

Supplemental material

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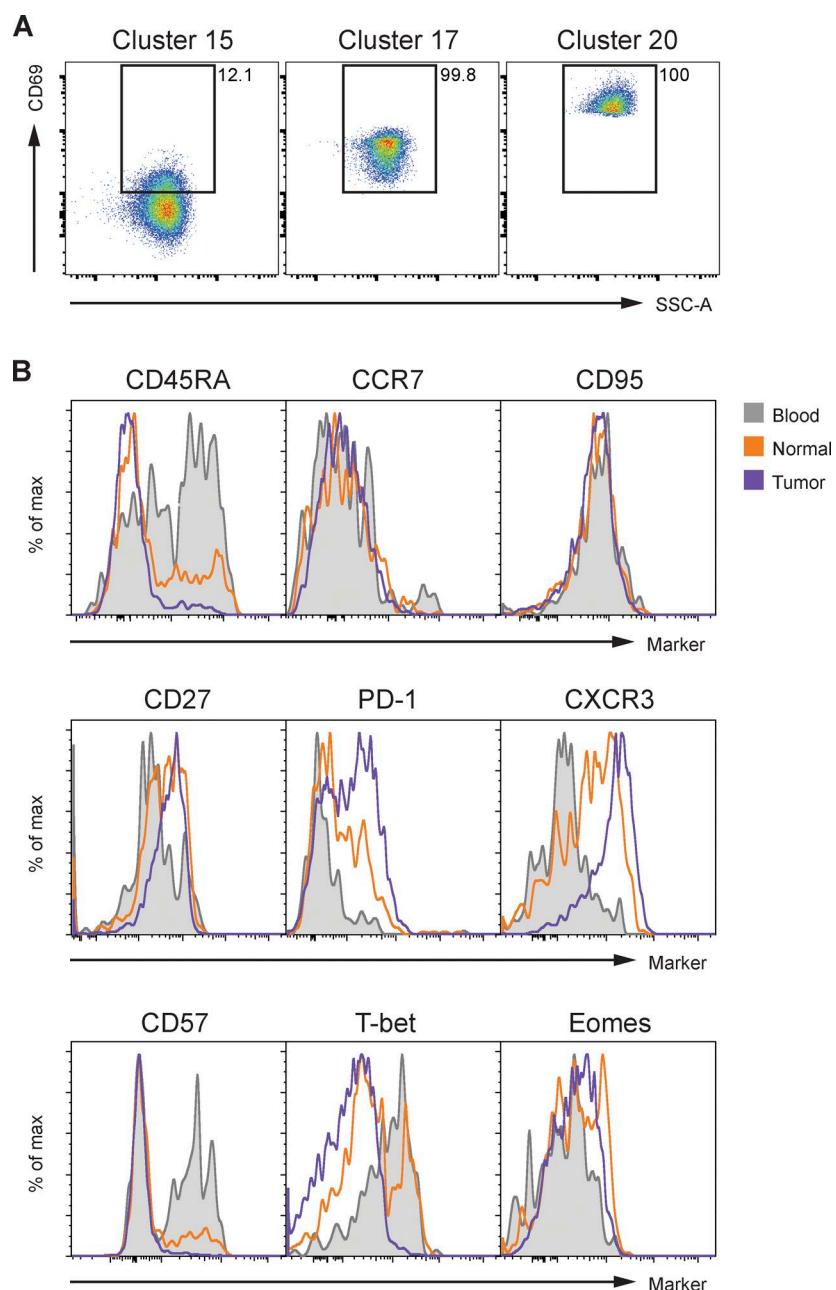


Figure S1. Phenotypic analysis of CXCR5⁺ CD8⁺ T cells. **(A)** Dot plots showing the expression of CD69 by CXCR5⁺ clusters 15 and 17 as defined in Fig. 1. Cluster 20, showing the highest CD69 iMFI in Fig. 1 D, is shown as reference. Numbers in the dot plots indicate the percentage of cells identified by the gates. **(B)** Representative histograms showing expression of the indicated markers by the CXCR5⁺ TIM-3⁻ CD8⁺ T cells from blood, normal tissue, and tumor samples, as determined by flow cytometry. Similar data were obtained from at least 22 additional samples (from six independent experiments).

