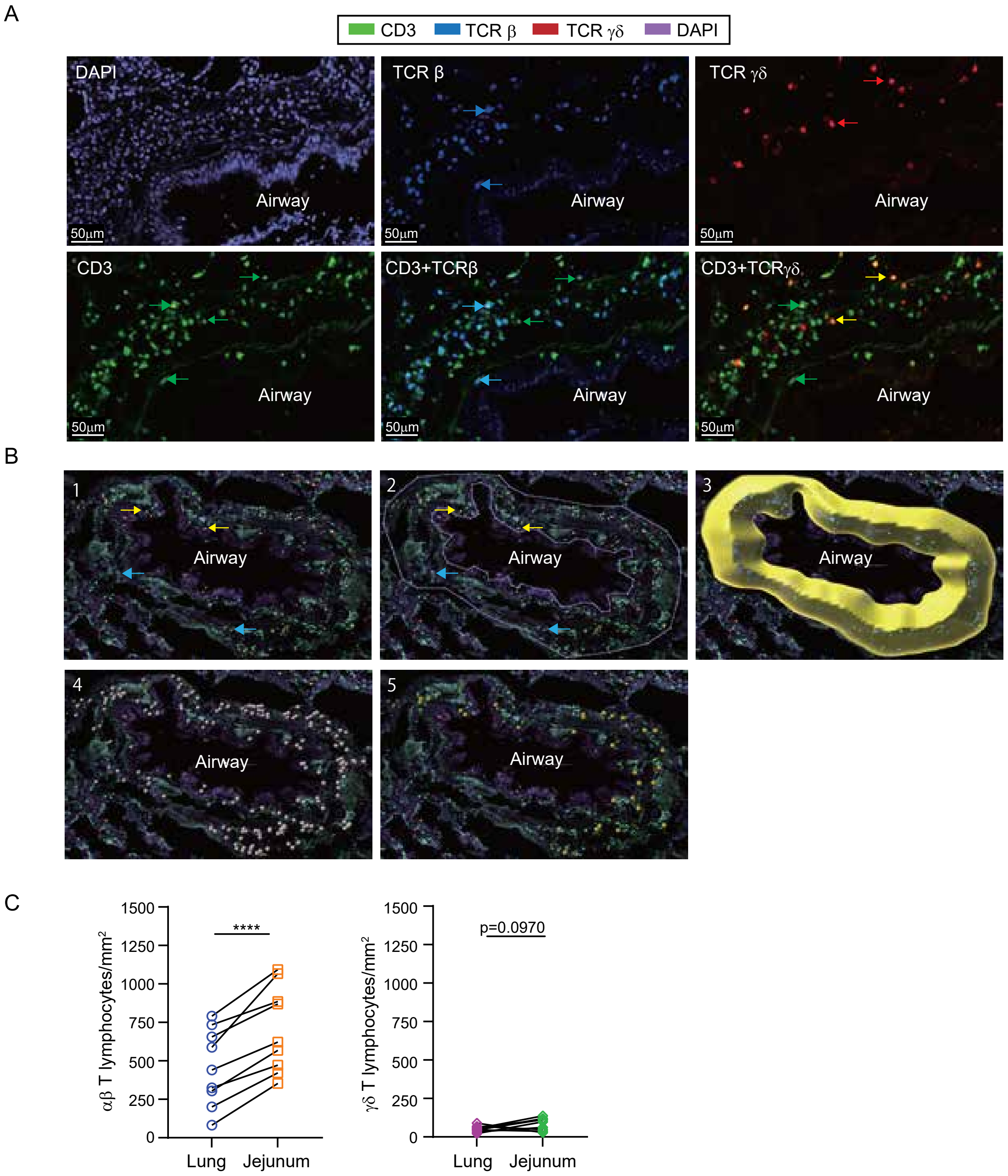

Figure S1: Immunofluorescence imaging to define T cell populations in pediatric organ donors. Related to Figure 1. (A) Representative images of the lung showing staining with single fluorescent labeled markers: DAPI to define cells and lung structures such as airways (top row left), TCRβ (top row middle), TCRγδ (top row right) and CD3 (lower row, left) and double staining combinations; CD3/TCRβ (bottom row middle), CD3 TCRγδ (bottom row right) shown. Arrows indicate cells staining positive for defined markers. (B) Representative images showing schematic for defining area and cellular composition within tissue. 1) Airway section stained with antibodies for DAPI, CD3, TCRβ, TCRγδ 2) Imaris software to define tissue adjacent to airway, 3) Imaris overlay to define total area within defined boundaries, 4) identification of all CD3$^+$ cells within defined area, 5) identification of CD3$^+$/TCRγδ$^+$/TCRβ$^-$ cells. Arrows indicate cells staining positive for defined markers. (C) Graphs showed paired analysis of αβ (left) and γδ (right)T cell density in the jejunum and lung for individual donors quantified by imaging; statistical testing done via paired T test, **** = p<0.0001.

