expressed on naïve/Tscm/Temra T cells), CD45RO (expressed on memory T cells), and CD27 and CCR7 (expressed on naïve and Tcm cells).

Figure S4

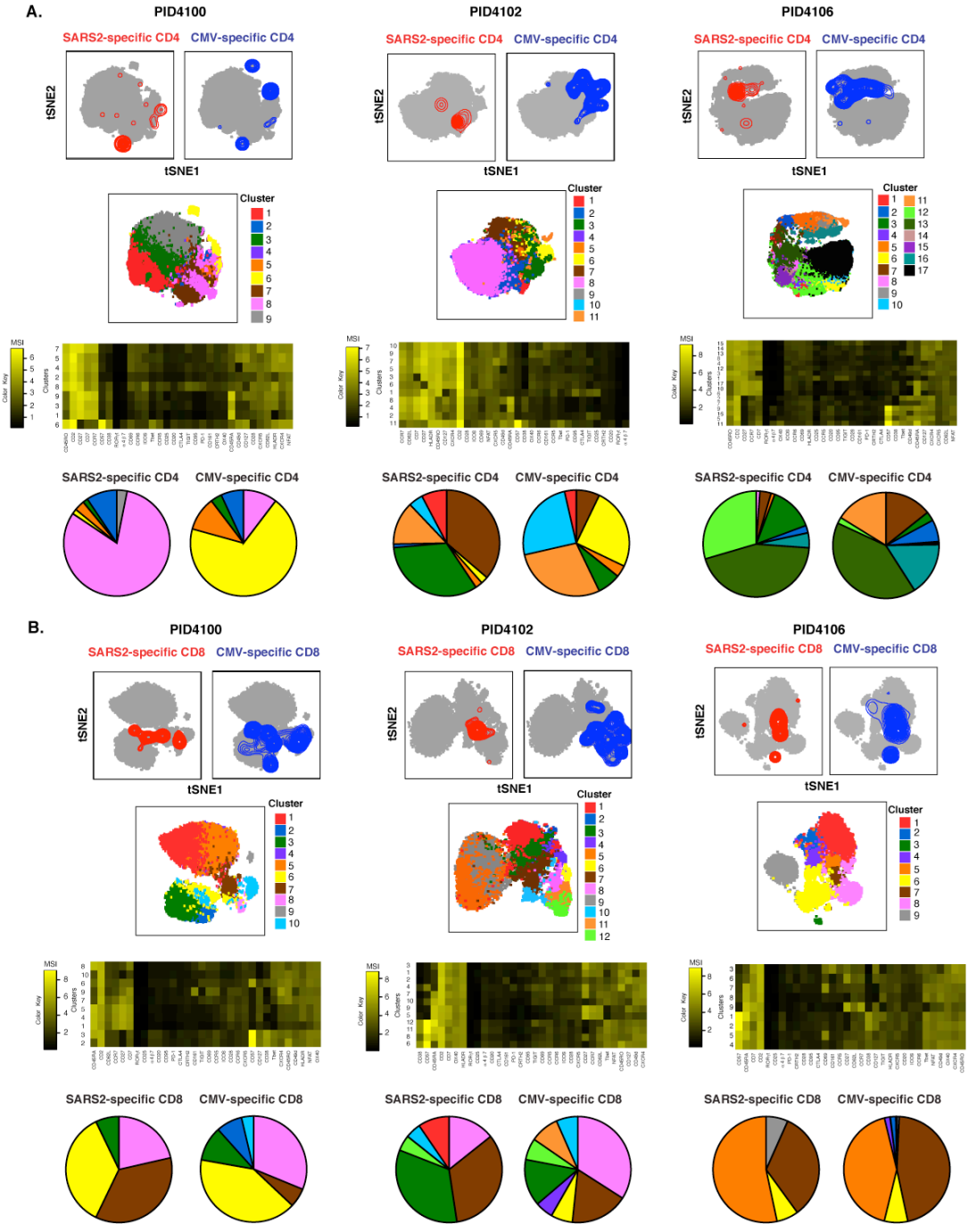

Figure S4. SARS-CoV-2 spike-specific T cells recognizing SARS-CoV-2 differ in phenotypes from those recognizing CMV – Related to Figure 2. Shown are t-SNE plots of CyTOF datasets reflecting CD4+ (**A**) or CD8+ (**B**) T cells from three CMV+ COVID-19 convalescent donors. The top pairs of plots are identical to the contour plots presented in Fig. 2 and serve as references for where in the t-SNE the SARS-CoV-2-specific and CMV-specific T cells are concentrated. The middle plots depict the same t-SNE colored by DensVM clusters. Shown underneath the clustered t-SNE plots are heatmaps showing relative expression levels (in mean signal intensity, or MSI) for each of the indicated antigens, hierarchically clustered based on Euclidean distances. The pie graphs at the bottom depict the proportions of SARS-CoV-2-specific or CMV-specific T cells that belong to each cluster. Note that each pair of pie graphs differs, with more pronounced differences observed among the CD4+ T cells.