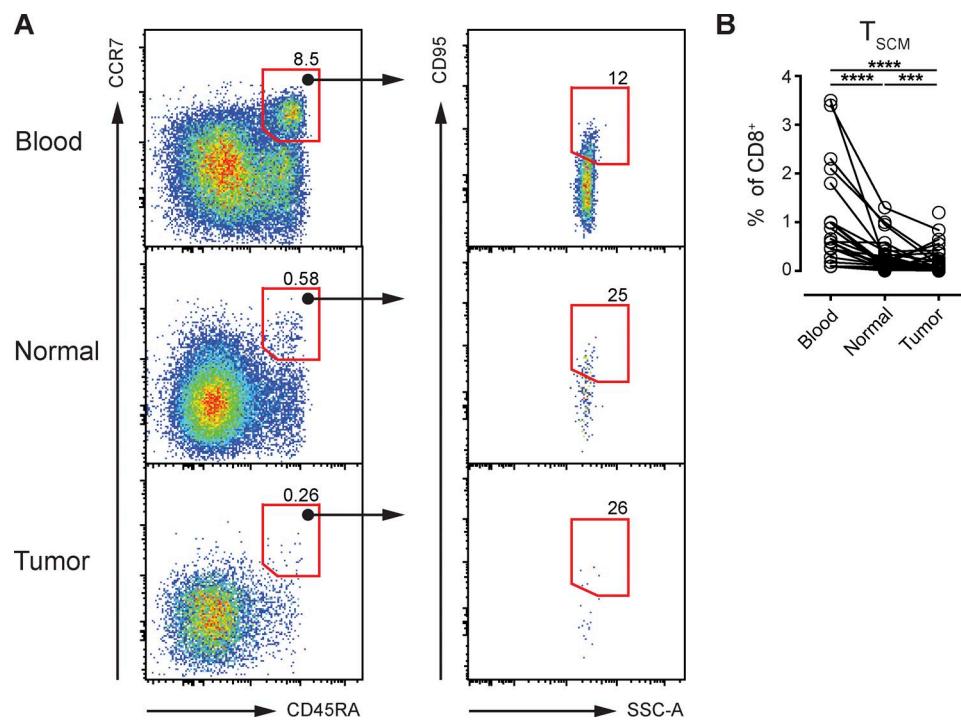


Figure S2. Bona fide CD8⁺ T_{SCM} cells are absent in lung tumors and adjacent tissues. **(A)** Representative dot plots showing the gating strategy used for the identification of T_{SCM} cells. Numbers in the dot plots indicate the percentage of cells identified by the gates. **(B)** Summary of the T_{SCM} frequency in blood ($n = 22$), normal lung tissues ($n = 45$), and NSCLC tumor ($n = 53$) samples obtained as in A. Data were obtained in six independent experiments. ***, P < 0.001; ****, P < 0.0001; paired Wilcoxon *t* test.

