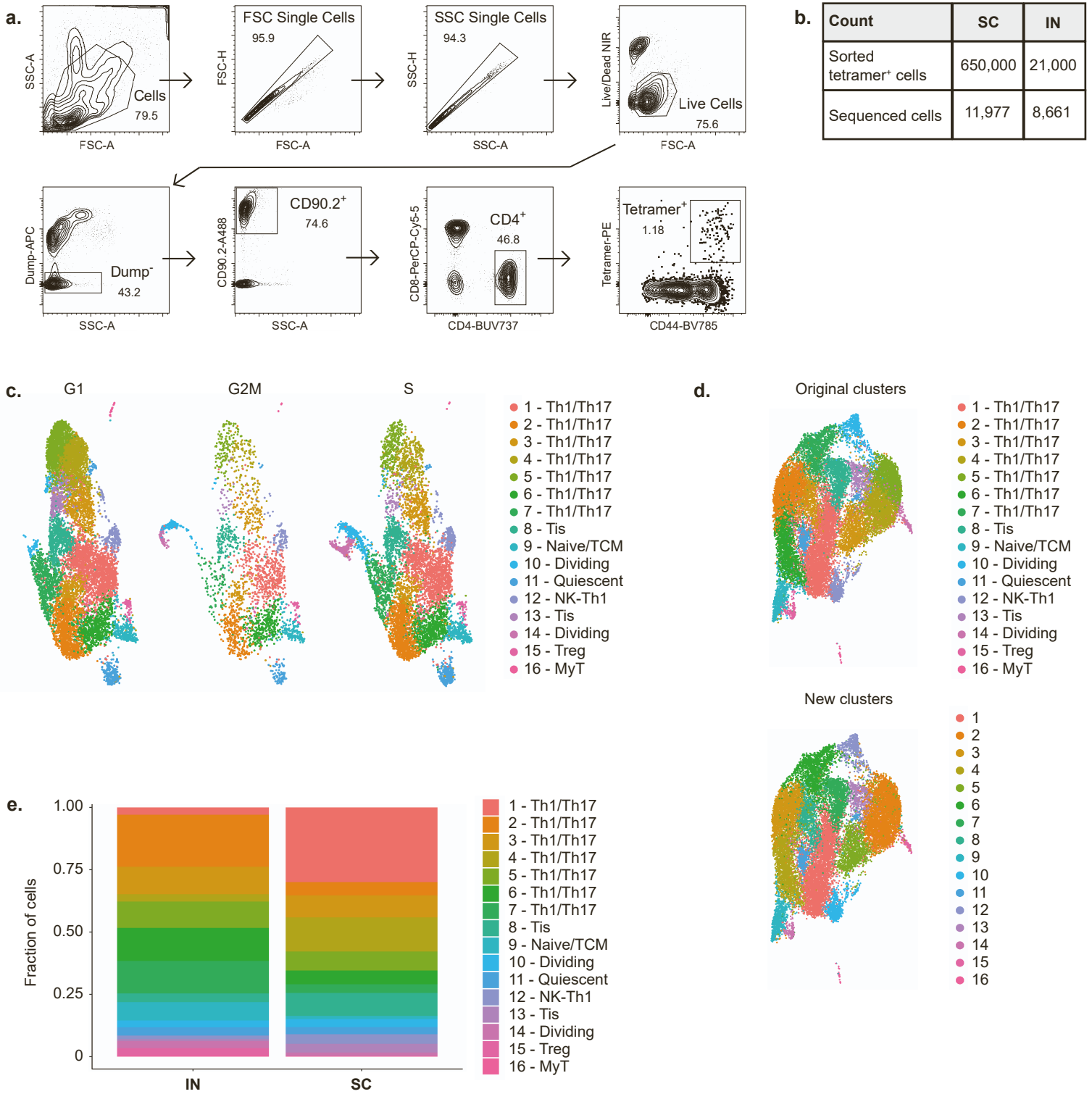


**iScience, Volume 25**

**Supplemental information**

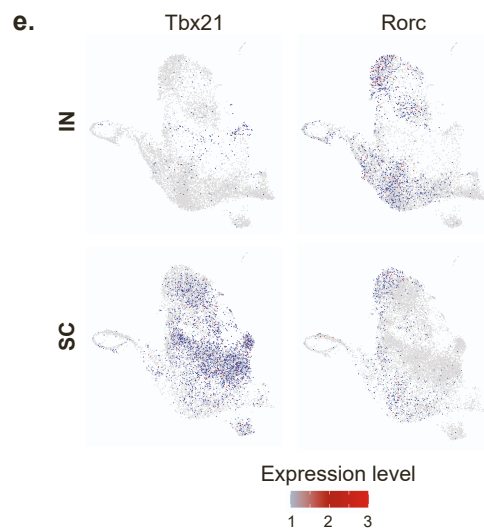
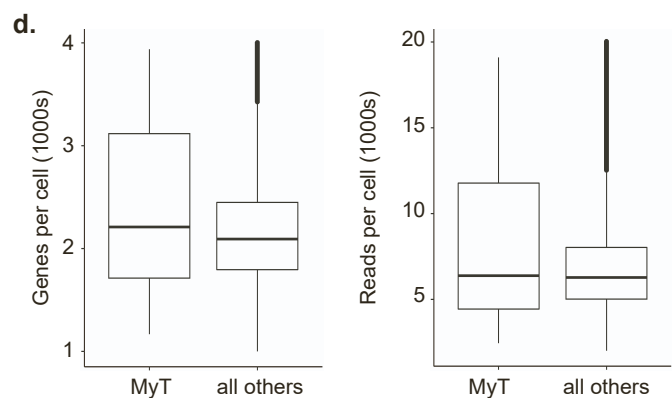
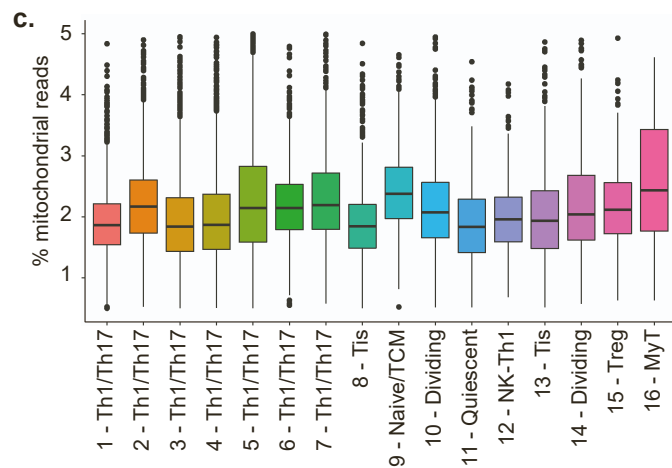
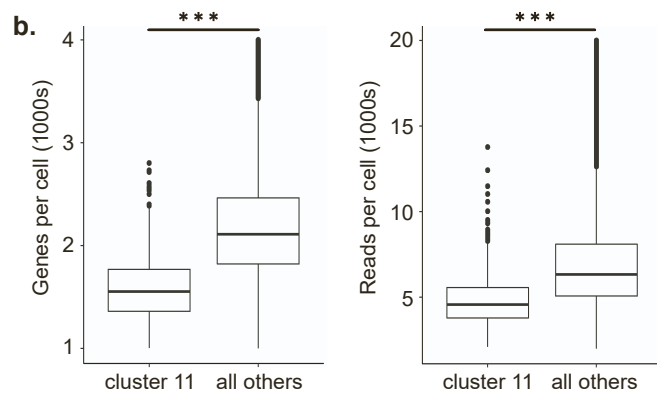
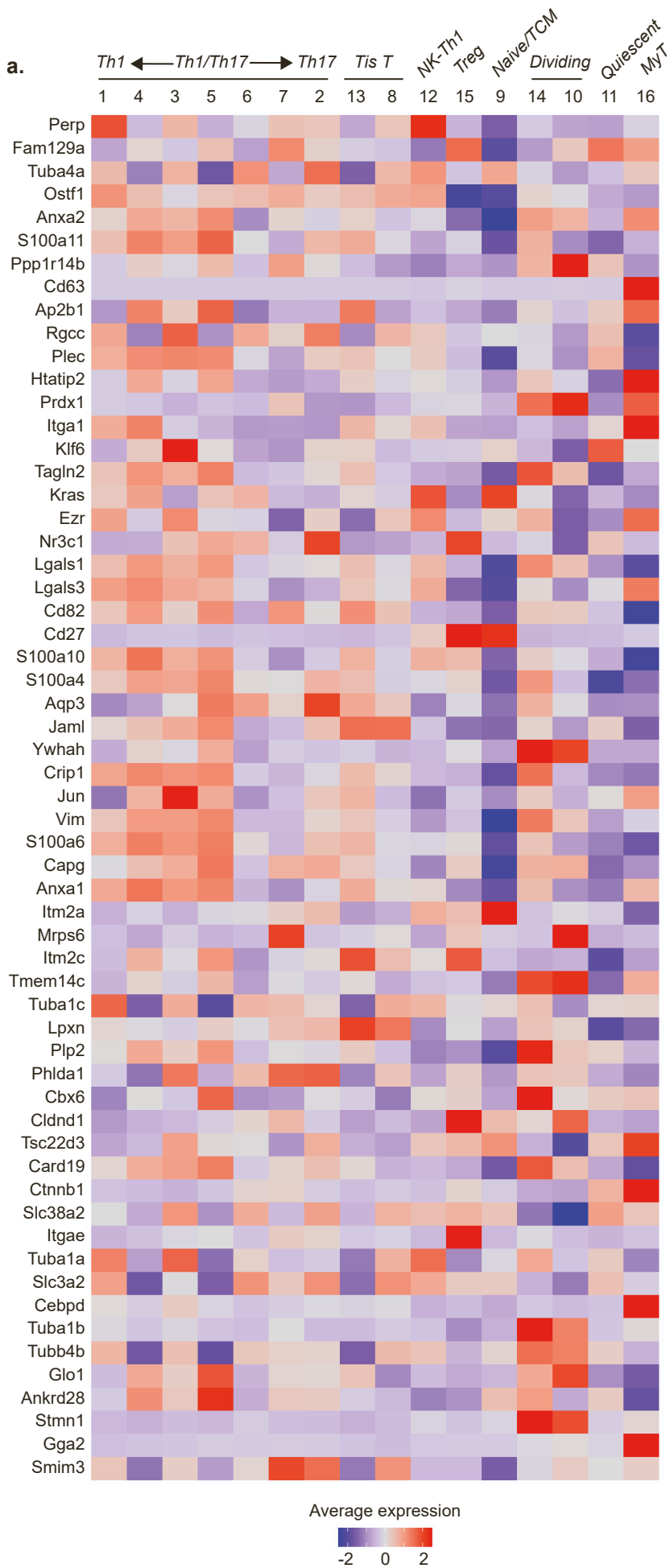
**Distinctive populations of CD4<sup>+</sup>T cells  
associated with vaccine efficacy**

**Therese Woodring, Colin N. Dewey, Lucas Dos Santos Dias, Xin He, Hannah E. Dobson, Marcel Wüthrich, and Bruce Klein**



**Supplemental Figure 1, related to Figure 3.**

**Cellular data and cluster assignment for scRNAseq.** (a) Flow cytometry plots for selection of tetramer+ CD4+ T cells from IN sample. (b) Counts of sorted and sequenced tetramer-positive CD4+ cells from SC and IN samples. (c) UMAP for cell cycle phases. Cells in all stages are distributed across all clusters. (d) UMAP after regression of cell cycle genes shows cohesive clusters with both the original cluster assignments prior to regression (top) and new cluster assignments after regression (bottom). (e) Stacked bar plot depicting relative abundance of each cluster within the IN and SC groups.

