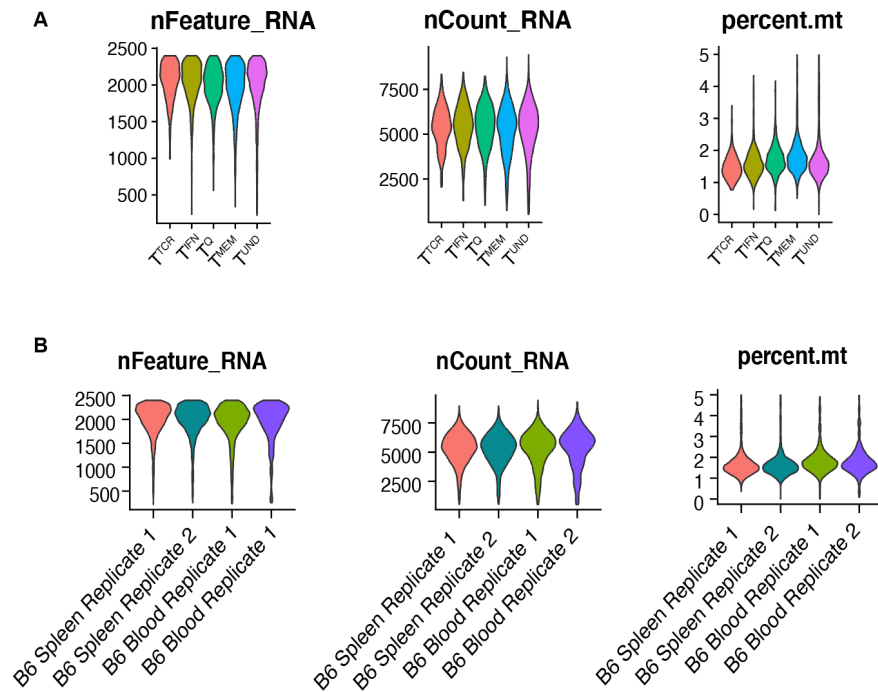




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Figure S1. Representative plots for mouse single cell quality control pipeline showing nFeature, nCount and mitochondrial content. A. nFeature, nCount and mitochondrial content per cluster after quality control filtering. **B.** nFeature, nCount and mitochondrial content separated out by tissue and donor.

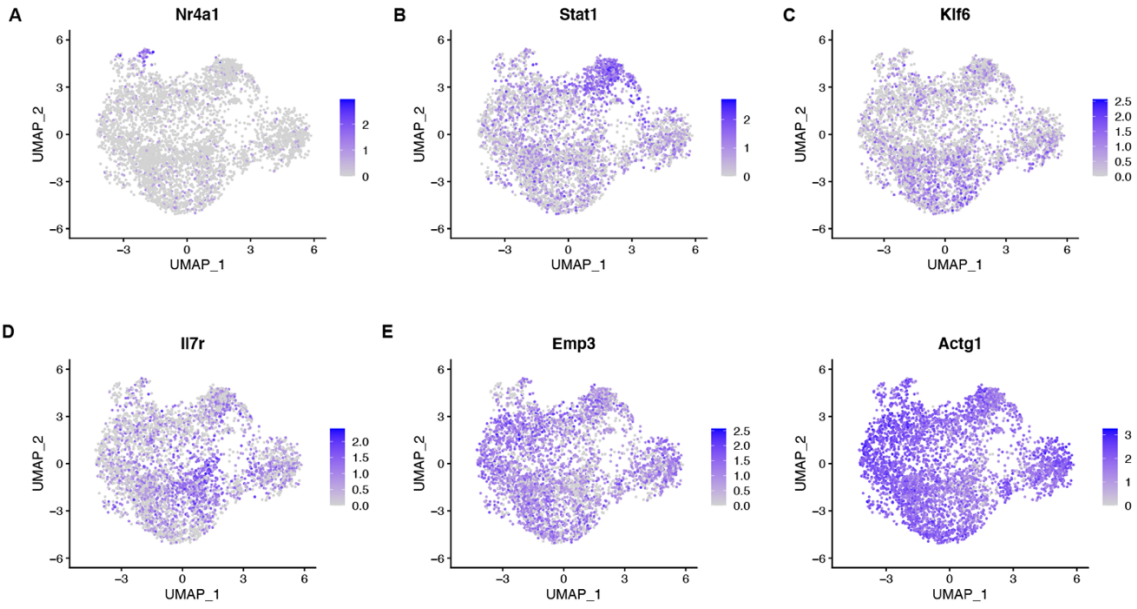
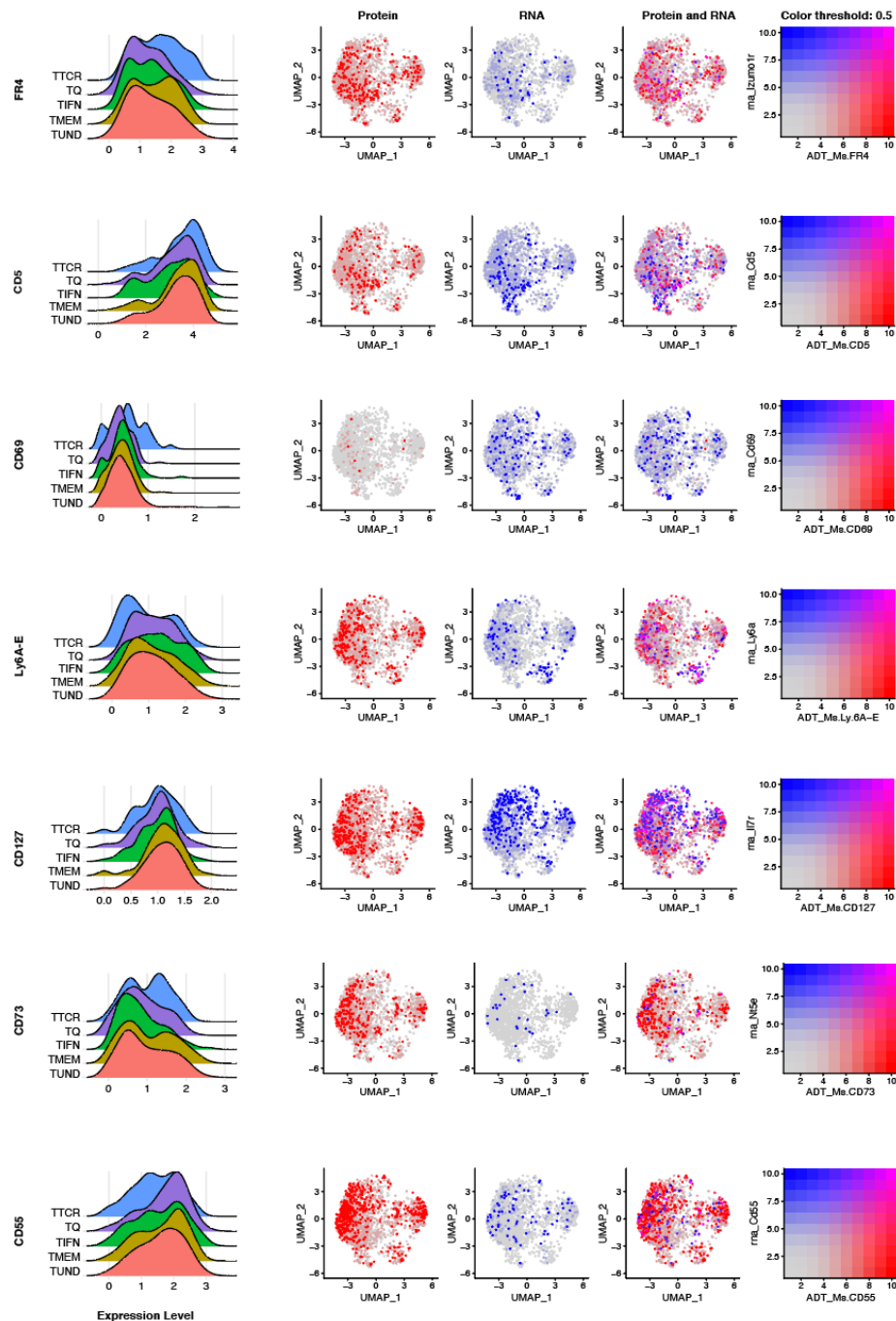


Figure S2. Feature plots of marker genes plots for mouse single cell data. Feature plot showing expression of *Nur77*, *Stat1*, *Klf6*, *Il7r*, *Emp3* and *Actg1* on UMAP.



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14 **Figure S3. CITE-seq of mouse naïve CD4⁺ T cells does not show overlap of surface markers**
 15 **with transcriptional clusters.** CITE-Seq performed on murine naïve CD4⁺ T cells isolated from
 16 C57BL/6J mice. FR4, CD5, CD69, Ly6A-E, CD127, CD73 and CD55 selected as representative
 17 markers of the CITE-Seq data. From left to right: Ridge plots showing expression of select
 18 protein markers by clusters (T^{TCR} in blue, T^Q in purple, T^{IFN} in green, T^{MEM} in brown and T^{UND} in
 19 red). Feature plots of RNA UMAP showing protein expression in red, RNA in blue and overlap of
 20 protein and RNA in purple.