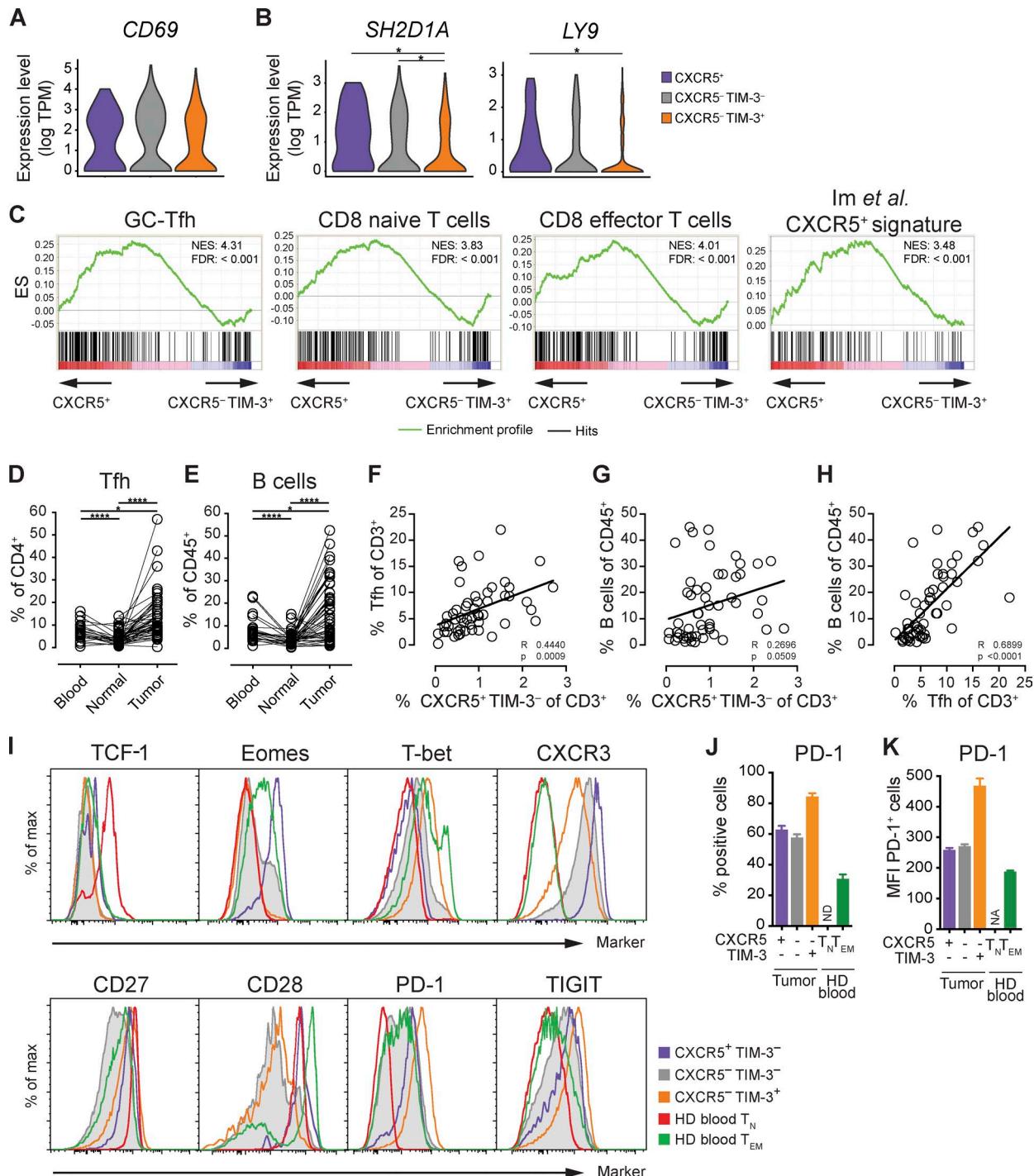


Figure S3. Gene signature of CXCR5⁺ CD8⁺ T cells resembles that of both CD4⁺ Tfh cells and memory CD8⁺ T cells. (A and B) Violin plots showing expression probability distributions of CD69 (A) and indicated Tfh-related genes (B) within the melanoma-infiltrating CXCR5⁺ ($n = 58$), CXCR5⁺ TIM-3⁻ ($n = 278$), and CXCR5⁺ TIM-3⁺ ($n = 297$) CD8⁺ T cells. *, $P < 0.05$; Wilcoxon rank sum test. (C) GSEA of the infiltrating CXCR5⁺ versus CXCR5⁺ TIM-3⁻ CD8⁺ T cells for the indicated gene sets. ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate. (D and E) Frequency of Tfh cells of CD4⁺ cells (D) and of CD19⁺ B cells out of CD45⁺ cells (E) within the blood ($n = 22$), normal lung tissues ($n = 45$), and NSCLC tumor ($n = 53$) samples. *, $P < 0.05$; ****, $P < 0.0001$; paired Wilcoxon t test. (F and G) Pearson correlation between the frequency of CXCR5⁺ TIM-3⁻ CD8⁺ T cells and that of CD4⁺ Tfh (C) and CD19⁺ B cells (D) in the NSCLC tumor samples ($n = 53$). (H) Pearson correlation between the frequency of CD4⁺ Tfh cell and that of CD19⁺ B cells in the NSCLC tumor samples ($n = 53$). (I) Representative histograms depicting expression of different markers on tumor-infiltrating CXCR5⁺ TIM-3⁻, CXCR5⁺ TIM-3⁻ and CXCR5⁺ TIM-3⁺ CD8⁺ T cells and T_N and T_{EM} CD8⁺ T cells from blood of a healthy donor (HD) as determined by flow cytometry. (J) Summary of the frequency of PD-1-expressing CXCR5⁺ TIM-3⁻, CXCR5⁺ TIM-3⁻, and CXCR5⁺ TIM-3⁺ CD8⁺ TILs ($n = 53$) and T_N and T_{EM} CD8⁺ T cells from HD blood ($n = 17$). Data are shown as mean \pm SEM. ND, not detectable. (K) Summary of the PD-1 MFI of the PD-1⁺ fraction within the CXCR5⁺ TIM-3⁻, CXCR5⁺ TIM-3⁻, and CXCR5⁺ TIM-3⁺ CD8⁺ TILs ($n = 53$) and T_N and T_{EM} CD8⁺ T cells from HD blood ($n = 17$). Data are shown as mean \pm SEM. NA, not applicable. Graphs in J and K report data performed in six independent experiments.