Figure S5

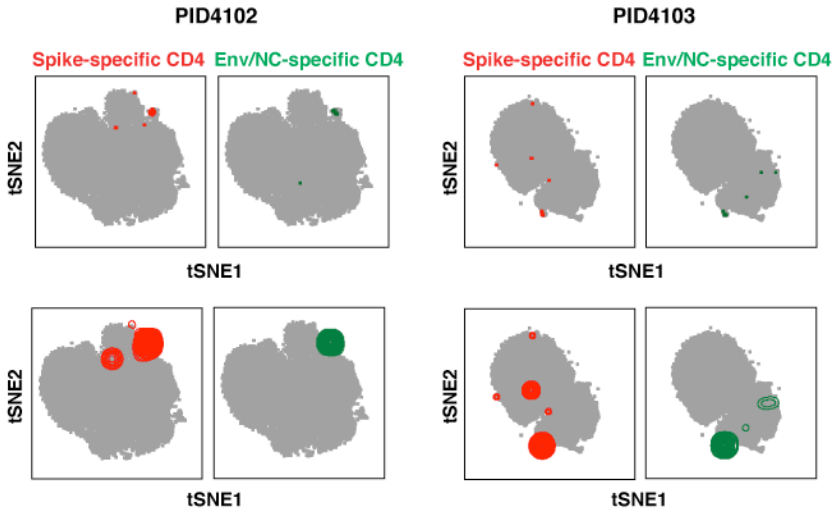

Figure S5. Antigen-specific CD4+ T cells against SARS-CoV-2 spike, envelope (env), and nucleocapsid (NC) are phenotypically similar – Related to Figure 2. Shown are t-SNE plots of CyTOF datasets reflecting CD4+ T cells from two COVID-19 convalescent individuals. Cells shown in grey correspond to CD4+ T cells from specimens stimulated with anti-CD49d/CD28 in the absence of any peptides. The top pairs of plots show SARS-CoV-2 spike-specific (*red*) or env/NC-specific (*green*) cells as individual dots. The bottom pairs of plots show the same data but with the antigen-specific cells shown as contours instead of dots, to better visualize regions with highest densities of antigen-specific cells. Note that the highest densities of antigen-specific cells reside in similar regions of the t-SNE, suggesting that CD4+ T cells recognizing spike, env, and NC are phenotypically similar.