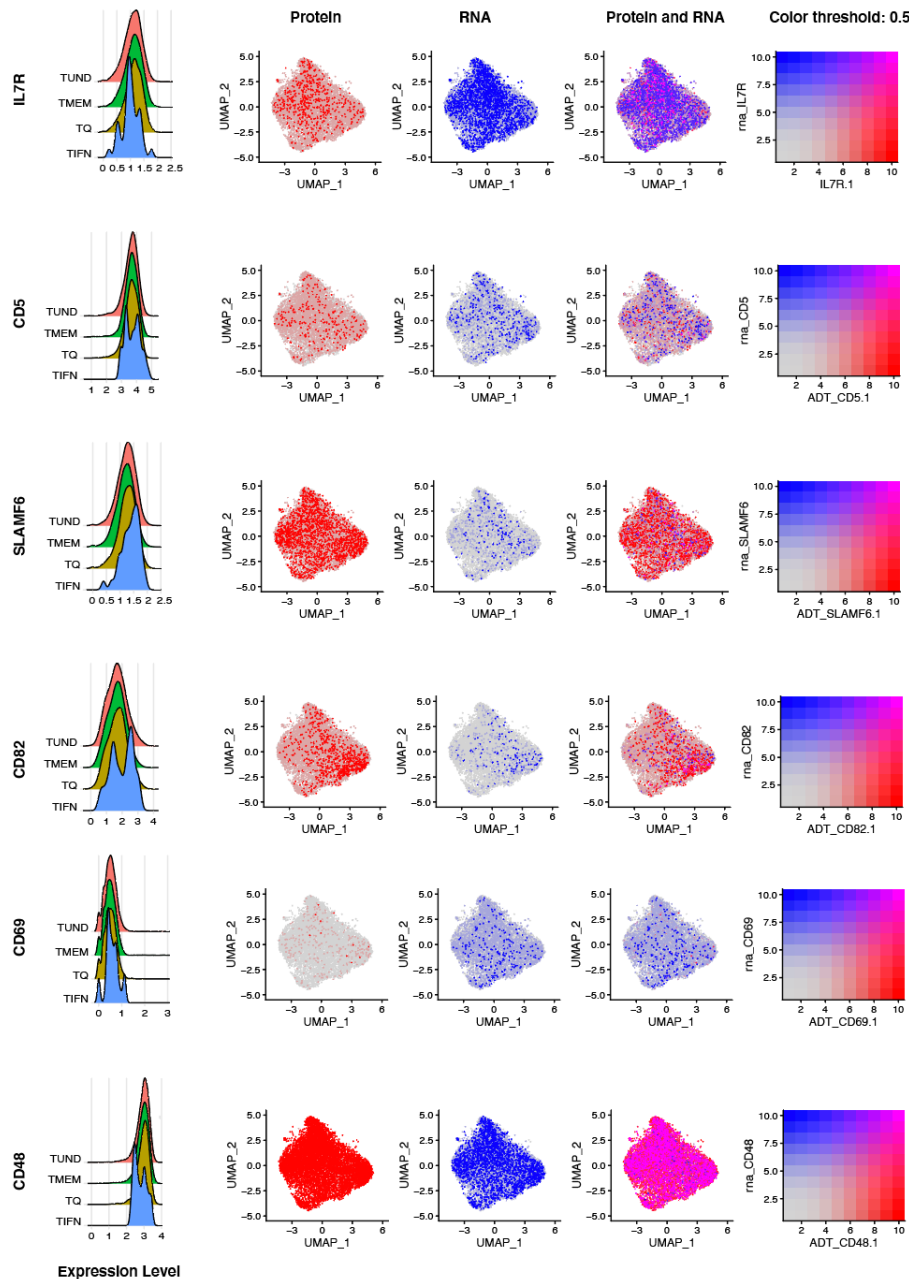
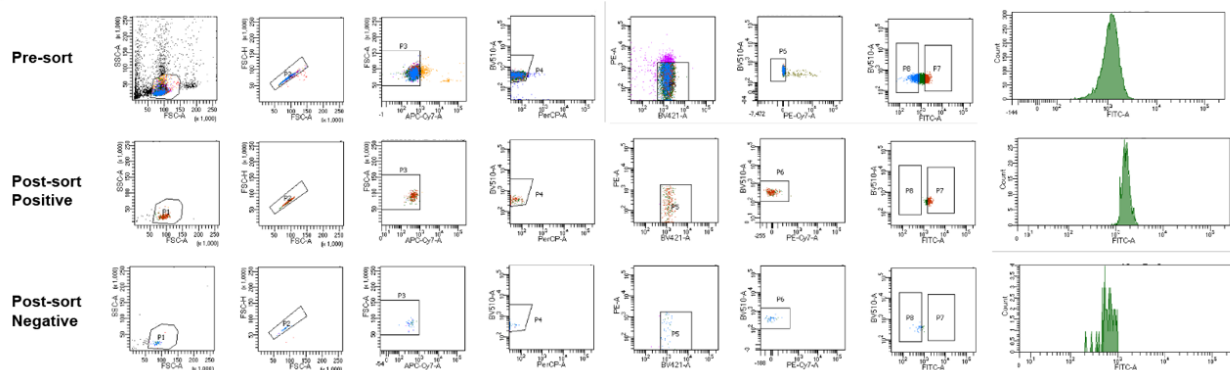


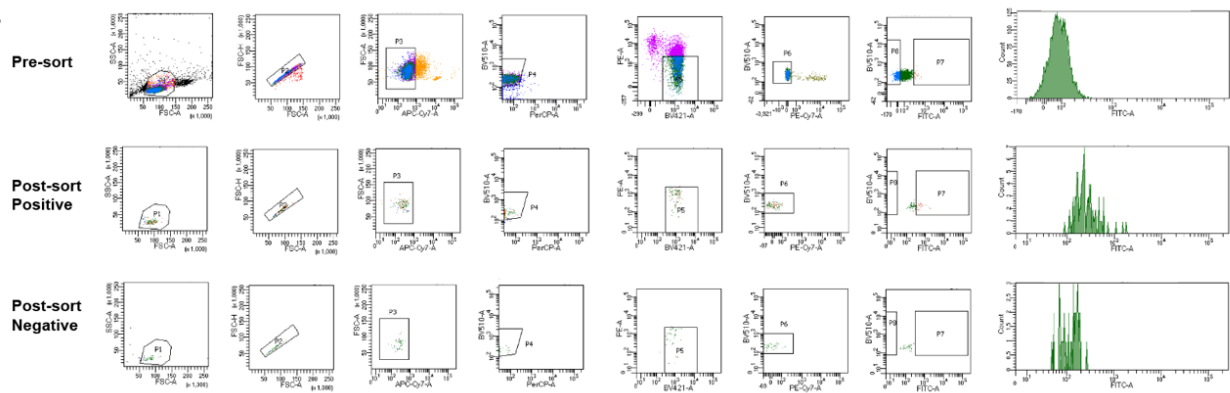
Figure S4. CITE-seq of human naïve CD4⁺ T cells doesn't show overlap of surface markers with transcriptional clusters. CITE-Seq performed on human naïve CD4⁺ T cells isolated from healthy donors. IL7R, CD5, SLAMF6, CD82, CD69 and CD48 selected as the best markers representative of the CITE-Seq data. From left to right: Ridge plots showing expression of select markers by cluster (T^Q in brown, T^{IFN} in blue, T^{MEM} in green and T^{UND} in red). Feature plots showing protein in red, RNA in blue and overlap of protein and RNA in purple.

A KLF2-GFP

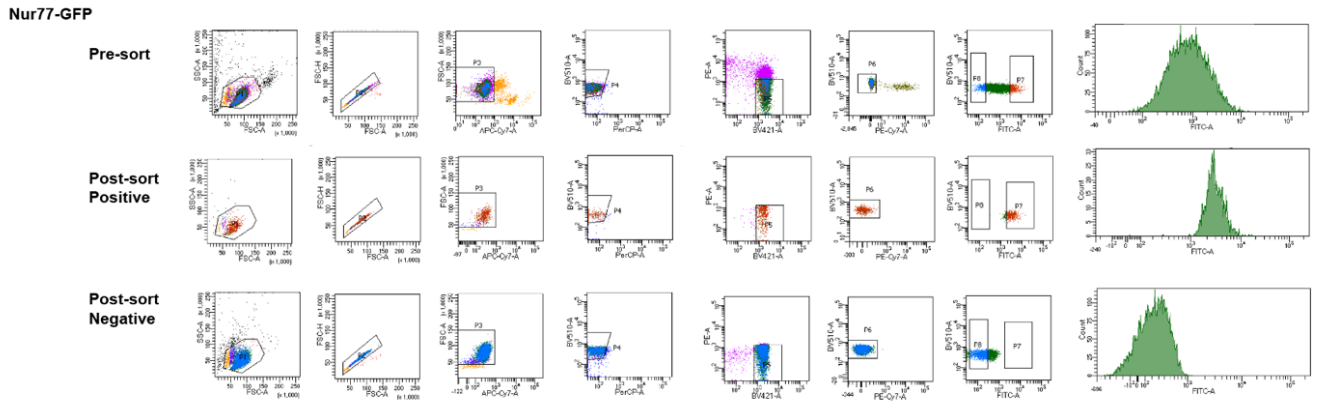


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Mx1-GFP



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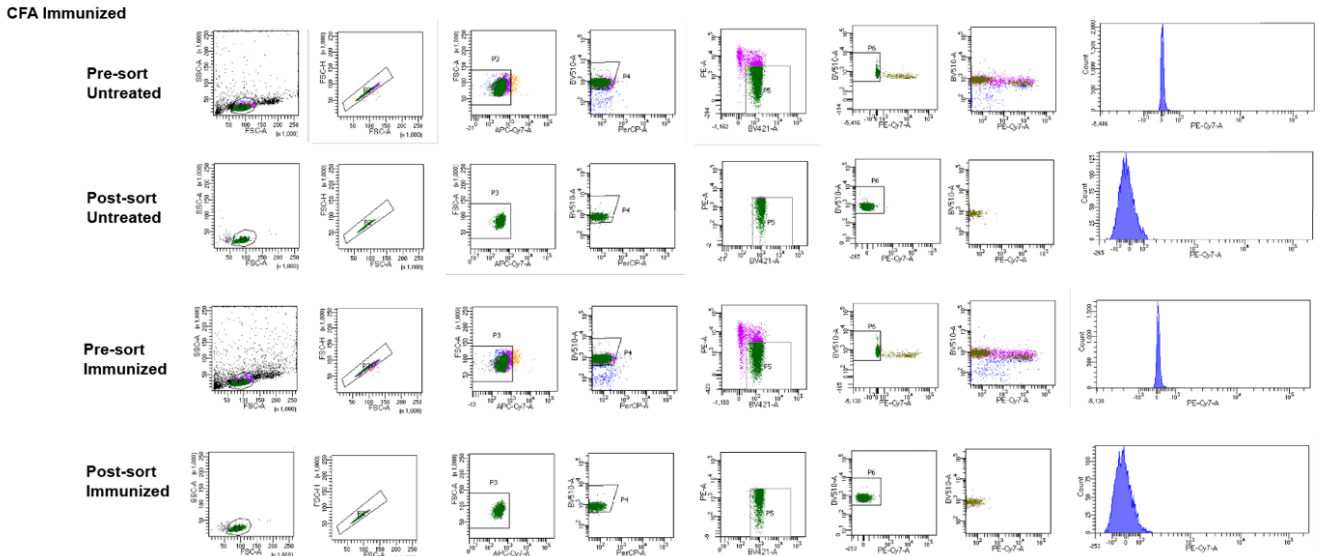