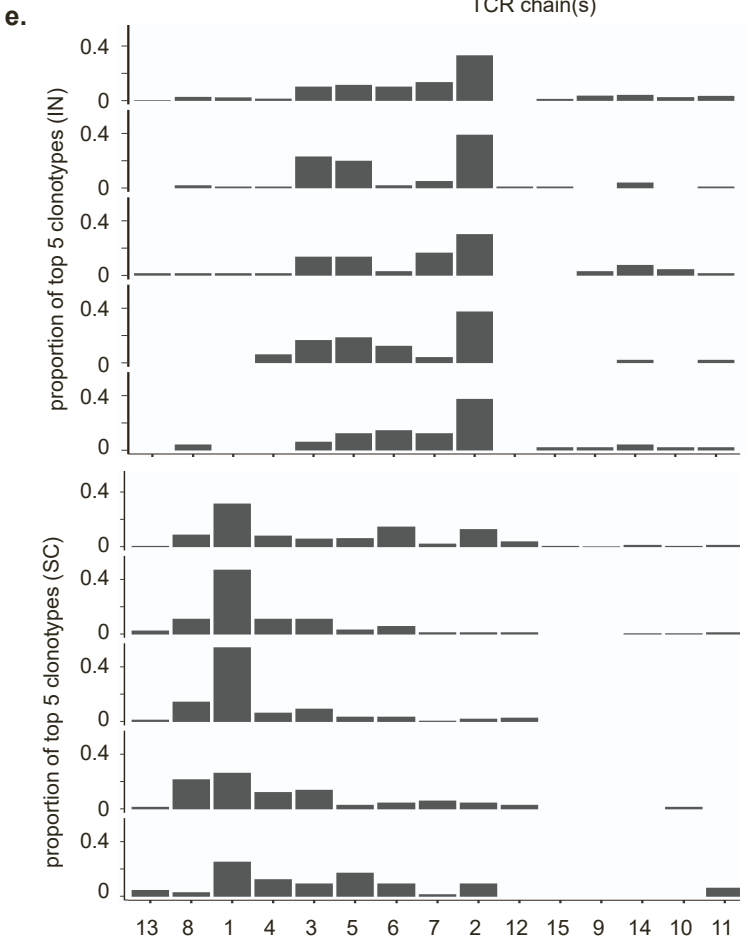
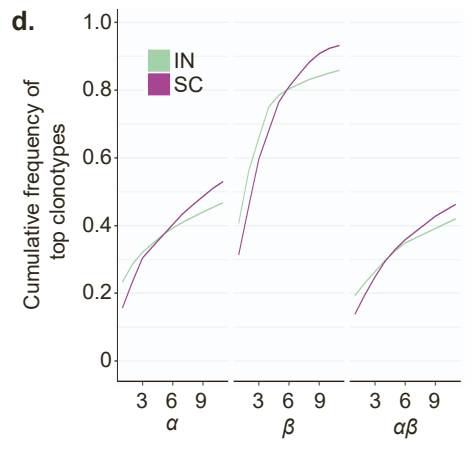
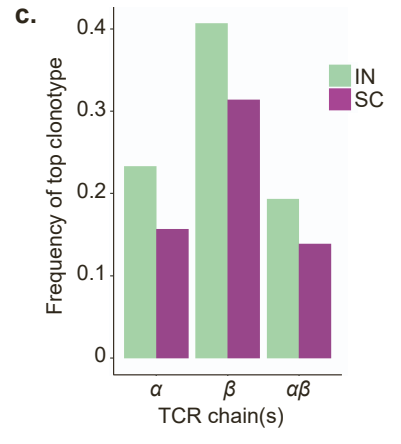
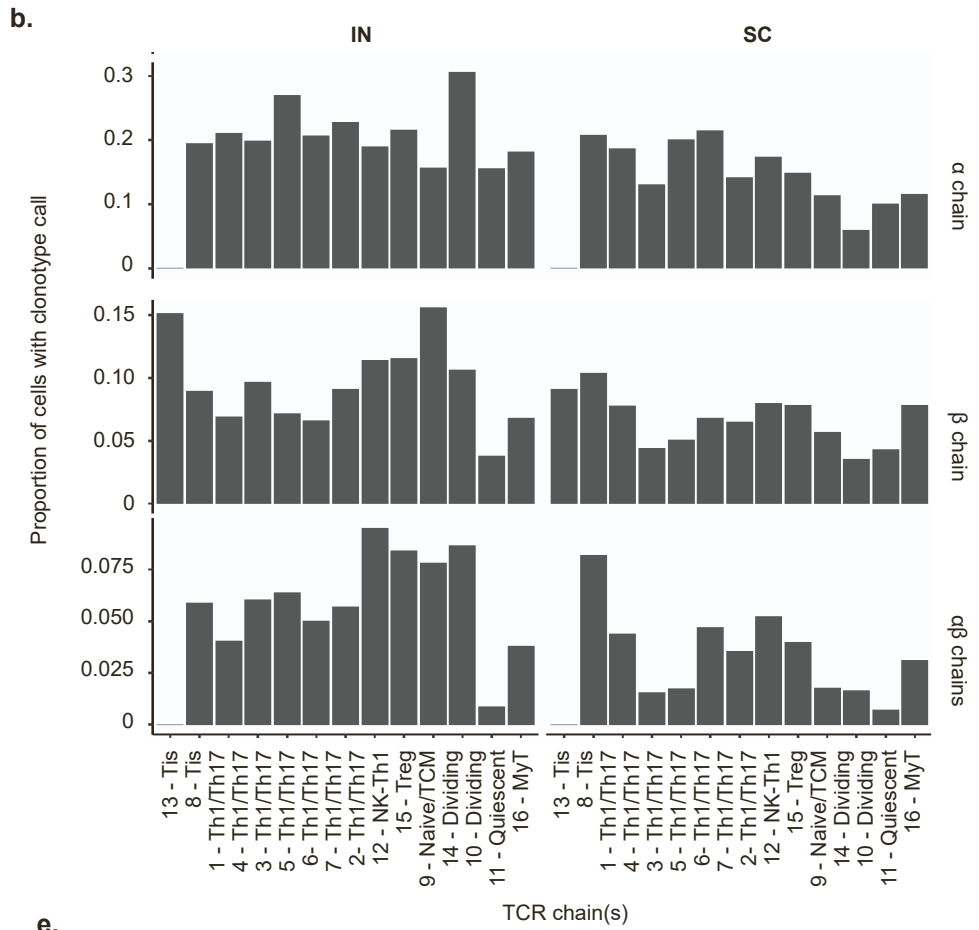


