Supplementary information for:

Differential T cell reactivity to seasonal coronaviruses and SARS-CoV-2 in community and health care workers

Authors

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Supplementary Tables

Supplementary Table 1a - List of megapools (MP) used in this study

MP name	Virus source	MP Type	Peptide number	Reference
CMV	CMV	Predicted	385	Carrasco Pro et al., J Immun Res (2015)
229E ≤ 60%	HCoV-229E	Predicted	205	Sup. Table 1b
HKU1 ≤ 60%	HCoV-HKU1	Predicted	272	Sup. Table 1b
NL63 ≤ 60%	HCoV-NL63	Predicted	252	Sup. Table 1b
OC43 ≤ 60%	HCoV-OC43	Predicted	258	Sup. Table 1b
S	SARS-CoV-2	Overlapping	253	Grifoni et al., Cell (2020)
CD4R	SARS-CoV-2	Predicted	221	Grifoni et al., Cell (2020)
CD8A	SARS-CoV-2	Predicted	314	Grifoni et al., Cell Host Microbe (2020)
CD8B	SARS-CoV-2	Predicted	314	Grifoni et al., Cell Host Microbe (2020)

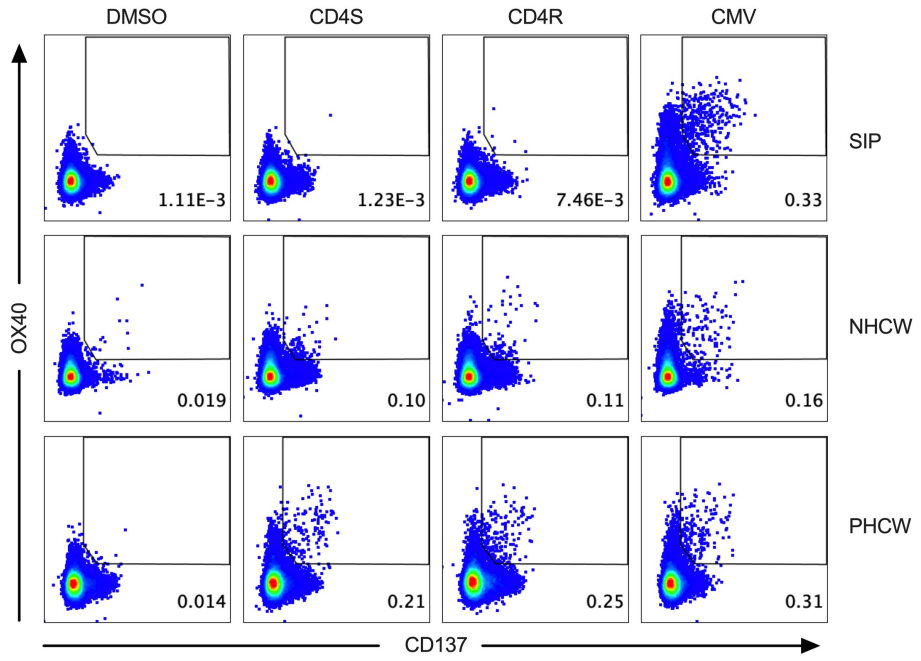
Supplementary Table 1b - List of individual peptide sequences for each HCoV-CCC MP

229E (205 peptides)	**HKU1** (272 peptides)	**NL63** (252 peptides)	**OC43** (258 peptides)
peptide 1	peptide 1	peptide 1	peptide 1
peptide 2	peptide 2	peptide 2	peptide 2
peptide 3	peptide 3	peptide 3	peptide 3
peptide …	peptide …	peptide …	peptide …