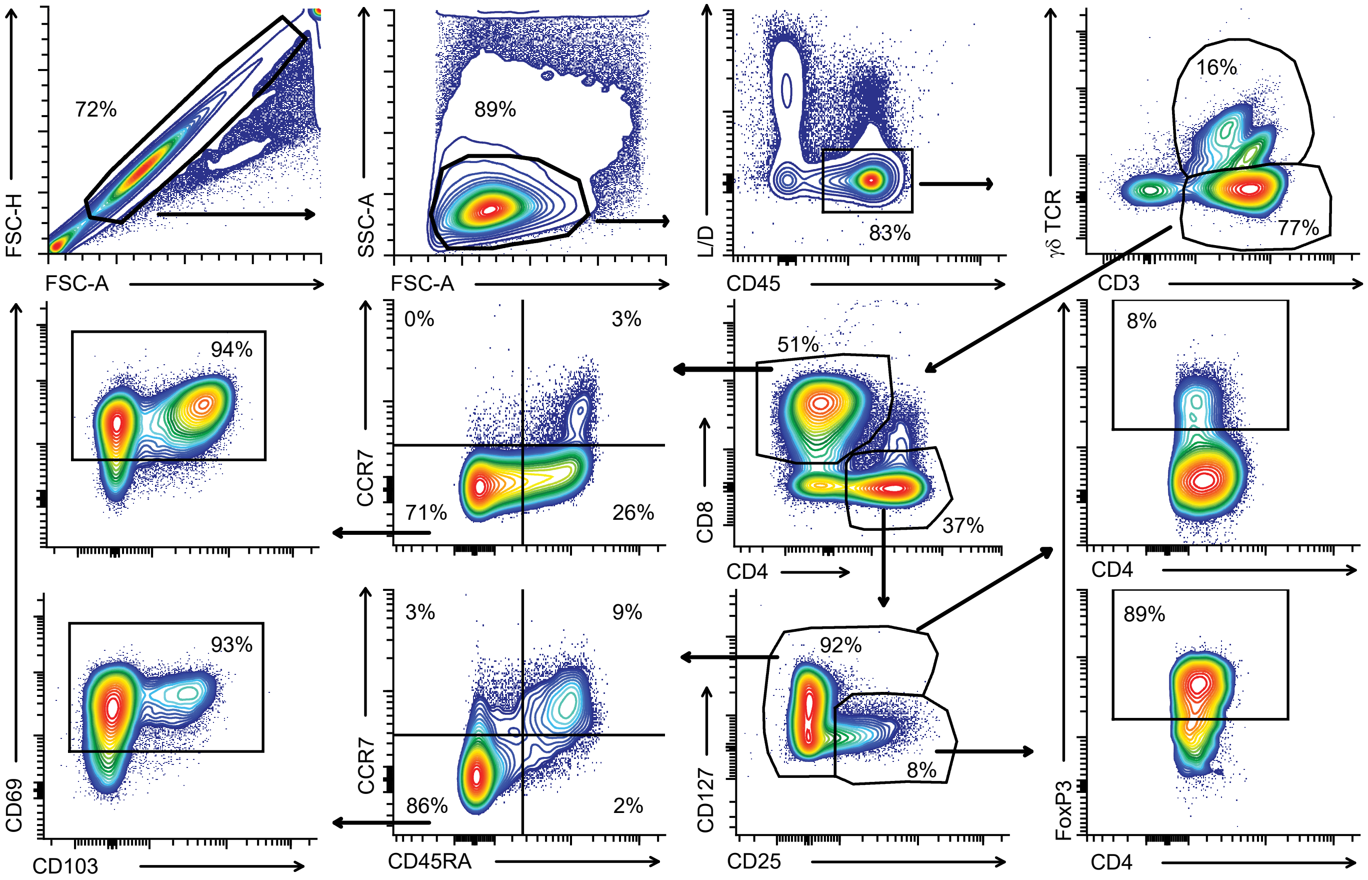

Figure S2: Flow cytometry gating strategy. Related to Figures 2, 3, 4, 5, 6. Representative flow cytometry plots showing gating strategy to identify T cells subsets within an intestine sample of 9-month old organ donor. Single cells were selected based on forward (FSC) and height (H) vs area (A), then lymphocytes were gated based on FSC vs side scatter (SSC) properties, followed by selection of CD45$^+$ live cells. T cells were gated based on expression of CD3; γδ T cells were gated based on expression of γδ TCR. CD3$^+$γδ- T cells were then gated based on expression of CD4 and CD8. CD4$^+$ T cells were subdivided based on expression of CD127 and CD25; CD25$^-$/CD127$^{+/-}$ were defined as conventional CD4 T cells (T$_{\mathrm{CONV}}$) and CD25$^+$/CD127$^-$ T cells were defined as regulatory T cells (T$_{\mathrm{REG}}$) if they further expressed FoxP3 with gating set in conjunction