**Supplemental Figure 2, related to Figure 3.**

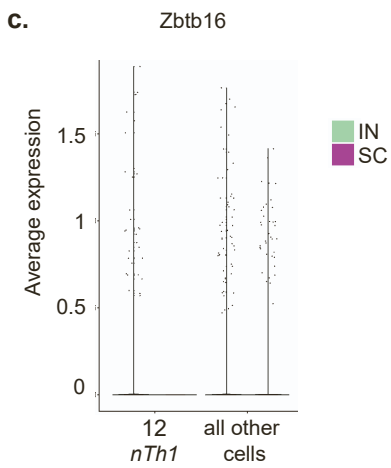
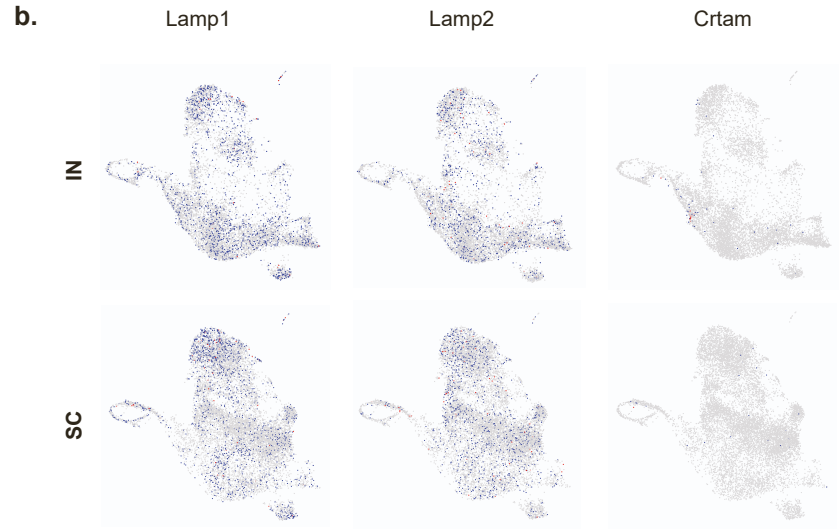
**Cluster abundance and gene expression between SC and IN vaccinated mice.** (a) Heatmap showing average expression of tissue-associated memory T cell genes involved in cytoskeleton, cell matrix, membrane scaffolding and adhesion. Gene list adapted from Szabo et al. (Szabo et al., 2019). The cluster with conspicuous downregulation of these tissue-associated genes corresponds to the n-äive/TCM population (cluster 9). (b) Comparison of the number of detected genes and reads in quiescent cells (cluster 11) compared to other clusters. Both were significantly lower in cluster 11 cells (genes,  $P=1.5 \times 10^{-174}$ ; reads,  $P=6.1 \times 10^{-103}$ ). P-values generated with Mann-Whitney test (\*\*\*) =  $P < 0.001$ ). (c) Less than 5% of reads mapped to mitochondrial genes across all clusters. Cluster 11 cells were comparable to other clusters for percent mitochondrial reads. (d) Comparison of the number of detected genes and reads in myeloid-like T cells (MyT, cluster 16) compared to other clusters. There was no significant increase in gene or read counts to suggest doublets within this population (genes,  $P=0.17$ ; reads,  $P=0.39$ ). P-values generated with Mann-Whitney test. (e) UMAP for Th1 transcription factor Tbx21 (Tbet) and Th17 transcription factor Rorc (ROR $\gamma$ t).