Figure S5. Representative plots showing sort gating strategy, and pre and post enrichment flow plots for KLF2-GFP, Mx1-GFP, Nur77-GFP and CFA immunized GFP^{Hi}, GFP^{Lo} and naïve CD4⁺ T cells. A. KLF2-GFP high vs low naïve CD4⁺ T cell sort gating and purity check. B. Mx1-GFP high vs low naïve CD4⁺ T cell sort gating and purity check. C. Nur77-GFP high vs low naïve CD4⁺ T cell sort gating and purity check. D. CFA immunized naïve CD4⁺ T cell sorting sort gating and purity check.

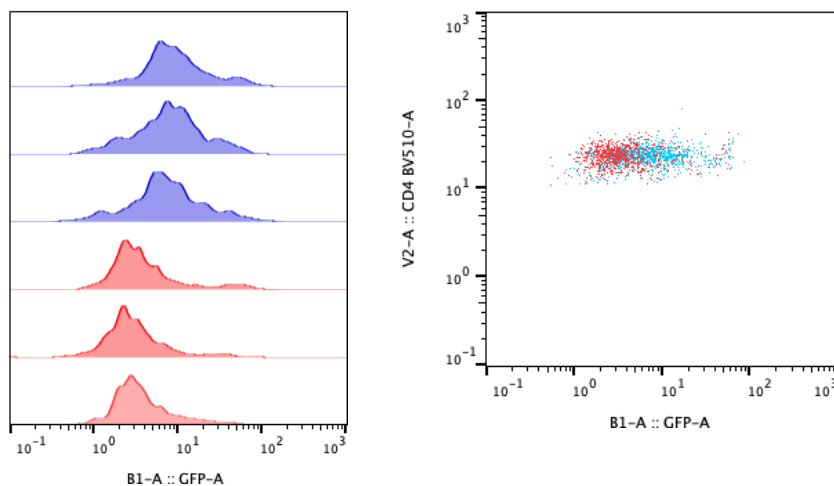


Figure S6. Adoptive transfer of Nur77^{HI} vs Nur77^{LO} cells shows GFP reporting stability. 300,000 naïve CD4s Nur77-GFP^{HI} and Nur77-GFP^{LO} (sorted using top 15% and bottom 15% GFP expression) were adoptive transferred into CD45.1 recipients. Nur77-GFP expression was assessed after 4 days. Blue = Nur77^{HI} and red = Nur77^{LO}.

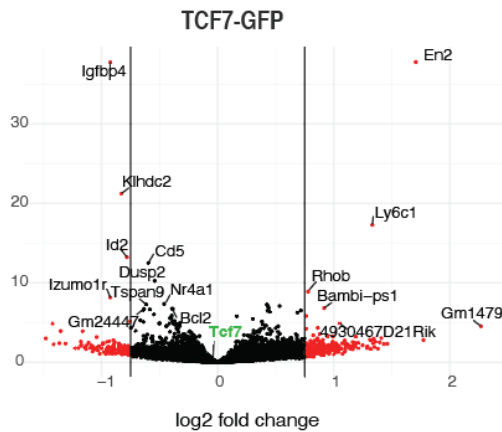
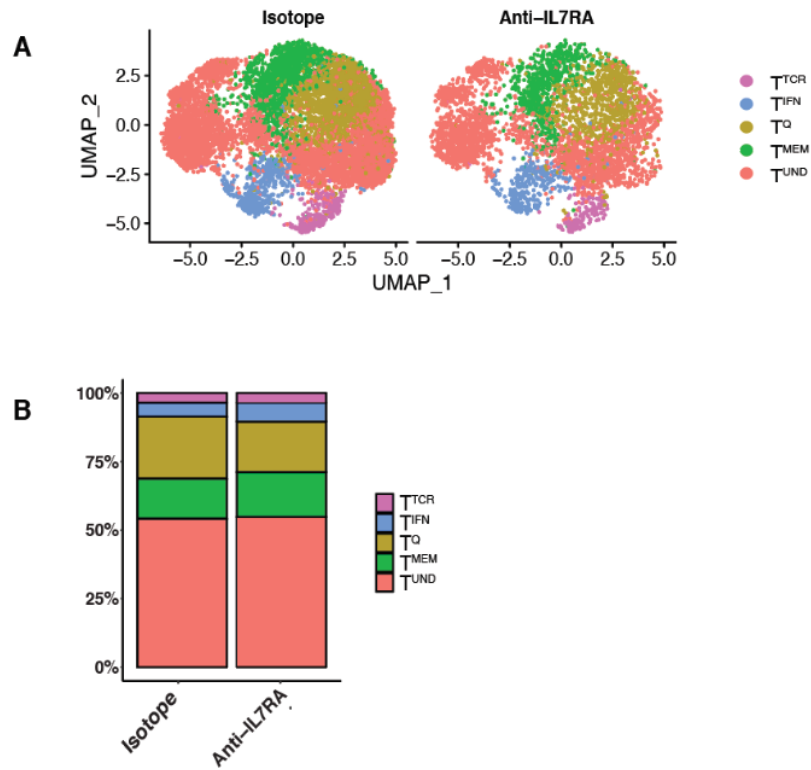


Figure S7. Bulk RNA sequencing of naïve CD4⁺ T cells from TCF7-GFP reporter mice shows no enrichment of T^{MEM}. Volcano plots showing bulk sequencing of sorted naïve CD4⁺ T cells sorted from top and bottom 10-15% GFP reporter mice, corresponding to TCF7^{HI} vs TCF7^{LO} naïve CD4⁺ T cells. The x axis is the log2fold change value and the y axis is -log10 of the p value. Genes labeled in green correspond to cluster markers. (n=4).



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69 **Figure S8. Naïve CD4⁺ T cell clusters don't change in anti-IL7RA or isotype treated mice. A:**
 70 UMAP naïve CD4⁺ in the spleens of anti-IL7RA or isotype treated mice. Mice were injected with
 71 300ug/mouse anti-IL7RA or isotype antibody every other day for a total of 3 treatments. **B:**
 72 Stacked bar plot of frequencies in (A), grouped by treatment. (n=2).

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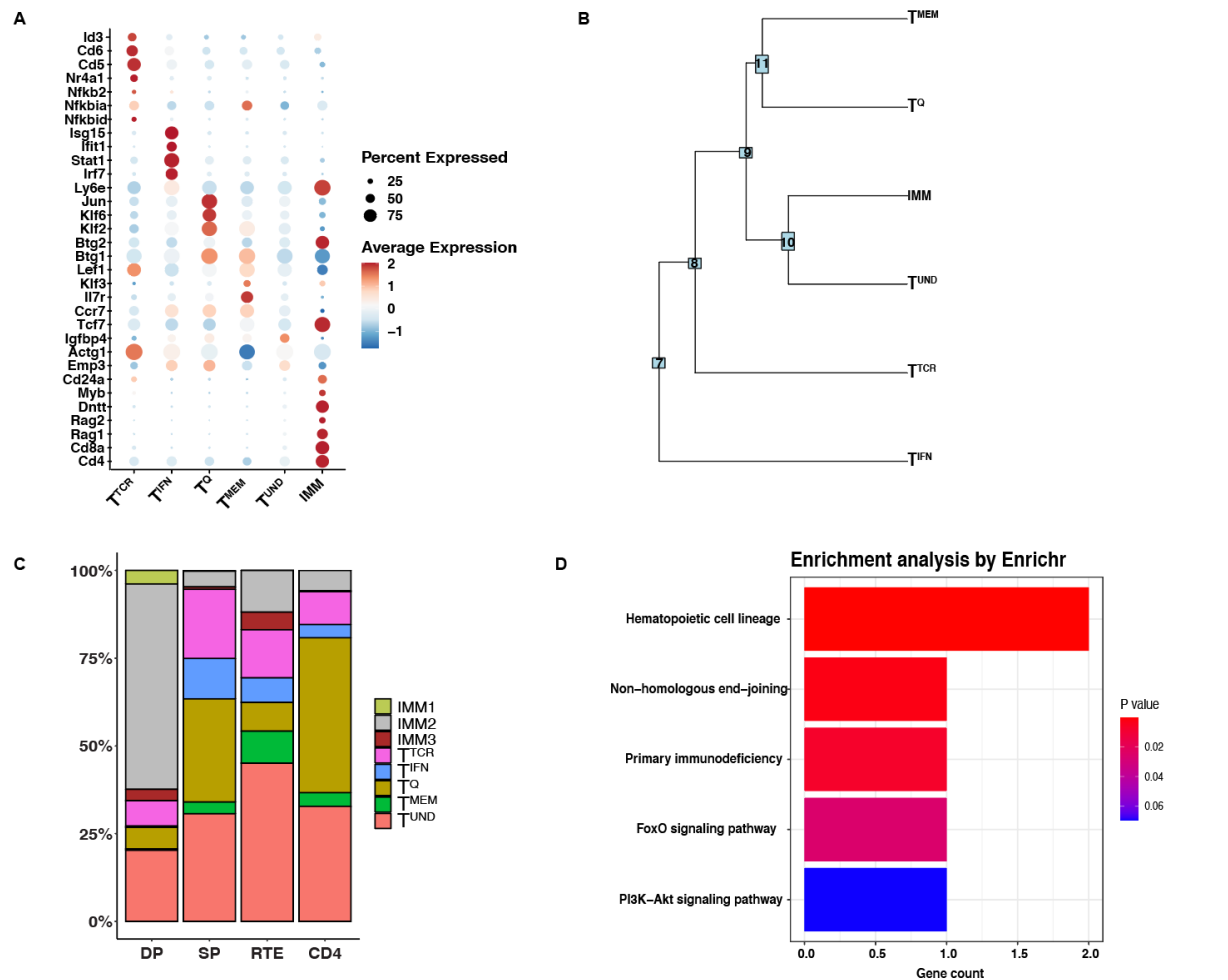