Figure S6

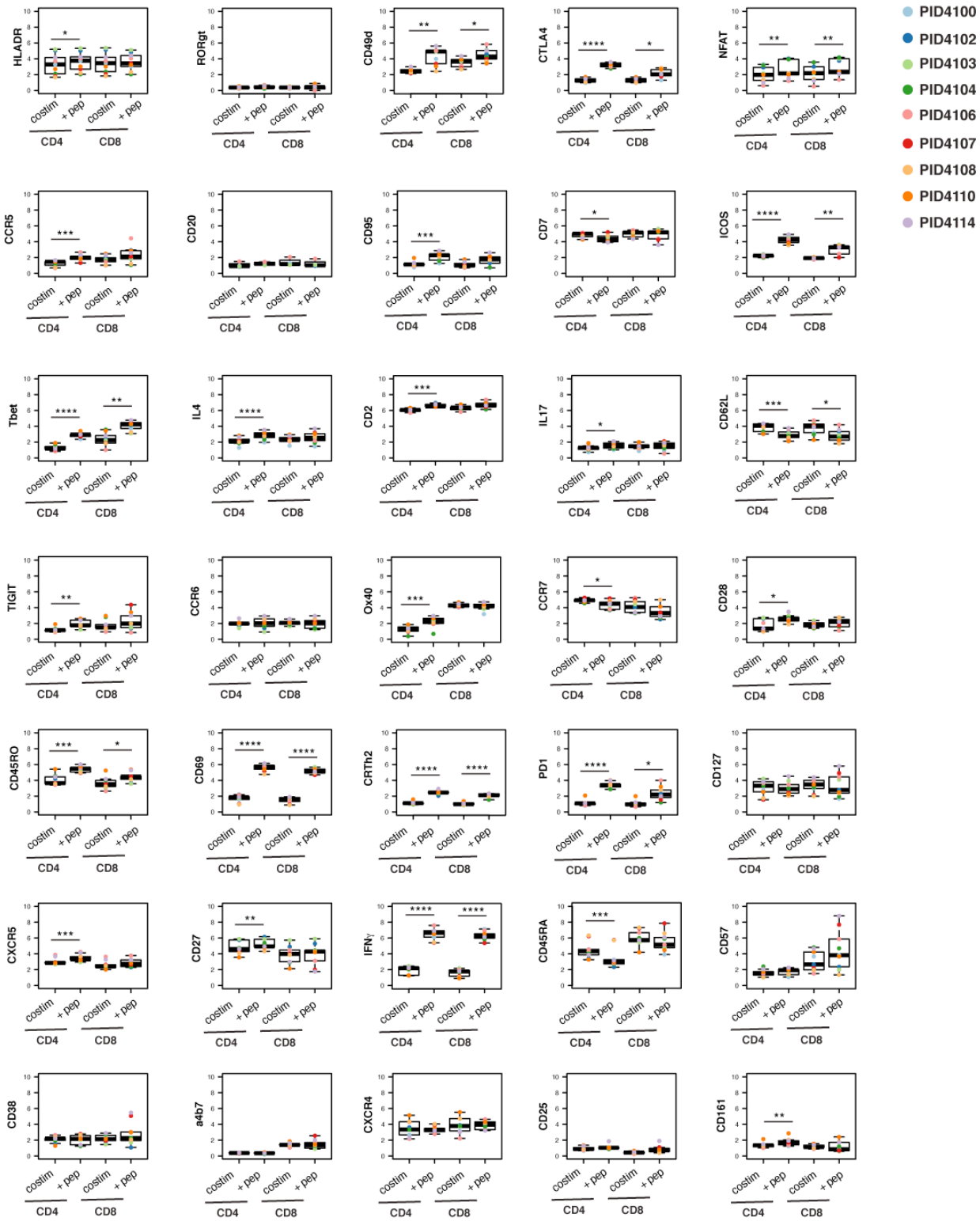
HLADR
RORgt
CD49d
CTLA4
NFAT
CCR5
CD20
CD95
CD7
ICOS
Tbet
IL4
CD2
IL17
CD62L
TIGIT
CCR6
Ox40
CCR7
CD28
CD45RO
CD69
CRTh2
PD1
CD127
CXCR5
CD27
IFNγ
CD45RA
CD57
CD38
a4b7
CXCR4
CD25
CD161
costim
+ pep
CD4
CD8
PID4100
PID4102
PID4103
PID4104
PID4106
PID4107
PID4108
PID4110
PID4114

Figure S6. Mean expression levels of 35 surface and intracellular antigens in CD4+ and CD8+ T cells from 9 convalescent individuals who had recovered from mild COVID-19 – Related to Figure 2. PBMCs were purified from freshly drawn blood specimens, treated with anti-CD49d/CD28 for 6 hours alone ("costim"), or in the additional presence of overlapping 15-mer peptides against SARS-CoV-2 spike ("+ pep"), and then phenotyped by CyTOF. Results are gated on live, singlet CD4+/CD8+ T cells for the "costim" conditions, and live, singlet CD4+/CD8+ T cells expressing IFN□ for the "+ pep" conditions. Shown are the mean signal intensity (MSI) values for arcsinh-transformed data. *$p < 0.05$, **$p < 0.01$ as assessed using the Student's paired t test and adjusted for multiple testing using the Benjamini-Hochberg for FDR.