Supplementary Table 2. List of antibodies used in the study

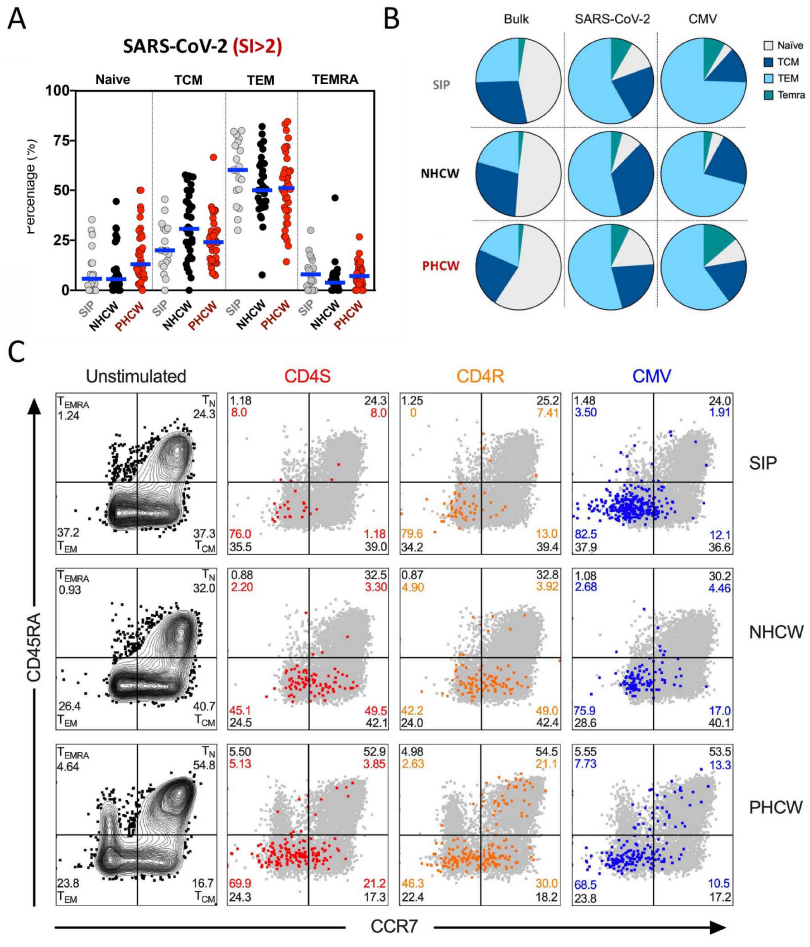
Antibody	Fluorochrome	Clone	Vendor	Catalog number
CD3	AF700	UCHT1	eBiosciences	56-0038-42
CD4	APCef780	RPA-T4	eBiosciences	47-0049-42
CD8	BV650	RPA-T8	Biolegend	301042
CD137	APC	4B4-1	Biolegend	309810
CD69	PE	FN50	BD Biosciences	555531
OX40	PE-Cy7	Ber-ACT35	Biolegend	350012
CD38	FITC	HB-7	Biolegend	356610
HLA-DR	PE-CF594	G46-6	BD Biosciences	562304
CD45RA	eF450	HI100	Invitrogen	48-0458-42
CCR7	PerCP/Cy5.5	G043H7	Biolegend	353220
CD14	V500	M5E2	BD Biosciences	561391
CD19	V500	HIB19	BD Biosciences	561121
Live/Dead Viability	eF506	-	Invitrogen	65-0866-18