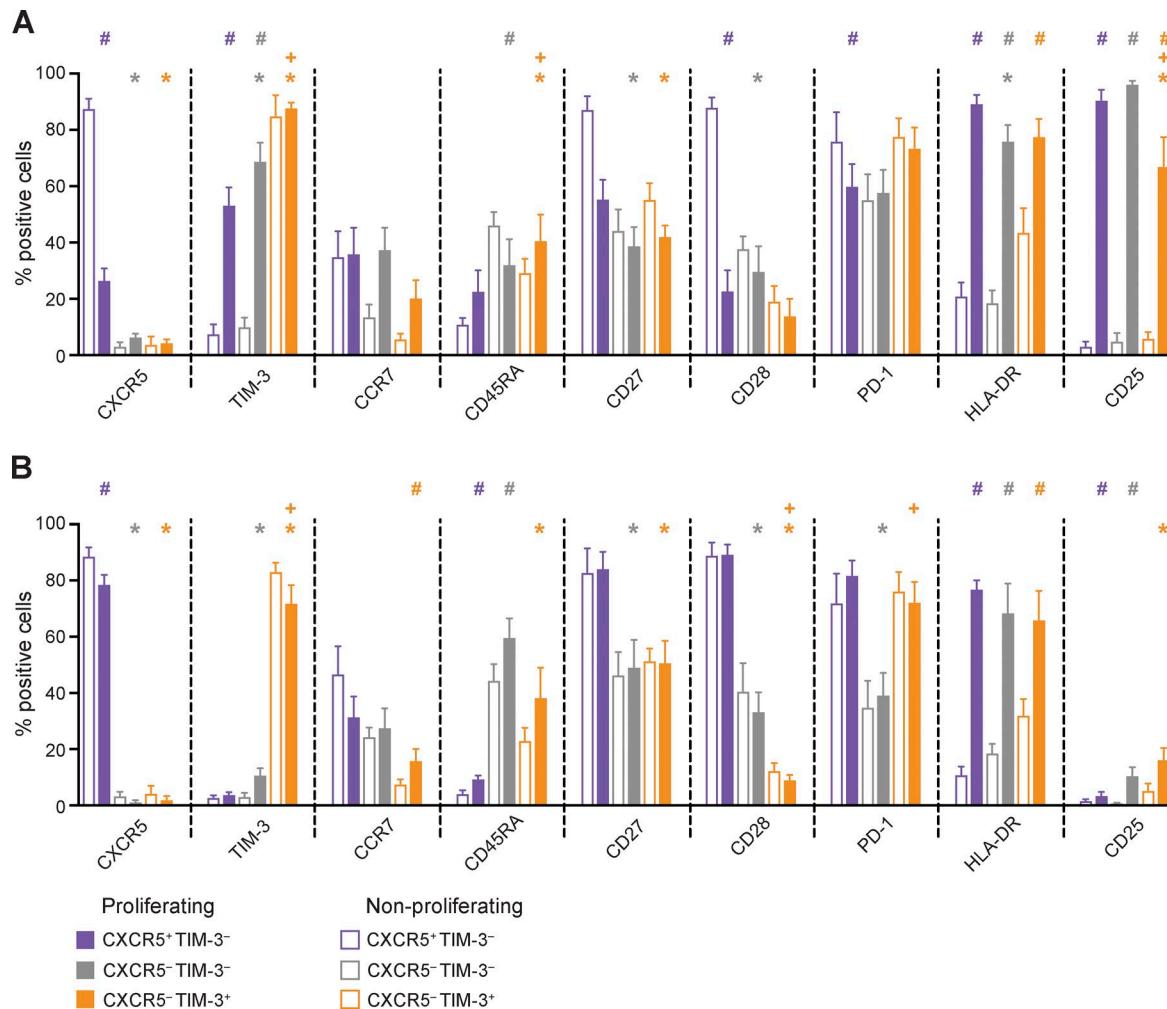


Figure S4. Proliferating CXCR5⁺ TIM-3⁻ CD8⁺ T cells are self-renewing and multipotent. (A and B) Percentage of cells expressing the indicated markers in the different subsets, as described in Fig. 5. Solid bars show the results of the proliferated cells following αCD3/CD28 + IL-2/IL-15 (A) or IL-15 (B) stimulation and open bars show the results of the non-proliferating cells of the negative control. Graphs show the summary of nine patients (nine independent experiments) after αCD3/CD28 + IL-2/IL-15 stimulation and of seven patients (six independent experiments) after IL-15 stimulation and data are shown as mean ± SEM; *, P < 0.05 versus proliferating CXCR5⁺ TIM-3⁻ CD8⁺ T cells; +, P < 0.05 versus proliferating CXCR5⁻ TIM-3⁻ CD8⁺ T cells; #, P < 0.05 versus non-proliferating cells of the same subset; paired Wilcoxon t test.

Table S1. List of antibodies used in the study

Specificity	Fluorochrome	Clone	Manufacturer	Cat. #	Lot. #	Panel
CXCR5	BV785	J252D4	BioLegend	356936	B223545	27-parameter
CCR7	BV711	150503	BD Biosciences	563921	6342963	27-parameter
CXCR3	APC	G025H7	BioLegend	353708	B208663	27-parameter
TIGIT	PerCP-eFluor710	MBSA43	eBioscience	46-9500-42	4290851	27-parameter
CD98	BB515	UM7F8	BD Biosciences	565103	6083847	27-parameter
CD73	APC-Cy7	AD2	BioLegend	344022	B221950	27-parameter
CD25	APC-R700	2A3	BD Biosciences	565106	7026658	27-parameter
TIM3	BV650	7D3	BD Biosciences	565564	6130935	27-parameter
CD57	BV605	NK-1	BD Biosciences	563895	5309627	27-parameter
PD-1	BV421	EH12.2H7	BioLegend	329919	B217576	27-parameter
CD8	BUV805	SK1	BD Biosciences	564912	6175655	27-parameter
CD95	BUV737	DX2	BD Biosciences	564710	7032582	27-parameter
HLA-DR	BUV661	G46-6	BD Biosciences	565073	6141596	27-parameter