Figure S7

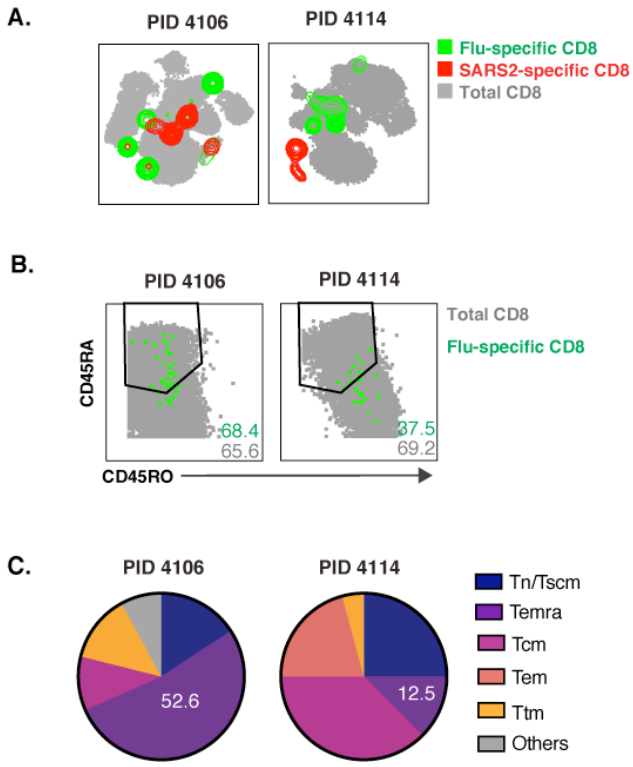

Figure S7. Flu-specific CD8+ T cells in convalescent individuals are phenotypically distinct from SARS-CoV-2-specific CD8+ T cells – Related to Figure 5. A) Shown are t-SNE plots of CyTOF datasets reflecting CD8+ T cells from two COVID-19 convalescent donors who harbored flu-specific CD8+ T cell responses. Cells shown in grey correspond to CD8+ T cells from specimens stimulated with anti-CD49d/CD28 in the absence of any peptides. Flu- and SARS-CoV-2-specific CD8+ T cells shown as green and red contours, respectively. **B)** Flu-specific CD8+ T cells include both CD45RA+CD45RO- and CD45RA-CD45RO+ cells. The phenotypes of total (*grey*) or flu-specific (*green*) CD8+ T cells are shown as dot plots for two COVID-19 convalescent donors for which flu-specific responses were detected. **C)** Temra cells can comprise a majority or minority of the flu-specific CD8+ T cell response. The proportions of flu-specific CD8+ T cells belonging to each subset are depicted as pie graphs. Numbers correspond to the percentages of cells belonging to the Temra subset.

Table S1: Participant Characteristics – Related to STAR Methods.