Supplementary Figures

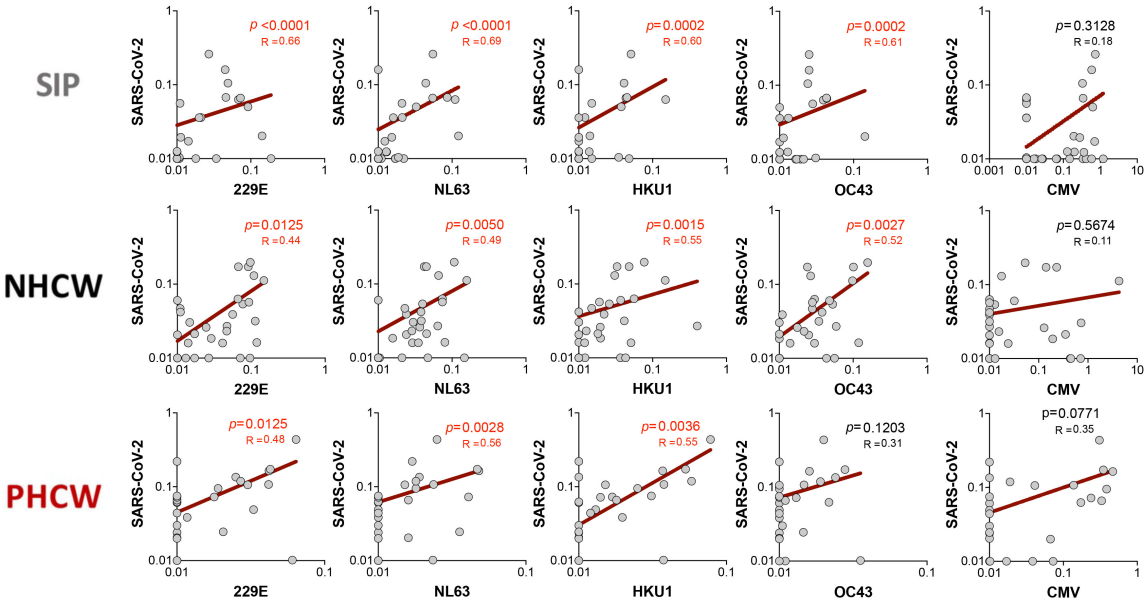


Supplementary Figure 1. Representative flow cytometry gating of AIM+ (OX40/CD137) CD4+ T cell responses against SARS-CoV-2 and CMV across all the cohorts.

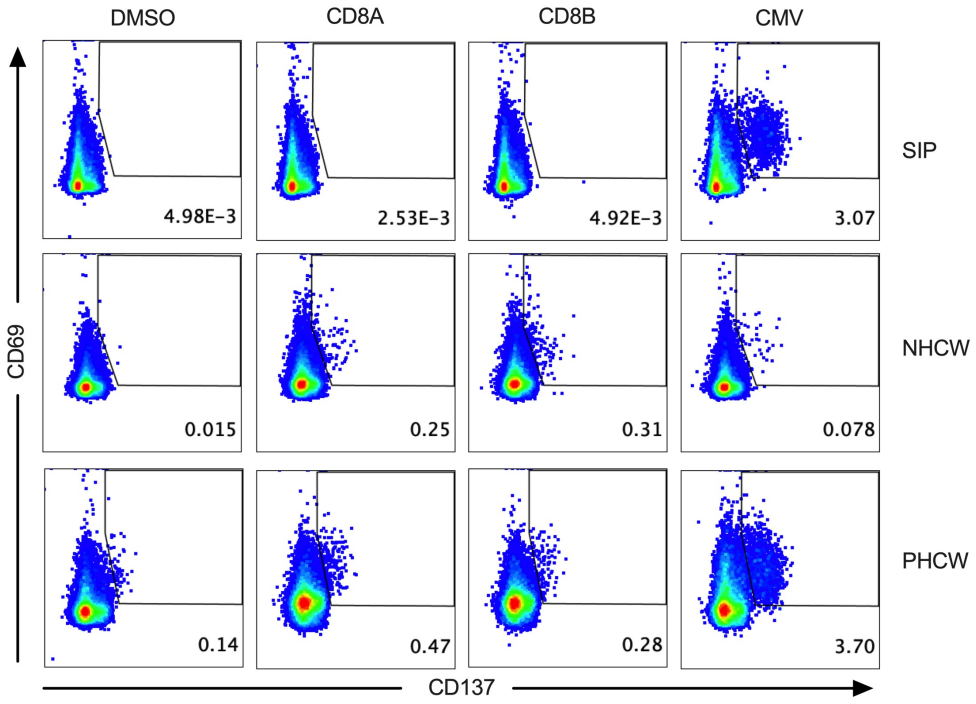
Representative FACS plots, gated on total CD4+ T cells for the SARS-CoV-2-specific CD4+ T cells measured as percentage of AIM+ (OX40+CD137+) after stimulation of PBMCs with peptide pools encompassing spike ("S") or the proteome without spike ("CD4R") in addition to the DMSO negative control and the unrelated ubiquitous pathogen CMV across all the cohorts. Cell frequency for AIM+ cells in the several conditions is indicated