CD4	BUV615	SK3	BD Biosciences	624297 – high parameter	6259508	27-parameter
CD45RA	BUV563	HI100	BD Biosciences	565702	6239733	27-parameter
CD69	BUV395	FN50	BD Biosciences	564364	6217783	27-parameter
CD27	BV570	O323	BioLegend	302825	B232183	27-parameter
CD71	PE-Cy5	M-A712	BD Biosciences	551143	6251869	27-parameter
Ki-67	BV480	B56	BD Biosciences	566109	6098705	27-parameter
CD3	BUV496	UCHT1	BD Biosciences	564809	6154666	27-parameter
FoxP3	PE-Cy5.5	PCH101	eBioscience	35-4776-42	4296471	27-parameter
T-bet	PE-Cy7	eBio4B10 (4B10)	eBioscience	25-5825-80	4277988	27-parameter
Eomes	PE-eFluor610	WD1928	eBioscience	61-4877-41	4288334	27-parameter
IRF4	PE	3.00E+04	eBioscience	12-9858-80	4303084	27-parameter
CD56	PE-CF594	B159	BD	562289	6203721	Immune populations
CD16	PE-Cy7	3G8	BD	557744	6237704	Immune populations
CD11b	PE	ICRF44	BD	555388	6133542	Immune populations
CD3	BV650	7D3	BioLegend	317324	B215924	Immune populations
CD19	APC-Vio770	LT19	Miltenyi Biotec	130-096-643	5161017055	Immune populations
NKp46	BV421	9E2	BioLegend	331914	B225814	Immune populations
CD45	PerCP-Cy5.5	HI30	eBioscience	45-0459-42	4291453	Immune populations
CXCR5	APC	J252D4	BioLegend	356908	B233003	Proliferation
CCR7	BV711	150503	BD	563921	6342963	Proliferation
TIGIT	PerCP-eF710	MBSA43	eBioscience	46-9500-42	4290851	Proliferation
CD27	PE	M-T271	BD	555441	6167952	Proliferation
HLA-DR	APC-H7	G46-6	BD	561358	6034926	Proliferation
CD25	APC-R700	2A3	BD	561506	7026658	Proliferation
CD8	BV786	RPA-T8	BD	563823	6154702	Proliferation
TIM3	BV650	7D3	BD	565564	7123522	Proliferation
CD57	BV605	NK-1	BD	563895	5309627	Proliferation
PD-1	BV421	EH12.2H7	BioLegend	329920	B225208	Proliferation
CD28	BUV737	Cd28.2	BD	564438	6319697	Proliferation
CD45RA	BUV563	HI100	BD	565702	7219651	Proliferation