with expression on T$_{\mathrm{CONV}}$ cells. Memory subsets of CD4$^+$ T$_{\mathrm{CONV}}$ and CD8$^+$ T cells were defined based on expression of CCR7 and CD45RA; Naïve – CCR7$^+$/CD45RA$^+$, central memory (T$_{\mathrm{CM}}$) - CCR7$^+$/CD45RA$^-$, effector memory (T$_{\mathrm{EM}}$) - CCR7$^-$/CD45RA$^-$, and effector memory RA$^+$ (T$_{\mathrm{EMRA}}$)- CCR7$^-$/CD45RA$^+$. CD4$^+$ and CD8$^+$ T$_{\mathrm{EM}}$ were further gated based on expression of CD69 and CD103 to define markers consistent with phenotype of resident memory (T$_{\mathrm{RM}}$) T cells. Numbers refer to percent of total cells gated as fraction of previous gate.

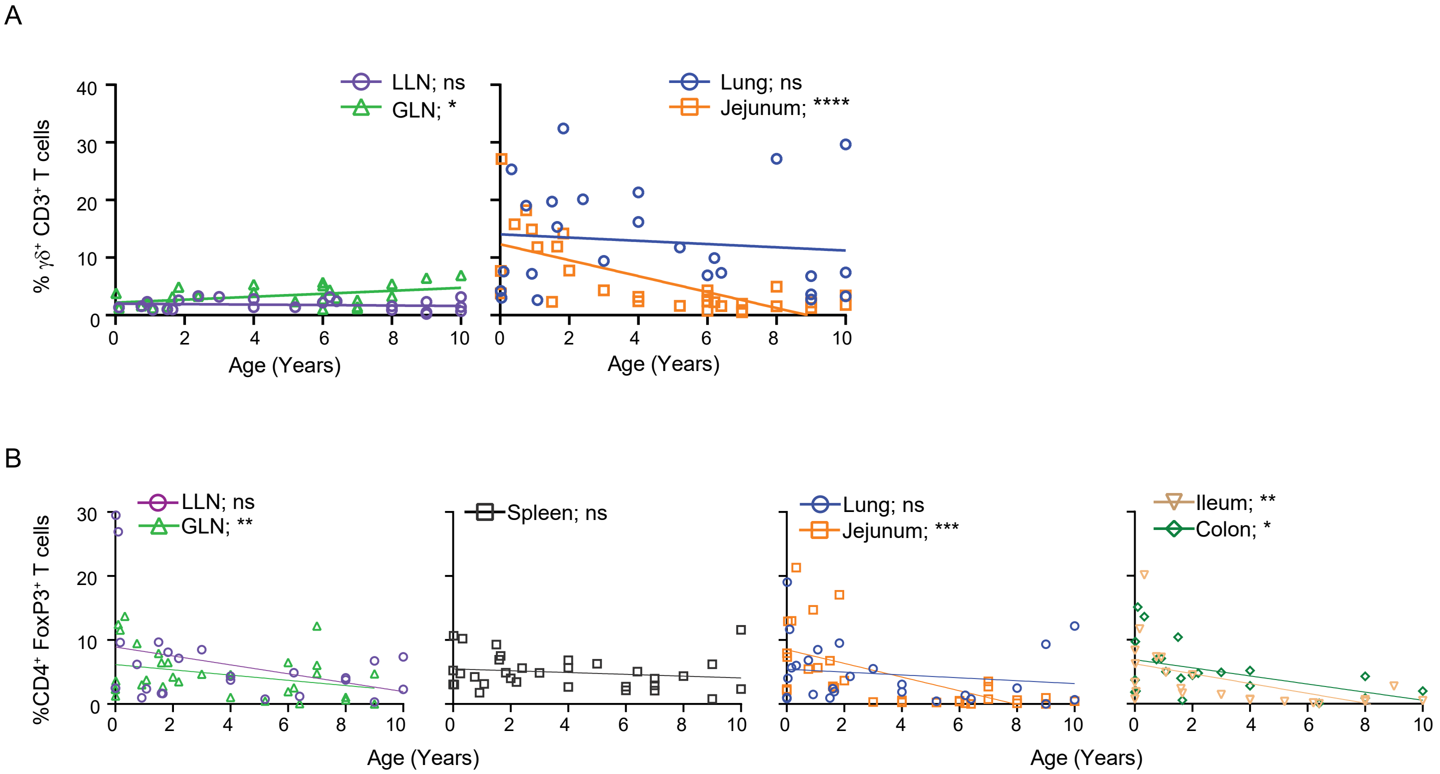

Figure S3: Altered frequency of γδ T cells and Tregs in specific sites across childhood. Related to Figures 1. (A) Graph depicts frequency of γδ T cells within lymphoid and mucosal tissues of individual donors of indicated ages compiled from flow