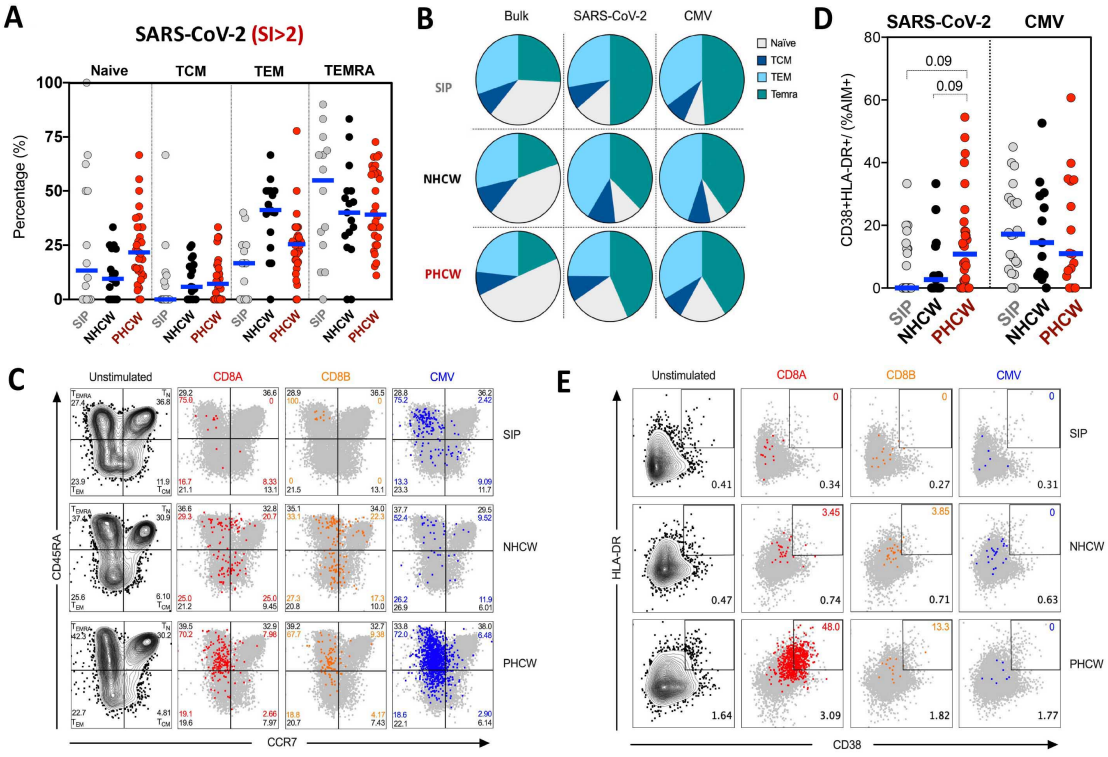
Supplementary Figure 2. CD4+ T cell reactivity to SARS-CoV-2 epitopes is mediated by memory cells. SARS-CoV-2-specific CD4+ T cell subsets (Tn: CD45RA+ CCR7+, Temra: CD45RA+ CCR7-, Tcm: CD45RA- CCR7+ and Tem: CD45RA- CCR7-) were measured after stimulation of PBMCs with peptide pools encompassing spike ("S") or the proteome without spike ("CD4R"). (A) Phenotype of antigen-specific CD4+ T cells (OX40+CD137+) responding to the indicated pools of SARS-CoV-2 and with SI>2 in each cohort. Each dot represents the response of an individual subject to an individual pool and geometric mean for the 3 different groups is shown (B) Overall averages for total CD4+ (Bulk) or antigen-specific CD4+ T cell subsets for SARS-CoV-2 or CMV detected in the 3 different cohorts. (C) Representative FACS plots, gated on total CD4+ T cells (grey) for the SARS-CoV-2-specific CD4+ T cells (colored) measured as percentage of AIM+ (OX40+CD137+) after stimulation of PBMCs with peptide pools encompassing spike ("S") or the proteome without spike ("CD4R") in addition to the DMSO negative control and the unrelated ubiquitous pathogen CMV across all the cohorts. Cell frequency for each of the subsets is indicated in each quadrant for AIM+ cells (colored) or total ("bulk") CD4+ T cells (black).