**a.**

Sample	Total $\alpha\beta$ T cells analyzed	# cells with $\alpha$ chain call	# cells with $\beta$ chain call	# cells with both chains called
IN	2421	1880	735	212
SC	2619	1933	822	173

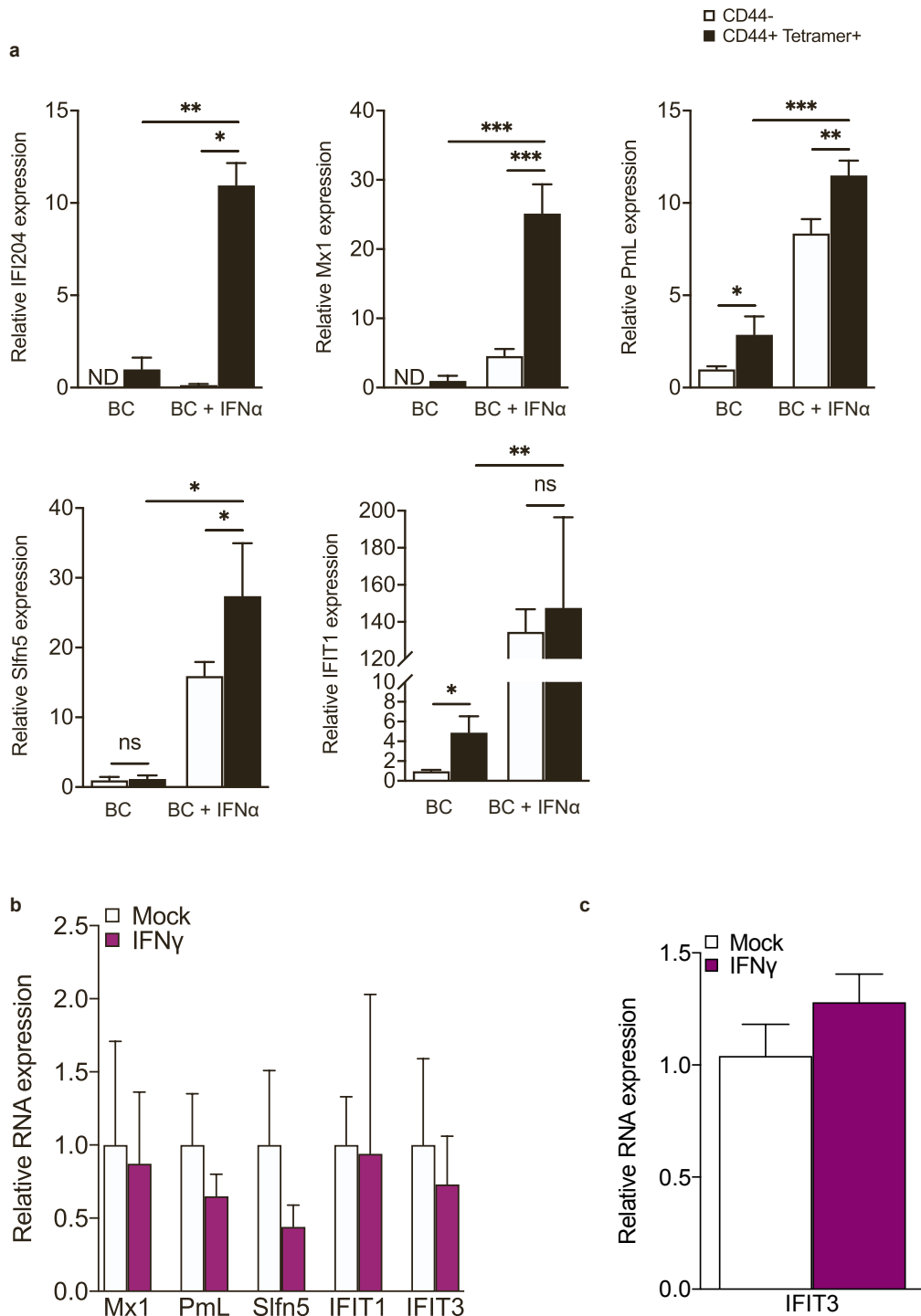


**Supplemental Figure 3, related to Figure 4.**  
**TCR clonotype diversity in IN and SC vaccinated mice.** (a) Total cells with  $\alpha$ ,  $\beta$ , or  $\alpha\beta$  TCR clonotype calls using TRUST4 in IN and SC samples. (b) Proportion of cells within each cluster with an  $\alpha$ ,  $\beta$ , or  $\alpha\beta$  TCR clonotype call. (c) Frequency of the most abundant  $\alpha$ ,  $\beta$ , or  $\alpha\beta$  TCR clonotype was higher IN than SC groups. (d) Cumulative frequency of the top  $\alpha$ ,  $\beta$ , or  $\alpha\beta$  TCR clonotypes. (e) The proportion of the 5 most abundant TCR  $\alpha$ -chain clonotypes within each cluster.



**Supplemental Figure 4, related to Figure 5.**

Tregs and NK-like Th1 cells in IN and SC vaccinated mice. (a) UMAP for expression of T cell anergy gene Rnf128 (GRAIL), separated by IN and SC samples. (b) Violin plot showing lack of expression of innate-like T cell gene Zbtb16 (PZLF) in NK-like Th1 and all other cells.



**Supplemental Figure 5, related to Figure 7.**

**RT-PCR Tis T cell signature.** (a) Quantitative reverse transcriptase PCR (RT-qPCR) detects increased expression of Tis T cell marker genes (*Ifi204*, *Mx1*, *Pml*, *Slfn5*, *Ifit1*) in tetramer-positive, CD44-positive (black) cells compared to control CD44-negative (white) cells from the spleen of *Blastomyces*-challenged, subcutaneously vaccinated mice. The addition of IFN $\alpha$  increased expression of these genes in both tetramer-positive and control cells. (b) RT-qPCR for Tis T cell marker genes in tetramer-positive, CD44-positive cells from the spleen of *Blastomyces*-challenged, subcutaneously vaccinated mice do not increase 12h after IFN $\gamma$  stimulation compared to those left unstimulated (Mock). (c) RT-qPCR for *Ifit3* in tetramer-positive, CD44-positive cells from the lungs of *Blastomyces*-challenged, subcutaneously vaccinated mice do not increase 12h after IFN $\gamma$  stimulation compared to those left unstimulated (Mock). The data in panels above were analyzed by two-tailed Student's t-test. Data are from one representative experiment of three performed and displayed as the mean  $\pm$  SEM. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