Figure S9. Thymic derived naïve CD4⁺ T cell clusters are maintained in recent thymic emigrants in the periphery. **A:** Dot plot showing thymic naïve DP, SP and peripheral splenic CD4⁺ T cell markers, including the markers for the thymic immature cluster. **B:** Cluster dendrogram showing similarities between clusters. **C:** Frequency plot of thymic DP and thymic SP, peripheral splenic naïve CD4⁺ T cells and peripheral RAG2-GFP⁺ naïve CD4⁺ T cells that represented Recent Thymic Emigrants (RTEs). RTEs were enriched using RAG2-GFP mice. **D:** EnrichR plot using DEGs in the Immature cluster of naïve DP, SP and peripheral naïve CD4⁺ T cells. Mice used for DP (thymic), SP (thymic) and CD4 (splenic) groups were VISTA CD4-Cre^{-/-}, representative of wild type mice. RTE refers to GFP⁺ splenic naïve CD4⁺ T cells from RAG2-GFP mice. (DP n=1, SP n=2, RTE n=2, CD4 n=3).

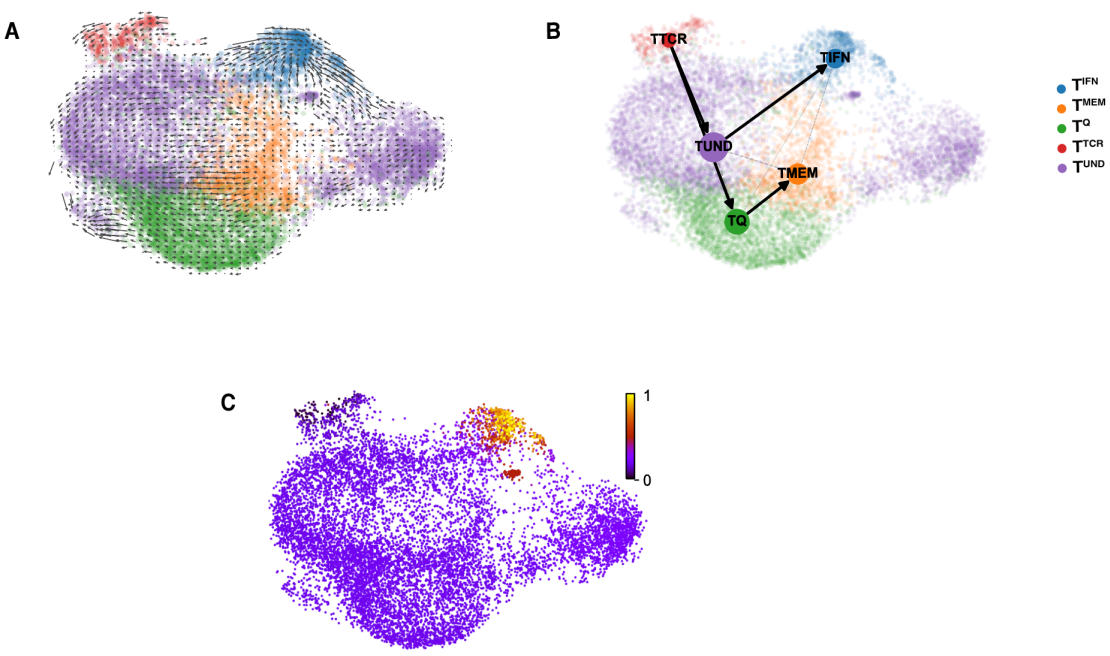


Figure S10. Pseudotime shows stability and plasticity of naïve CD4⁺ T cell clusters. A: RNA velocity of splenic naïve CD4⁺ T cells. **B:** PAGA plot from RNA velocity analysis of splenic naïve CD4⁺ T cells. **C:** Pseudotime plot showing the lineage trajectory of splenic naïve CD4⁺ T cells.

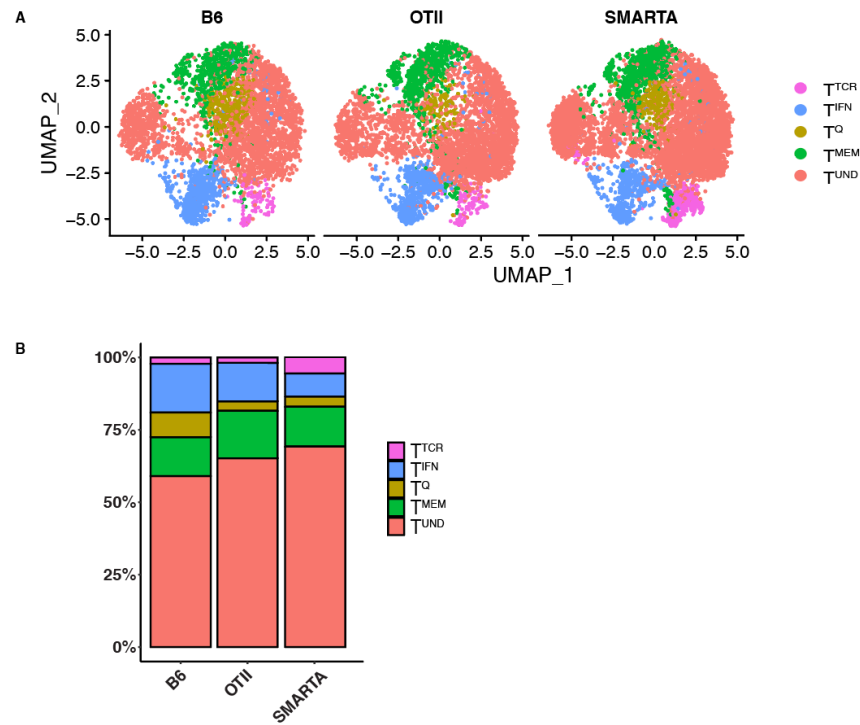


Figure S11. Naïve CD4⁺ T cell clusters from monoclonal vs polyclonal T cells are not transcriptionally distinct. A: UMAPs showing naïve CD4⁺ transcriptional clusters from TCR transgenics OT-II and SMARTA and control B6 mice. (n=2). **B:** Frequency plots quantifying clusters from TCR transgenics OT-II and SMARTA and control B6 mice.

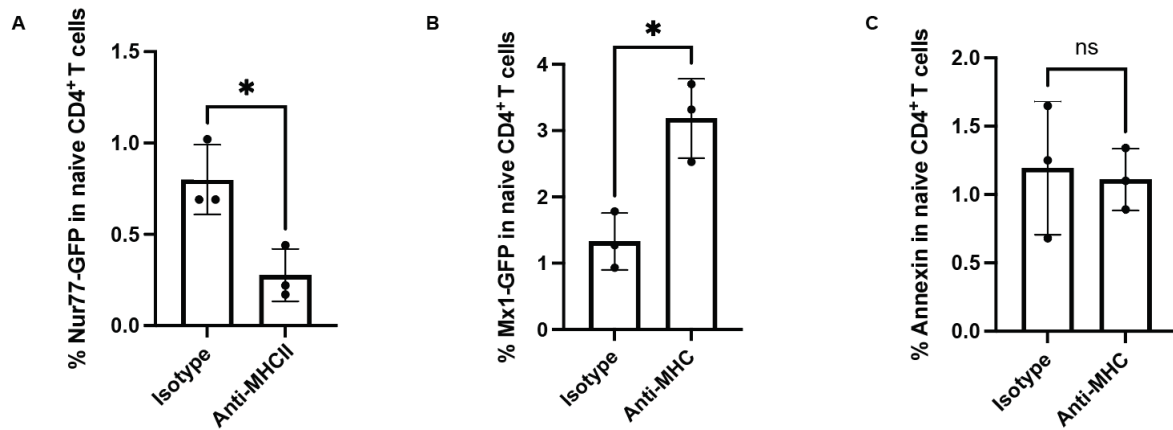


Figure S12. Naïve CD4⁺ T cells from MHCII or control isotype treated mice. A. Nur77-GFP, **B.** Mx1-GFP and **C.** Annexin staining in naïve CD4⁺ T cells treated with anti-MHCII or isotype control. Significance testing performed using unpaired t-test. * <0.05, ** <0.01, ***<0.001. (n=3).