Table S1. List of antibodies used in the study (Continued)

Specificity	Fluorochrome	Clone	Manufacturer	Cat. #	Lot. #	Panel
CD38	BUV496	HIT2	BD	564657	7125745	Proliferation
CD69	BUV395	FN50	BD	564364	7108931	Proliferation
CD127	PE-Cy5	eBioRDR5	eBioscience	15-1278-42	4303360	Proliferation
CXCR5	APC	J252D4	BioLegend	356908	B233003	Additional characterization
CCR7	BV711	150503	BD	563921	6342963	Additional characterization
CD27	APC eFluor780	O323	eBioscience	E08451-1637	E08451-1637	Additional characterization
CD28	BV786	CD28.2	BioLegend	B233916	B233916	Additional characterization
TIM3	BV650	7D3	BD	565564	6130935	Additional characterization
CD4	BV570	RPA-T4	Biolegend	300534	B222263	Additional characterization
CD45RA	BV421	HI-100	BD	562885	5355746	Additional characterization
CD8	BUV805	SK1	BD	564912	6175655	Additional characterization
CD95	BUV737	DX2	BD	564710	7032582	Additional characterization
CD127	PECy5	eBioRDR5	eBioscience	15-1278-42	4303360	Additional characterization
PD-1	PeCy7	EH12.2H7	Biolegend	329918	B203832	Additional characterization
CD3	BUV496	UCHT1	BD	564809	6254666	Additional characterization
TCF1	PE	S33-966	BD	564217	7005823	Additional characterization
CXCR5	APC	J252D4	BioLegend	356908	B233003	Effector functions
TIM3	BV650	7D3	BD	565564	6130935	Effector functions
CD4	BV570	RPA-T4	Biolegend	300534	B222263	Effector functions
CD8	BV786	RPA-T8	BD	563823	6154702	Effector functions
CD45RA	APC-H7	HI-100	BD	560674	7047723	Effector functions
CD107	PE-Cy5	H4A3	BD	555802	5023614	Effector functions
IL-2	BV421	5344111	BD	562914	4324884	Effector functions
IFNy	PE-Cy7	B27	BioLegend	506518	B182253	Effector functions
TNFα	BUV395	MAB11	BD	563996	7089866	Effector functions