Cluster	All cells	Percent (All)	IN	Percent (IN)	SC	Percent (SC)
1	3842	18.6%	254	2.9%	3588	30.0%
2	2453	11.9%	1807	20.9%	646	5.4%
3	2005	9.7%	956	11.0%	1049	8.8%
4	1910	9.3%	259	3.0%	1651	13.8%
5	1829	8.9%	916	10.6%	913	7.6%
6	1799	8.7%	1143	13.2%	656	5.5%
7	1556	7.5%	1133	13.1%	423	3.5%
8	1394	6.8%	301	3.5%	1093	9.1%
9	795	3.9%	636	7.3%	159	1.3%
10	632	3.1%	242	2.8%	390	3.3%
11	627	3.0%	285	3.3%	342	2.9%
12	577	2.8%	125	1.4%	452	3.8%
13	485	2.4%	65	0.8%	420	3.5%
14	416	2.0%	253	2.9%	163	1.4%
15	274	1.3%	253	2.9%	21	0.2%
16	44	0.2%	33	0.4%	11	0.1%
<b>Total</b>	<b>20,638</b>	<b>100%</b>	<b>8661</b>	<b>100%</b>	<b>11,977</b>	<b>100%</b>

**Supplemental Table 1, related to Fig. 1.** Number and percent of cells in each cluster overall (All cells) and in intranasally vaccinated (IN) and subcutaneously vaccinated (SC) mice.



gene symbol	avg_log2FC	pct.SC	pct.IN	gene symbol	avg_log2FC	pct.SC	pct.IN
Il17a	-2.939	0.166	0.71	Nkg7	1.944	0.668	0.118
Il17f	-1.876	0.062	0.398	Rps17	-0.532	0.92	0.972
Il18rap	0.858	0.532	0.208	Ifitm2	1.788	0.437	0.047
Ctla4	-1.262	0.483	0.75	Ifitm3	2.028	0.453	0.04
Ramp1	-1.120	0.396	0.709	Ifngr1	0.595	0.919	0.823
Rgs1	-0.875	0.908	0.983	Rps12	-0.406	0.998	0.999
Rgs16	-1.117	0.217	0.472	Lilr4b	0.859	0.429	0.175
Vim	0.685	0.988	0.939	Rps15	-0.640	0.981	0.996
Rpl12	-0.549	0.995	0.998	Gadd45b	-0.961	0.745	0.88
Itga4	-1.062	0.246	0.659	Ifng	2.193	0.63	0.142
AA467197	1.762	0.73	0.164	Rps26	-0.449	0.996	0.998
Atp8b4	0.707	0.345	0.04	Dusp4	-1.012	0.118	0.496
Dusp2	1.303	0.77	0.604	Uba52	-0.361	0.982	0.996
1110034G24Rik	-0.528	0.065	0.277	Junb	-0.632	0.979	0.996
Pmepa1	-0.653	0.194	0.471	Znrf1	-0.665	0.605	0.817
Ar	-0.551	0.153	0.386	Gabarapl2	0.576	0.898	0.793
Car5b	0.579	0.319	0.077	Itgb1	1.457	0.909	0.582
Sema4a	0.594	0.374	0.113	Lgals3	1.996	0.81	0.284
S100a13	0.572	0.916	0.823	Dnajc15	1.188	0.799	0.427
S100a6	0.915	0.973	0.86	Epsti1	1.277	0.759	0.345
S100a10	0.787	0.992	0.966	Rps25	-0.362	0.99	0.997
Cd2	0.645	0.889	0.753	Nptn	0.740	0.65	0.377
Csf1	0.690	0.266	0.035	Rplp1	-0.506	0.999	1
Rps20	-0.423	0.998	0.999	Nt5e	-1.121	0.155	0.571
Tox	-0.772	0.058	0.375	Chst2	-0.586	0.098	0.343
Rps8	-0.308	0.999	1	Gpx1	-0.856	0.62	0.834
Sh3bgrl3	0.678	0.993	0.972	Ccr4	-0.547	0.053	0.284
Rcc2	-0.676	0.412	0.644	Cxcr6	0.701	0.881	0.674
Fosl2	-0.784	0.41	0.655	Ccr2	0.900	0.69	0.327
Arap2	0.626	0.66	0.435	Ramp3	-0.708	0.11	0.354
Rpl5	-0.548	0.989	0.997	Hs3st3b1	0.530	0.294	0.083
Selplg	0.604	0.876	0.736	Rpl23a	-0.461	0.969	0.988
Ndufa4	-0.893	0.468	0.801	Ccl5	2.212	0.327	0.071
1810058I24Rik	0.697	0.709	0.5	Hlf	-0.931	0.113	0.45
Zyx	0.768	0.581	0.306	Tbx21	0.841	0.397	0.052
Arl6ip5	0.724	0.842	0.662	Ikzf3	-1.184	0.153	0.597
Slc2a3	0.727	0.488	0.198	Gna13	-0.812	0.769	0.912
Klrd1	1.109	0.339	0.04	Timp2	-0.473	0.103	0.319
Klrk1	1.137	0.552	0.15	Serpinb9	0.692	0.313	0.097
Klrc2	0.842	0.313	0.016	Odc1	-0.732	0.811	0.935
Klrc1	1.344	0.479	0.026	Ahr	-0.605	0.104	0.354
Ybx3	0.663	0.71	0.487	Tspan13	-0.813	0.255	0.588
Kcnn4	0.662	0.694	0.471	Rps29	-0.335	1	1
Zfp36	-1.032	0.499	0.76	Zfp36l1	-0.753	0.758	0.893
Fxyd5	0.584	0.976	0.949	Fos	-0.864	0.567	0.812