Supplementary Figure 3. Correlation of SIP, NHCW and PHCW cohort responses to CCC and SARS-CoV-2. Correlation between SARS-CoV-2-specific CD4+ T cells and each of the 4 CCC-specific CD4+ T cells (HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43) as well as CMV. Total SARS-CoV-2 MP responses per donor were used in each case ("Spike" + "Non-spike" (CD4-total). Statistical comparisons were performed using Spearman correlations. Non-linear fit curve and P and R values are shown.

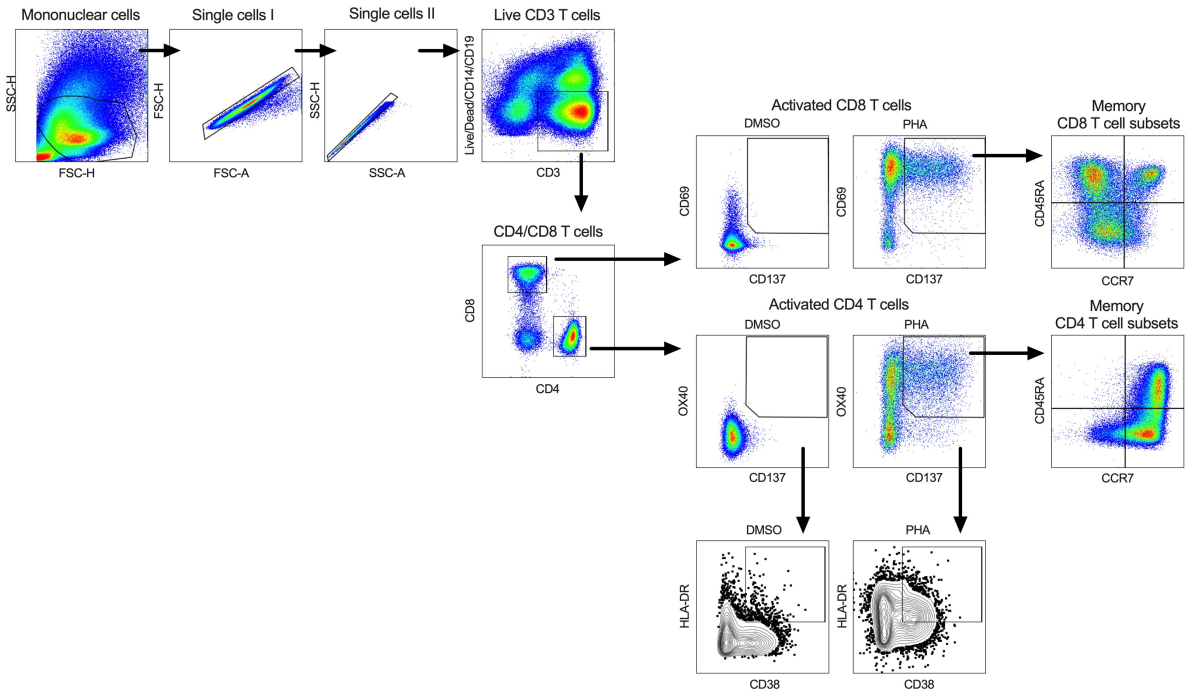


Supplementary Figure 4. Representative flow cytometry gating of AIM+ (CD69/CD137) CD8+ T cell responses against SARS-CoV-2 and CMV across all the cohorts. Representative FACS plots, gated on total CD8+ T cells for the SARS-CoV-2-specific CD8+ T cells measured as percentage of AIM+ (CD69+CD137+) after stimulation of PBMCs with class I MPs (CD8A, CD8B) in addition to the DMSO negative control and the unrelated ubiquitous pathogen CMV across all the cohorts. Cell frequency for AIM+ cells in the several conditions is indicated



Supplementary Figure 5. CD8+ T cell reactivity to SARS-CoV-2 epitopes is mediated by memory cells and associated with recent infection in PHCW. SARS-CoV-2-specific CD8+ T cell subsets (Tn: CD45RA+ CCR7+, Temra: CD45RA+ CCR7-, Tcm: CD45RA- CCR7+ and Tem: CD45RA- CCR7-) were measured after stimulation of PBMCs with class I MPs (CD8A, CD8B). (A) Phenotype of antigen-specific CD4+ T cells (CD69+CD137+) responding to the indicated pools of SARS-CoV-2 and with SI>2 in each cohort. Each dot represents the response of an individual subject to an individual pool and geometric mean for the 3 different groups is shown (B) Overall averages for total CD8+ (Bulk) or antigen-specific CD8+ T cell subsets for SARS-CoV-2 or CMV detected in the 3 different cohorts. (C) Representative FACS plots, gated on total CD8+ T cells (grey) for the SARS-CoV-2-specific CD8+ T cells (colored) measured