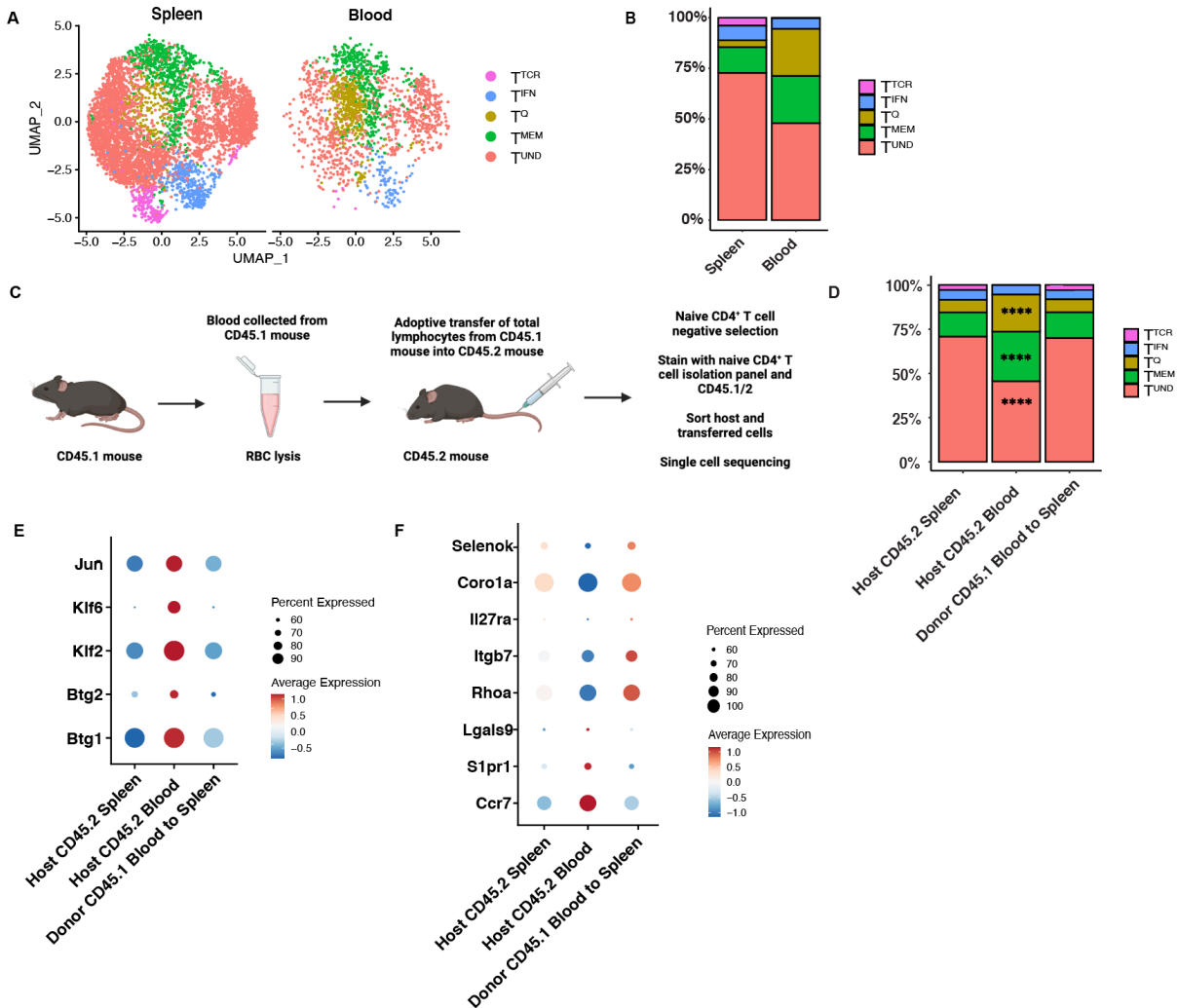


Figure S13. Tissue microenvironment dependent homeostasis of naïve CD4⁺ T cells in the spleen and blood. **A:** UMAP of naïve CD4⁺ T cells from C57BL/6J mice spleens, lymph nodes and blood. (n=2). **B:** Stacked bar plot of frequencies in (A). **C:** Experimental outline of adoptive transfer of lymphocytes from blood into host mouse, followed by isolation and sorting of transferred blood naïve CD4⁺ T cells from host and donor spleen and blood after 3 days. (n=2). **D:** Stacked bar plot of frequencies in from adoptive transfer experiment in (C). All statistical significance testing in figure was performed using 2-way ANOVA. * <0.05, ** <0.01, ***<0.001. **E:** Dot plot of T_Q genes in adoptive transfer experiment. **F:** Dot plot of migratory genes in adoptive transfer experiment.

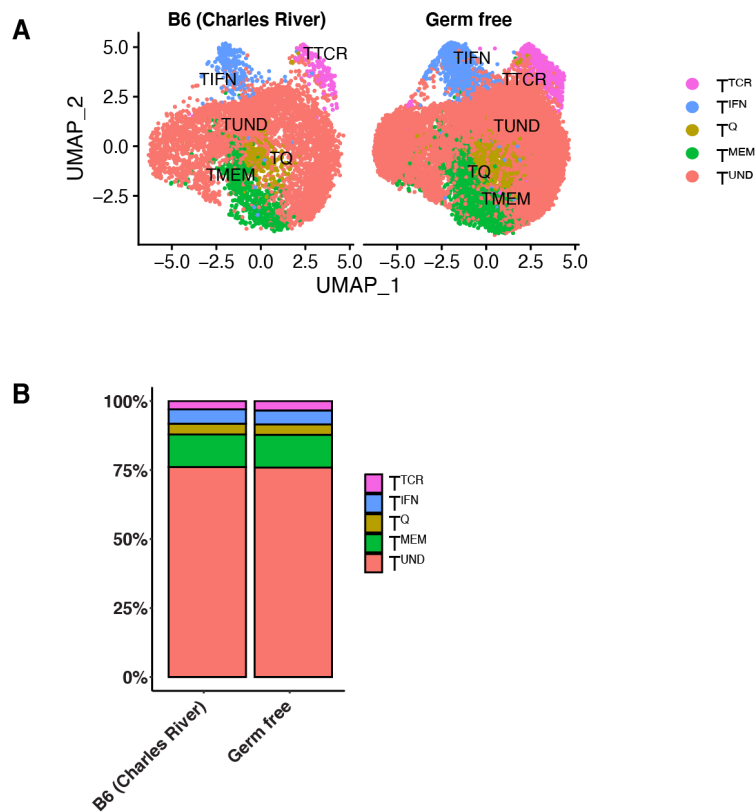


Figure S14. Microbiome experience does not influence naïve CD4⁺ T cell transcriptional heterogeneity. **A:** UMAP showing clustering of naïve CD4⁺ T cells from germ free and specific pathogen free mice. (n=2). **B:** Frequency plot qualifying the UMAP frequencies in (A).

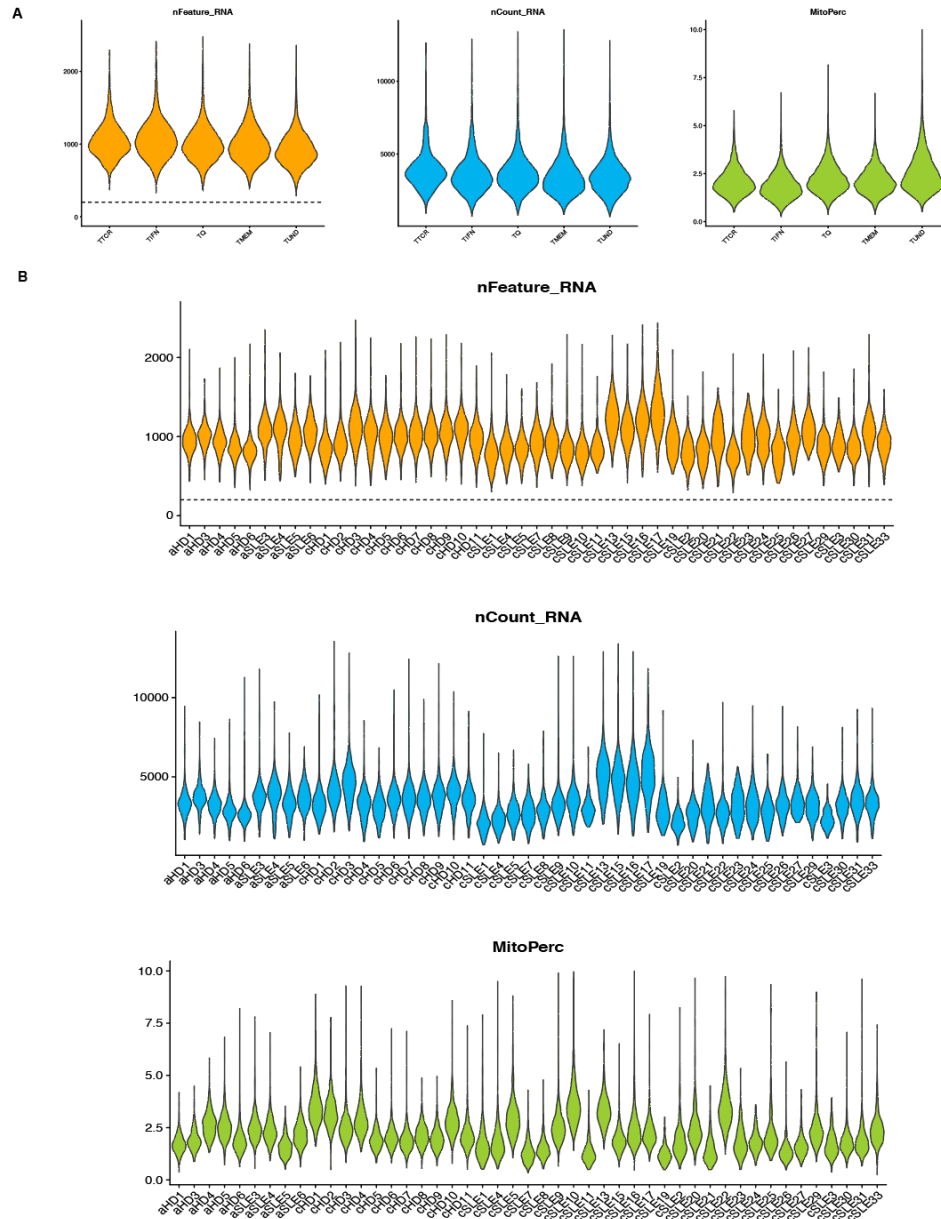


Figure S15. Representative plots for human single cell RNA sequencing quality control pipeline showing nFeature, nCount and mitochondrial content. A. nFeature, nCount and mitochondrial content per cluster after quality control filtering. **B.** nFeature, nCount and mitochondrial content separated out donor.