**Supplemental Table 2, related to Fig. 3.** Top differentially expressed genes in subcutaneously inoculated mice (SC) compared to intranasally inoculated mice (IN). All differences in expression are statistically significant ( $P < 0.01$ ). avg\_log2FC = average log2 fold change, where positive denotes genes upregulated SC. Pct.SC = percent of cells expressing gene SC. Pct.IN = percent of cells expressing gene IN.

gene symbol	avg_log2FC	pct.Tis	pct.other	gene symbol	avg_log2FC	pct.Tis	pct.other
Stat1	1.463588	0.874	0.43	Dhx58	0.552192	0.271	0.034
Sp100	1.021642	0.845	0.538	Ifi35	0.956346	0.698	0.314
Ifi206	1.364668	0.678	0.143	Lgals3bp	1.005164	0.608	0.206
Ifi214	1.158886	0.49	0.057	Rnf213	1.640465	0.742	0.163
Ifi213	1.474788	0.673	0.102	Rsad2	1.154667	0.393	0.025
Ifi209	1.280381	0.672	0.194	Cmpk2	1.025174	0.437	0.046
Ifi208	1.29585	0.605	0.12	Ly6a	1.384124	0.957	0.654
Ifi204	1.342273	0.426	0.025	Rtp4	1.447903	0.635	0.068
Mndal	1.548596	0.935	0.601	Parp14	1.16424	0.624	0.203
Ifi211	0.920783	0.309	0.043	Dtx3l	0.876607	0.518	0.146
Ifi203	1.643232	0.941	0.567	Parp9	0.847324	0.507	0.13
Ifih1	0.869165	0.407	0.061	Mx1	0.997256	0.318	0.023
B2m	0.671886	1	0.999	H2-T23	0.947757	0.987	0.902
Samhd1	1.457596	0.89	0.557	Eif2ak2	0.607137	0.297	0.054
Zbp1	1.701802	0.831	0.235	AW112010	1.148043	0.988	0.867
Gbp7	1.141752	0.65	0.229	Ms4a4c	1.125778	0.44	0.099
Gbp2	1.330516	0.529	0.141	Ms4a4b	1.308764	0.972	0.758
Ddx58	0.906377	0.54	0.158	Ms4a6b	1.070097	0.972	0.789
lsg15	2.651938	0.891	0.252	Ifit3	1.700427	0.434	0.02
Gbp9	0.653588	0.332	0.07	Ifit1	1.757272	0.53	0.032
Gbp6	0.927166	0.502	0.124	Psmb8	0.797364	0.98	0.893
Oasl2	0.964412	0.379	0.028	Stat2	0.674791	0.383	0.098
Oas3	0.925822	0.461	0.058	Ube2l6	0.770356	0.356	0.089
Trafd1	1.021619	0.578	0.18	Psmb10	0.93669	0.79	0.461
Samd9l	1.105671	0.72	0.339	Slfm2	0.957202	0.92	0.696
Herc6	1.191133	0.596	0.137	Irf9	0.744718	0.542	0.201
Usp18	0.842146	0.324	0.017	Daxx	0.686932	0.388	0.109
lsg20	1.766755	0.779	0.219	Psme1	0.798974	0.944	0.782
Trim30a	1.47597	0.761	0.21	Gbp4	0.901133	0.565	0.226
Trim30d	0.584551	0.266	0.04	H2-T22	0.911738	0.836	0.586
Ifitm3	2.196072	0.63	0.245	H2-K1	0.49828	1	0.999
Irf7	1.619405	0.811	0.229	9930111J21Rik2	0.925375	0.751	0.42
Ddx60	0.655933	0.3	0.043	Tapbp	0.888208	0.798	0.541
Bst2	2.361482	0.96	0.521	Parp10	0.597853	0.401	0.125
Phf11b	1.275133	0.654	0.219	Helz2	0.610078	0.371	0.11
Epsti1	1.175369	0.898	0.554	Chmp4b	0.756142	0.923	0.777
Pml	0.653543	0.36	0.083	Psme2	0.722376	0.897	0.714
Shisa5	0.872388	0.994	0.942	Sp110	0.807667	0.626	0.325
Irgm1	1.015191	0.607	0.177	H2-Q4	0.668293	0.966	0.844
Ifi47	1.28474	0.726	0.266	Trim12c	0.632304	0.373	0.123
Igtp	1.217419	0.73	0.277	Trim12a	0.723719	0.563	0.26
Xaf1	1.258989	0.65	0.125	H2-D1	0.394261	1	1
Slfm5	2.008012	0.675	0.136	Ogfr	0.639643	0.544	0.257
Slfm8	0.983883	0.494	0.111	Tap1	0.693251	0.741	0.472
Slfm1	1.470985	0.75	0.227	Nampt	0.518962	0.348	0.118

**Supplemental Table 3, related to Fig. 6.** Top differentially expressed genes between Tis T cells (combined cluster 8 and 13) and all other cells. All differences in expression are statistically significant ( $P < 0.01$ ). avg\_log2FC = average log2 fold change, where positive denotes genes upregulated in Tis T cells compared to other cells. Pct.Tis = percent of cells in clusters 8 & 13 expressing gene. Pct.other = percent of cells in remaining clusters expressing gene.