as percentage of AIM+ (CD69+CD137+) after stimulation of PBMCs with class I MPs (CD8A, CD8B) in addition to the DMSO negative control and the unrelated ubiquitous pathogen CMV across all the cohorts. Cell frequency for each of the subsets is indicated in each quadrant for AIM+ cells (colored) or total ("bulk") CD4+ T cells (black). (D) Recently activated SARS-CoV-2-specific CD8+ T cells were measured as percentage of CD38+/HLA-DR+ cells in AIM+ (CD69+CD137+) CD8+ T cells after stimulation of PBMCs

with class I MPs (CD8A, CD8B). Graphs show data for specific responses against SARS-CoV-2 and CMV responses with SI>2. Each dot represents the response of an individual subject to an individual pool. Geometric mean for the 3 different groups is shown. Non-parametric Kruskal-Wallis multiple comparison test was applied. P values are shown with $p<0.05$ defined as statistical significant. (E) Representative FACS plots of HLA-DR/CD38+ cells in AIM+ (CD69+CD137+) CD8+ T cells (colored) overlapped with total HLA-DR/CD38 expression (grey) for all the cohorts in the different unstimulated or stimulated conditions. Cell frequency of HLA-DR/CD38+ in AIM+ cells or total CD8+ T cells is indicated on the top and bottom right corner respectively.



Supplementary Figure 6. Gating strategy for CD4+ T cell and CD8+ T cell AIM assays used in this study. Example of flow cytometry gating strategy. Briefly, mononuclear cells were gated out of all events followed by subsequent singlet gating. Live CD3+ T cells were gated out from dead cells, B cells and Monocytes. T cells were then divided as CD4+ or CD8+ and each population further subdivided into either AIM_CD4+ (OX40/CD137) or AIM_CD8+ (CD69/CD137) respectively or alternatively as HLA-DR/CD38. AIM+ cells were further mapped into memory subsets as a function of CCR7 and CD45RA expression (Tn: CD45RA+ CCR7+, Temra: CD45RA+ CCR7-, Tcm: CD45RA- CCR7+ and Tem: CD45RA- CCR7-).