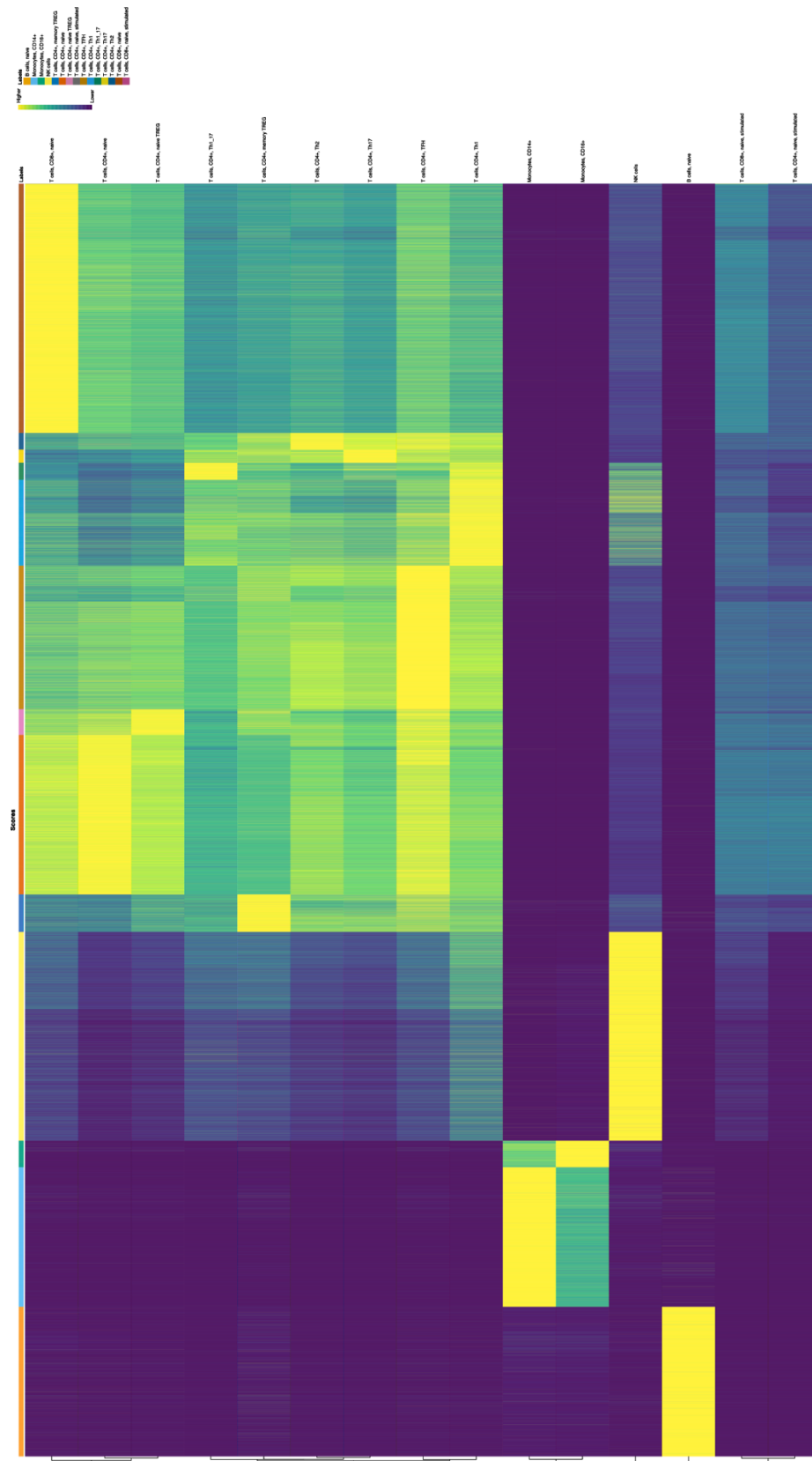


Figure S17. SingleR predictions for immune cell subsets in lupus dataset.

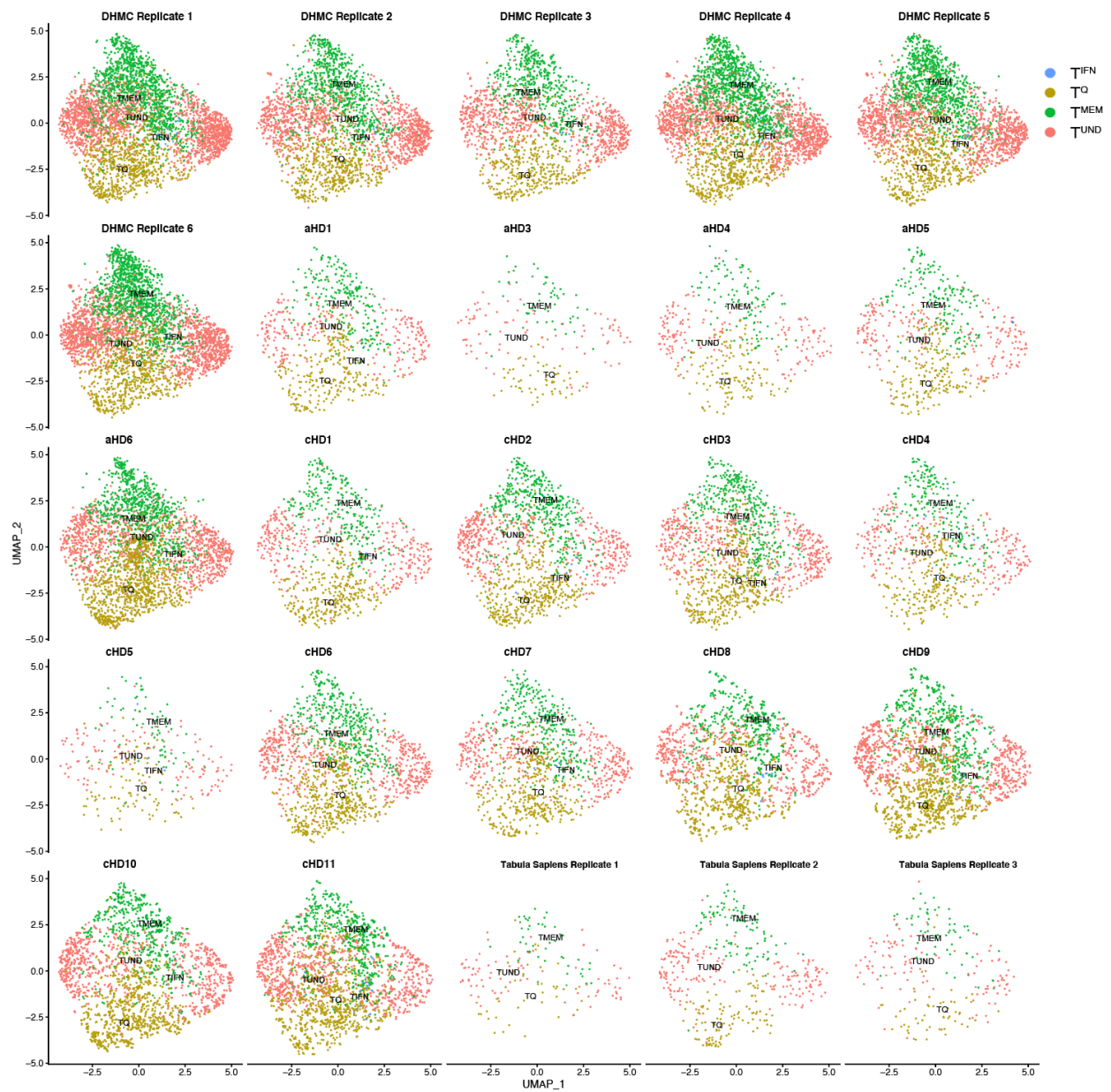


Figure S19. UMAPs separated by donor for human healthy dataset (DHMC, lupus and tabula sapiens datasets).