gene symbol	avg_log2FC	pct.Tis.8	pct.Tis.13	gene symbol	avg_log2FC	pct.Tis.8	pct.Tis.13
H3f3b	1.736125	1	0.992	Sqstm1	1.104345	0.808	0.381
Junb	1.946537	1	0.948	Nfkbid	1.15721	0.666	0.208
Vps37b	2.251496	0.939	0.324	Irf2bp2	0.955399	0.876	0.6
Bhlhe40	2.005815	0.981	0.79	Ptp4a1	1.018083	0.758	0.338
Nfkbia	2.075163	0.995	0.808	Ctla4	1.332767	0.7	0.268
Pim1	1.994649	0.986	0.74	Srgn	0.722013	0.999	0.973
Tnfaip3	1.977649	0.958	0.48	Coq10b	0.929079	0.793	0.359
Odc1	2.346535	0.963	0.555	Cxcr4	1.266615	0.639	0.198
Pnrc1	1.484511	0.981	0.68	Hif1a	0.998846	0.913	0.612
Csrnp1	1.757204	0.877	0.247	Hilpda	1.225128	0.496	0.072
Ubc	1.303885	0.999	0.899	Rrad	1.24763	0.679	0.254
Kdm6b	1.875581	0.902	0.328	Stk17b	0.821825	0.959	0.81
Ifrd1	1.673152	0.918	0.439	Bcl2a1d	1.063756	0.841	0.544
Dusp5	1.559009	0.928	0.528	Gadd45b	1.333962	0.91	0.637
Btg1	1.071251	1	0.986	Nfkbiz	1.015138	0.653	0.235
Cdkn1a	1.759169	0.83	0.272	Dnajb9	0.948277	0.613	0.186
Rgs2	1.845596	0.925	0.526	Zfp622	0.872833	0.728	0.313
Nr4a1	1.766735	0.804	0.266	Dennd4a	1.057691	0.791	0.41
Zc3h12a	1.443751	0.758	0.192	Sub1	0.718955	0.976	0.864
Tgif1	1.413129	0.714	0.144	Wsb1	0.844474	0.742	0.33
Bcl2a1b	1.476229	0.938	0.658	Fth1	0.624196	1	0.994
Sh2d2a	1.393376	0.914	0.61	Gpr183	1.041411	0.803	0.476
Ubald2	1.533704	0.831	0.357	Tnfrsf4	1.420604	0.605	0.221
Ier5	1.233939	0.963	0.616	Atf3	1.224609	0.627	0.233
Srsf5	1.00658	0.981	0.771	Ly6a	-0.83014	0.949	0.981
Gna13	1.362324	0.912	0.548	Fosl2	1.00056	0.533	0.142
Hspa5	1.019739	0.993	0.891	Sdhaf1	0.989205	0.587	0.19
Eif1	0.612944	1	0.994	Dusp2	1.372155	0.859	0.604
Rgs1	1.595625	0.961	0.808	Mknk2	0.806657	0.808	0.449
Ddx5	0.676566	1	0.981	Skil	0.77867	0.933	0.672
Tob1	1.164373	0.795	0.278	Rinl	-0.87748	0.573	0.802
Smad7	1.081028	0.95	0.693	Sertad1	0.808728	0.652	0.247
Cish	1.082261	0.918	0.614	Gramd3	1.019667	0.732	0.379
Socs3	1.362927	0.707	0.212	Birc2	0.801778	0.807	0.478
Prdx6	1.057144	0.938	0.711	Lmnb1	0.927639	0.734	0.357
Zfp3612	1.328891	0.941	0.691	Arl6ip1	-0.7051	0.863	0.942
Hist1h1c	1.982579	0.73	0.318	Tubb4b	0.795374	0.83	0.507
Rgcc	1.262302	0.896	0.538	Ube2s	0.714336	0.881	0.623
Ier2	1.064392	0.944	0.676	Rora	0.850761	0.919	0.765
Slc3a2	1.048187	0.877	0.53	Ppp1r15a	0.802229	0.863	0.52
Nr4a3	1.165843	0.628	0.151	Arid5a	0.834068	0.702	0.322
Gpr132	1.059531	0.828	0.431	Vgll4	0.802229	0.761	0.406
Arf4	0.89394	0.966	0.736	Pde4b	0.911252	0.709	0.379
Per1	1.036051	0.613	0.146	Syt13	0.881117	0.788	0.458
Zfp3611	1.050164	0.905	0.588	Ccn1	0.774097	0.89	0.635

**Supplemental Table 4, related to Fig. 6.** Top differentially expressed genes between cluster 8 and cluster 13 Tis T cells. All differences in expression are statistically significant ( $P < 0.01$ ). avg\_log2FC = average log2 fold change, where positive denotes genes upregulated in cluster 8 compared to cluster 13. Pct.Tis.8 = percent of cells in cluster 8 expressing gene. Pct.Tis.13 = percent of cells in cluster 13 expressing gene.

Cluster 8 Tis T cells	Cluster 13 Tis T cells
Ifi213	Epsti1
Stat2	Sp100
Parp14	Nxpe3
Il18rap	Rnf213
Serpinb9	Smyd3
Gm4070	Ddx60
Slfn1	Slco3a1
Tnfrsf4	Herc6
Ddx60	Skap1
Ptp4a2	Samhd1

**Supplemental Table 5, related to Fig. 6.** Genes with the most significantly different velocities in Tis T cells, identified from analysis of unspliced reads with Velocyto. The only gene that also appears among the top differentially expressed genes between cluster 8 and 13 is *Tnfrsf4*, which is already more highly expressed in cluster 8. Data shown for Tis T cell-enriched SC sample only.