Patient ID	Gender	Age	SARS-CoV-2 Status	CMV status	Date of symptom onset	Date of PCR+ test	Date of blood draw(s)	Time between PCR+ test and analysis
PID4100	Female	28	Infected	Positive	3/17/20	3/19/20	4/24/20	36 days
PID4102	Male	46	Infected	Positive	3/11/20	3/13/20	4/29/20 5/6/20* 5/20/20*	47 days, 54 days* 69 days*
PID4103	Female	41	Infected	Negative	3/13/20	4/9/20	4/29/20 5/6/20* 5/20/20*	20 days, 27 days* 41 days*
PID4104	Female	33	Infected	Negative	3/11/20	3/14/20	5/13/20	60 days
PID4106	Female	67	Infected	Positive	3/6/20	3/26/20	5/4/20	39 days
PID4114	Female	45	Infected	Positive	4/15/20	4/17/20	6/15/20	59 days
PID4107	Female	38	Infected	Positive	3/25/20	4/1/20	5/13/20	42 days
PID4108	Female	18	Infected	Positive	3/16/20	NA	5/13/20	NA
PID4110	Male	57	Infected	Positive	4/19/20	4/24/20	5/15/20	21 days
PID4101	Female	42	Uninfected	Positive	NA	NA	4/24/20	NA
PID4105	Male	57	Uninfected	Negative	NA	NA	5/4/20	NA
PID4109	Male	30	Uninfected	Negative	NA	NA	5/13/20	NA

*Used in longitudinal analysis

Table S2: List of CyTOF antibodies used in study – Related to STAR Methods. Antibodies were either purchased from the indicated vendor or prepared in-house using commercially available MaxPAR conjugation kits per manufacturer's instructions (Fluidigm).

Antigen Target	Clone	Elemental Isotope	Vendor
HLADR	TÜ36	Qdot (112Cd)	Thermofisher
RORγt*	AFKJS-9	115 In	In-house
CD49d (α4)	9F10	141Pr	Fluidigm
CTLA4*	14D3	142Nd	In-house
NFAT*	D43B1	143Nd	Fluidigm
CCR5	NP6G4	144Nd	Fluidigm
CD20	2H7	145Nd	In-house
CD95	BX2	146Nd	In-house
CD7	CD76B7	147Sm	Fluidigm
ICOS	C398.4A	148Nd	Fluidigm
Tbet*	4B10	149Sm	In-house
IL4*	MP4-25D2	150Nd	In-house
CD2	TS1/8	151Eu	Fluidigm
IL17*	BL168	152Sm	In-house
CD62L	DREG56	153Eu	Fluidigm
TIGIT	MBSA43	154Sm	Fluidigm
CCR6	11A9	155Gd	In-house
IL6*	MQ2-13A5	156 Gd	In-house
CD8	RPA-T8	157Gd	In-house
CD19	HIB19	157Gd	In-house
CD14	M5E2	157Gd	In-house
OX40	ACT35	158Gd	Fluidigm
CCR7	G043H7	159Tb	Fluidigm
CD28	CD28.2	160Gd	Fluidigm
CD45RO	UCHL1	161Dy	In-house
CD69	FN50	162Dy	Fluidigm
CRTH2	BM16	163Dy	Fluidigm
PD-1	EH12.1	164Dy	In-house
CD127	A019D5	165Ho	Fluidigm
CXCR5	RF8B2	166Er	In-house
CD27	L128	167Er	Fluidigm
IFNγ*	B27	168Er	Fluidigm
CD45RA	HI100	169Tm	Fluidigm
CD3	UCHT1	170Er	Fluidigm
CD57	HNK-1	171Yb	In-house
CD38	HIT2	172Yb	Fluidigm
α4β7	Act1	173Yb	In-house
CD4	SK3	174Yb	Fluidigm
CXCR4	12G5	175Lu	Fluidigm
CD25	M-A251	176Yb	In-house
CD161	NKR-P1A	209 Bi	In-house

*Intracellular antibodies

Table S3: Frequencies of SARS-CoV-2- and CMV-specific T cells in convalescent individuals – Related to Figure 1.

Patient ID	SARS2 CD4*	SARS2 CD8#	CMV CD4*	CMV CD8#
PID4100	0.067	0.05	0.36	0.66
PID4102	0.28	0.21	0.061	0.47
PID4103	0.054	0.038	0	0
PID4104	0.068	0.009	0	0
PID4106	0.14	0.07	0.18	6.9
PID4107	0.030	0.14	0.016	0.049
PID4108	0.029	0.015	0.026	0.027
PID4110	0.14	0.026	0.048	0.0052
PID4114	0.076	0.010	0.13	0.037

***** Reported as frequency of live, singlet CD4+ T cells
[#] Reported as frequency of live, singlet CD8+ T cells