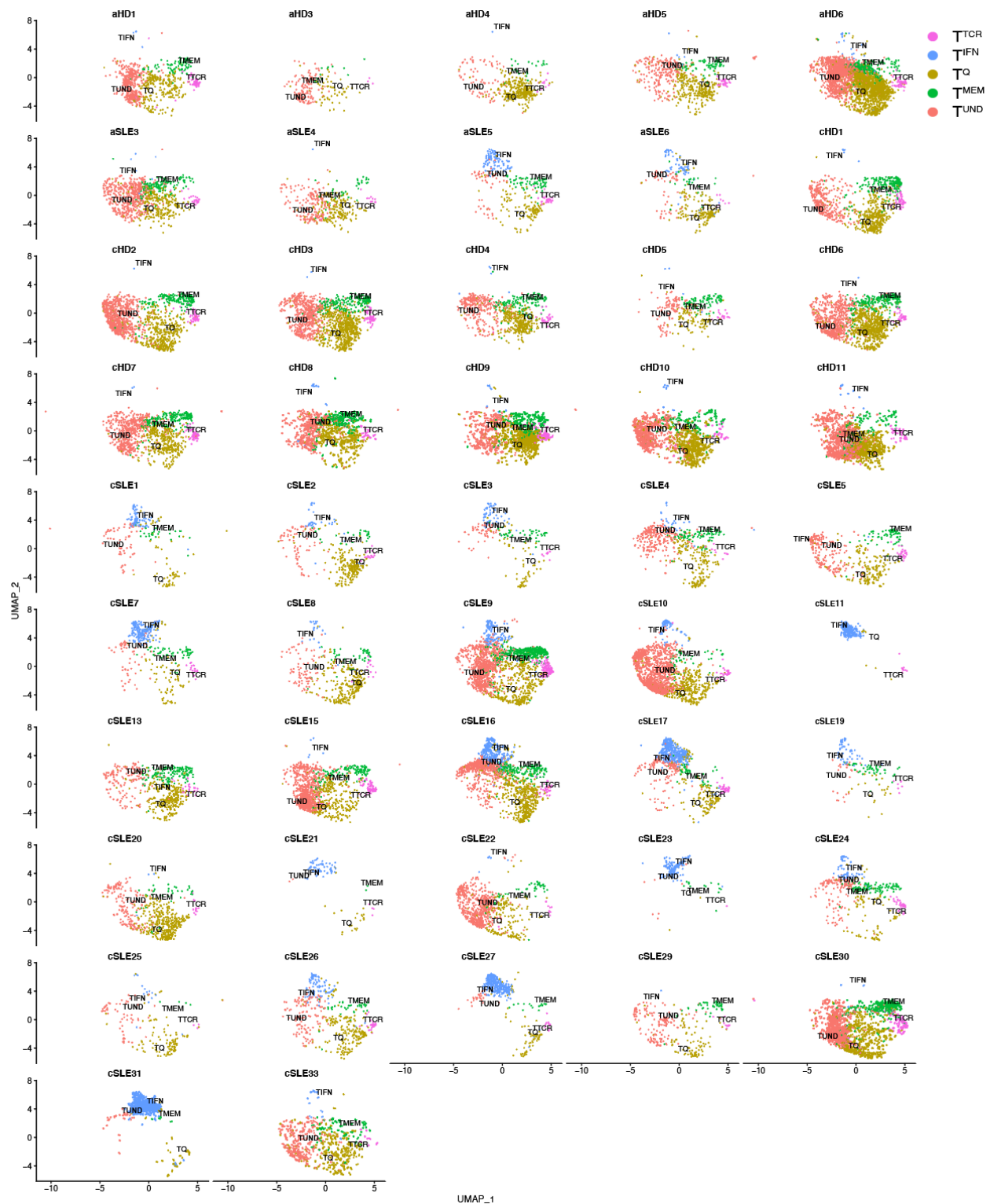


Figure S20. UMAPs separated by donor for lupus dataset.

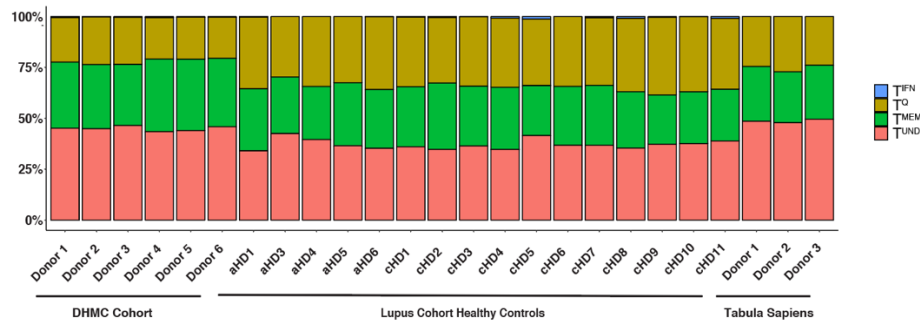


Figure S21. Stability of naïve CD4⁺ T cell clusters in healthy donors. Naïve CD4⁺ T cell transcriptional clusters by donor in 3 healthy datasets - our DHMC cohort, healthy controls from the lupus dataset and Tabula Sapiens dataset.

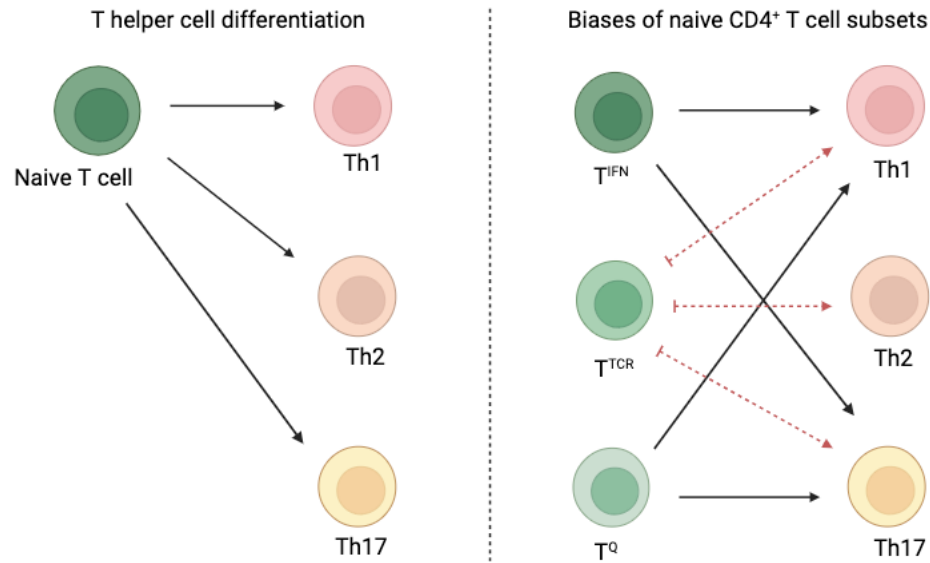


Figure S22. Model depicting the redefined lineage biases dependent on naïve CD4⁺ T cell cluster identity. Black arrows represent skewing biases and red dotted arrows represent inhibition of differentiation trajectories. Figure created in BioRender.

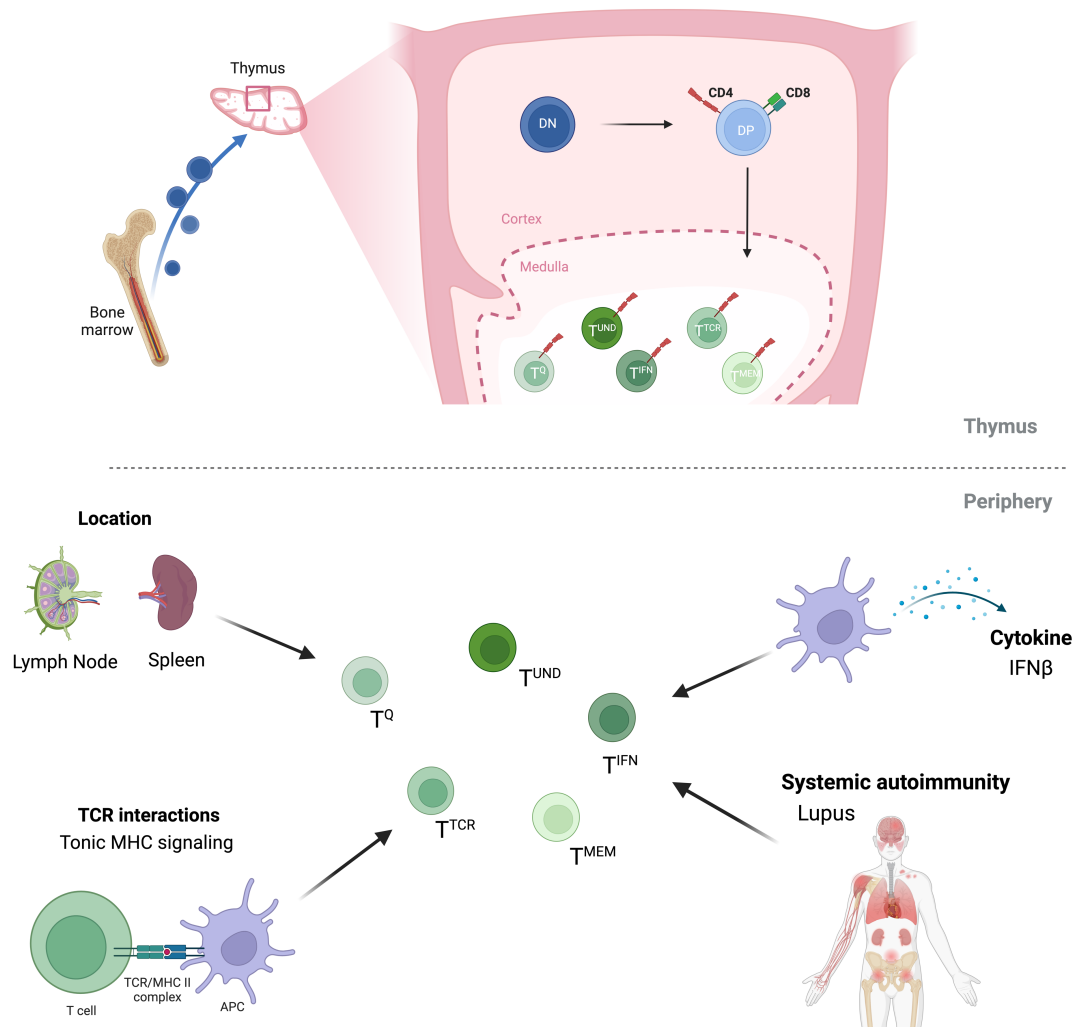


Figure S23. Summary of the environmental cues that impact naïve CD4⁺ T cell subsets. The thymus impacts the emerge of all clusters during T cell development. In the periphery, tonic signaling controls T^{CR}, tissue microenvironment impacts T^Q, while lupus and IFN β influence T^{IFN}. Figure created in BioRender.

	avg_log2FC	p_val_adj	cluster	gene
lsg15.2	1.954369158	0	TIFN	lsg15
Ifi2712a.2	1.650667835	1.52E-205	TIFN	Ifi2712a
Bst2.3	1.588458876	2.47E-224	TIFN	Bst2
Irf7.3	1.526434888	1.31E-240	TIFN	Irf7
Ly6a.3	1.50364345	3.28E-186	TIFN	Ly6a
Ifit3	1.250361205	0	TIFN	Ifit3
Stat1.3	1.206833125	2.44E-158	TIFN	Stat1
Zbp1.3	1.005371713	4.72E-176	TIFN	Zbp1
Slfn5	0.980556081	1.98E-167	TIFN	Slfn5
lsg20	0.938249423	2.87E-181	TIFN	lsg20
Ifit1	0.916530793	1.64E-249	TIFN	Ifit1
Ly6c1.1	0.91420587	1.28E-52	TIFN	Ly6c1
Slfn1.1	0.889972139	4.56E-102	TIFN	Slfn1
Rnf213.1	0.853788163	6.93E-136	TIFN	Rnf213
Ifi47.1	0.844785083	1.21E-85	TIFN	Ifi47
Ly6e.1	0.842306812	5.11E-95	TIFN	Ly6e
Ifi203.1	0.802468333	1.94E-87	TIFN	Ifi203
H2-T23	0.777907907	2.33E-87	TIFN	H2-T23
Rtp4	0.777299303	9.01E-148	TIFN	Rtp4
Daxx	0.771964491	1.58E-107	TIFN	Daxx
Usp18	0.763124998	9.28E-177	TIFN	Usp18
Samhd1.1	0.695226717	2.54E-52	TIFN	Samhd1
Ifi213	0.684699729	1.69E-93	TIFN	Ifi213
Phf11b	0.676366775	2.99E-98	TIFN	Phf11b
Xaf1	0.67295841	1.69E-115	TIFN	Xaf1
H2-T22	0.661115955	4.59E-63	TIFN	H2-T22
Ms4a4b.2	0.619795816	1.76E-51	TIFN	Ms4a4b
AW112010	0.607360432	8.41E-34	TIFN	AW112010
Gbp6	0.600023679	1.69E-136	TIFN	Gbp6
Oasl2	0.596644306	9.56E-154	TIFN	Oasl2
Mndal	0.589671837	9.94E-52	TIFN	Mndal
Igtp	0.577020786	3.79E-74	TIFN	Igtp
Sp100	0.559817726	1.9E-42	TIFN	Sp100
Tapbp	0.559738193	1.36E-49	TIFN	Tapbp
Trim30a	0.554844423	8.12E-77	TIFN	Trim30a
Irgm1	0.542305958	5.66E-85	TIFN	Irgm1
Epsti1	0.535521203	4.29E-49	TIFN	Epsti1
Psmb8.1	0.530759273	2.75E-44	TIFN	Psmb8
Psme1	0.515398826	4.11E-47	TIFN	Psme1
Shisa5	0.514877376	4.43E-60	TIFN	Shisa5
Lgals9	0.510713142	2.2E-39	TIFN	Lgals9
Ifi208	0.508911793	5.06E-53	TIFN	Ifi208
Il7r	0.665623842	8.08E-53	TMEM	Il7r
Crif3	0.561841005	3.22E-54	TMEM	Crif3
Cnn2	-0.454840783	5.46E-29	TMEM	Cnn2
Pfn1	-0.568768864	9.63E-78	TMEM	Pfn1
Cd52	-0.60276123	6.36E-63	TMEM	Cd52
Actg1	-0.684903763	4.79E-73	TMEM	Actg1
Gm42418	-0.693510769	9.29E-05	TMEM	Gm42418
Actb	-0.705063757	5.86E-142	TMEM	Actb

Jun.2	0.892844921	9.28E-103	TQ	Jun
Bst2.2	-0.48556722	7.24E-14	TQ	Bst2
lsg15.1	-0.626217104	1.18E-18	TQ	lsg15
Ifi2712a.1	-0.646707547	3.66E-23	TQ	Ifi2712a
Gm42418.2	-0.807795476	9.41E-15	TQ	Gm42418
Nr4a1	1.381368859	5.31E-33	TTCR	Nr4a1
Nfkbia.2	1.10989336	1.41E-08	TTCR	Nfkbia
Cd5	0.964813922	1.61E-15	TTCR	Cd5
Itm2a	0.923743811	7.92E-06	TTCR	Itm2a
Nfkbid	0.909659257	2.1E-28	TTCR	Nfkbid
Egr1	0.852550171	7.65E-10	TTCR	Egr1
Eif5a	0.846708958	1.91E-12	TTCR	Eif5a
Id3	0.816114947	1.87E-10	TTCR	Id3
Ptma	0.707212947	6.03E-13	TTCR	Ptma
Srgn.1	0.660628171	0.012093841	TTCR	Srgn
Eif4a1	0.654819458	4.06E-07	TTCR	Eif4a1
Cd69.1	0.65216419	0.066856179	TTCR	Cd69
Ptpn6.1	0.629952363	1E-11	TTCR	Ptpn6
Hsp90ab1	0.61920352	9.7E-11	TTCR	Hsp90ab1
Ncl	0.540566264	1	TTCR	Ncl
Cd6	0.540028559	2.7E-08	TTCR	Cd6
Ppp1r14b	0.535649395	2.67E-07	TTCR	Ppp1r14b
Myc	0.529589526	1	TTCR	Myc
Foxp4	0.527072885	3.59E-12	TTCR	Foxp4
Ldha	0.526681471	5.76E-05	TTCR	Ldha
Dusp2	0.525934497	0.009528252	TTCR	Dusp2
Npm1	0.522404946	0.195582211	TTCR	Npm1
Zfp36	0.519290129	1	TTCR	Zfp36
Il2rg	0.506001938	0.002012912	TTCR	Il2rg
Cd82	0.501199636	1	TTCR	Cd82
Nme1	0.500127736	0.007269548	TTCR	Nme1
Slfn1.2	-0.463631773	0.327414274	TTCR	Slfn1
Rflnb.1	-0.465624184	0.020700893	TTCR	Rflnb
Ighm	-0.473082704	0.03214052	TTCR	Ighm
Igfbp4.3	-0.603243782	0.464699796	TTCR	Igfbp4
Klf2.2	-0.641160193	0.000214479	TTCR	Klf2
Tsc22d3.1	-0.662586831	9.26E-06	TTCR	Tsc22d3
Txnip	-0.811289022	8.08E-07	TTCR	Txnip
Gm42418.1	0.823264781	3.3E-29	TUNDIFF	Gm42418
Jun.1	-0.532774216	9.79E-41	TUNDIFF	Jun
Ly6a.1	-0.567234612	1.24E-26	TUNDIFF	Ly6a
Irf7.1	-0.569221822	1.39E-33	TUNDIFF	Irf7
Bst2.1	-0.575451906	7.52E-22	TUNDIFF	Bst2
Ifi2712a	-0.596935882	3.87E-22	TUNDIFF	Ifi2712a
lsg15	-0.742313603	1.98E-35	TUNDIFF	lsg15

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	avg_log2FC	p_val_adj	cluster	gene
IFI6	1.155015875	4.9E-45	TIFN	IFI6
ISG15	1.079970094	2.48E-35	TIFN	ISG15
STAT1	1.049918715	2.35E-37	TIFN	STAT1
IFITM1.1	0.866528588	3.39E-07	TIFN	IFITM1
MX1	0.792242784	2.59E-42	TIFN	MX1
IFI44L	0.75779067	8.05E-118	TIFN	IFI44L
LY6E	0.752273832	7.78E-12	TIFN	LY6E
MT2A	0.711845438	5.72E-16	TIFN	MT2A
XAF1	0.703793409	6.76E-33	TIFN	XAF1
TRIM22	0.632261399	8.65E-12	TIFN	TRIM22
UBE2L6	0.506859548	3.23E-13	TIFN	UBE2L6
FOS.1	-0.575702234	2.92E-191	TMEM	FOS
JUNB.1	-0.879717655	0	TMEM	JUNB
JUNB.2	1.249266644	0	TQ	JUNB
FOS.2	0.931396173	0	TQ	FOS
JUN.2	0.919902759	0	TQ	JUN
DUSP1.2	0.693638663	0	TQ	DUSP1
NFKBIA.2	0.559120839	0	TQ	NFKBIA
CD69.1	0.553869139	0	TQ	CD69
CXCR4.1	0.543796782	0	TQ	CXCR4
ZFP36.1	0.503991548	0	TQ	ZFP36
MTRNR2L12.1	-0.500473614	4.33E-154	TQ	MTRNR2L12
JUNB	-0.563059958	7.32E-193	TUND	JUNB

Table S2: Markers for human single cell clusters.

		Mouse				
		TIFN	TMEM	TQ	TTCR	TUND
Human	TIFN	0.35615371	-0.2257231	-0.24556111	0.06411807	-0.21393918
	TMEM	0.04725379	0.4243488	0.06068659	-0.32741842	0.11802961
	TQ	-0.2650143	0.0552417	0.28617781	0.04471883	-0.06048239
	TUND	-0.25504152	-0.1611469	-0.0390069	0.18210937	0.2643629

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233 **Table S3: R values for Mouse-Human naïve CD4⁺ T cell cluster correlation.**

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