cytometry results and gating strategy in Fig. S1. (B) Graph shows frequency of Tregs ($CD4^+/CD127^-/CD25^+/FoxP3^+$) within indicated sites for each individual donors by age. Number of donors for each site: LLN, n=19-25; GLN, n=18-35, spleen = 32, lung = 23-26, jejunum = 23-31, ileum = 23, colon = 21. Statistical testing by Pearson's correlation. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

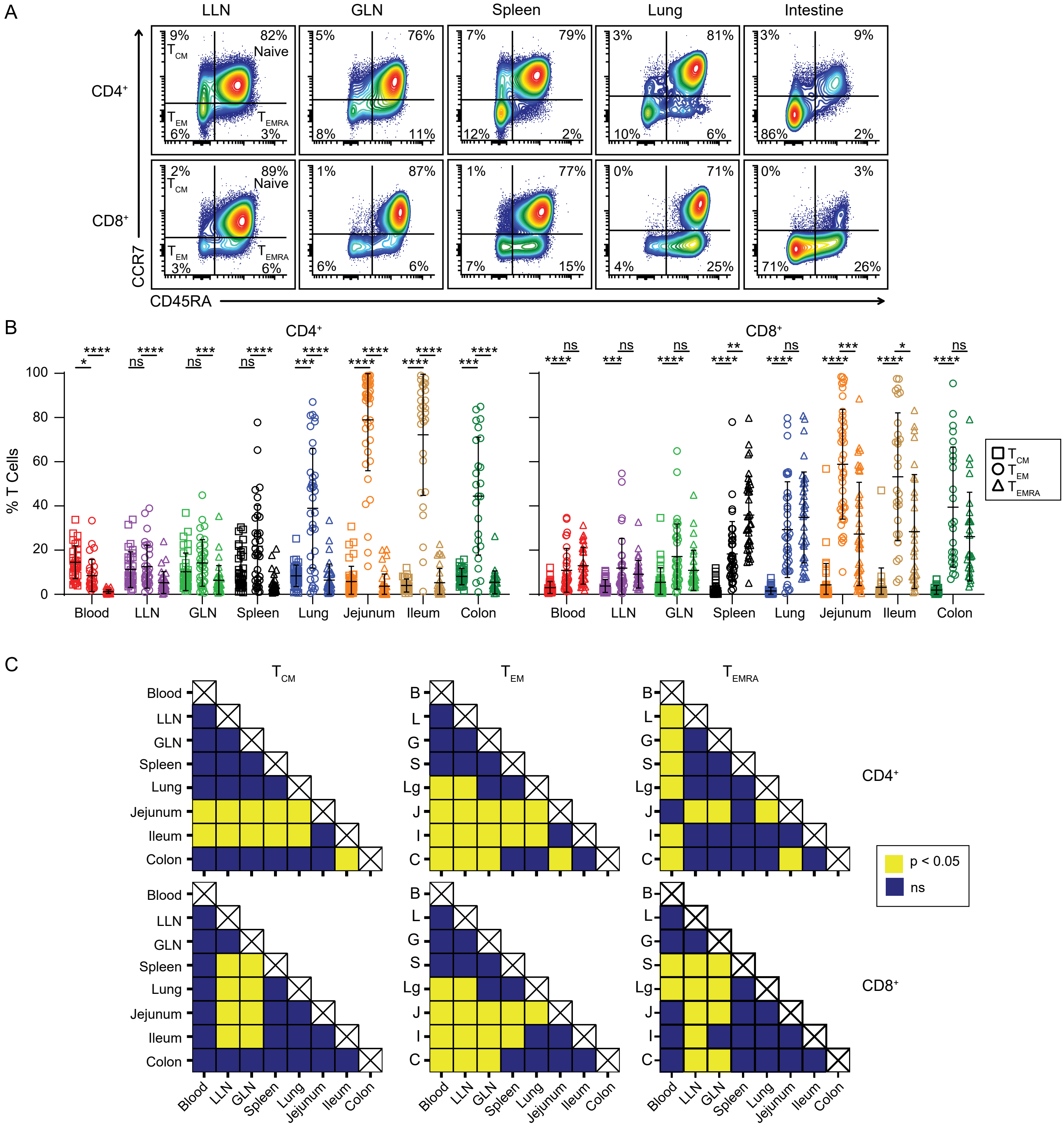

Figure S4: T cell subset composition in pediatric tissues. Related to Figure 2. (A) Representative flow cytometry plots showing CD45RA and CCR7 expression by CD4^{+} (top) and CD8^{+} (bottom) T cells isolated from indicated tissues of a 9-month organ donor. Abbreviations: Lung-associated lymph node (LLN), Gut-associated lymph node (GLN). T cell subsets are designated as Naïve (CCR7^{+}CD45RA^{+}), Central memory (T$_{\mathrm{CM}}$, CCR7^{+}/CD45RA^{-}), effector memory (T$_{\mathrm{EM}}$, CCR7^{-}/CD45RA^{-}), and terminal effector (T$_{\mathrm{EMRA}}$, CCR7^{-}/CD45RA^{+}). Numbers in each quadrant refer to % each subset comprises of total CD4^{+} T$_{\mathrm{CONV}}$ or CD8^{+} T cells. (B) Graph shows frequency of memory T cell subsets (squares, T$_{\mathrm{CM}}$; circles, T$_{\mathrm{EM}}$; triangles, T$_{\mathrm{EMRA}}$) in each site for the following numbers of donors: blood, n= 31; LLN, n=33; GLN, n= 35; spleen, n= 36; lung, n= 36; jejunum, n= 41; ileum, n= 29; colon, n= 25. Statistical testing by Kruskal-wallis one-way anova with Dunn's multiple comparisons. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001. (C) Analysis of subset frequencies (T$_{\mathrm{CM}}$, T$_{\mathrm{EM}}$, T$_{\mathrm{EMRA}}$) as in (B) comparing between sites for CD4^{+} (top) and CD8^{+} (bottom) with yellow squares representing p<0.05. Statistical testing by Kruskal-Wallis one-way anova with Dunn's multiple comparisons.

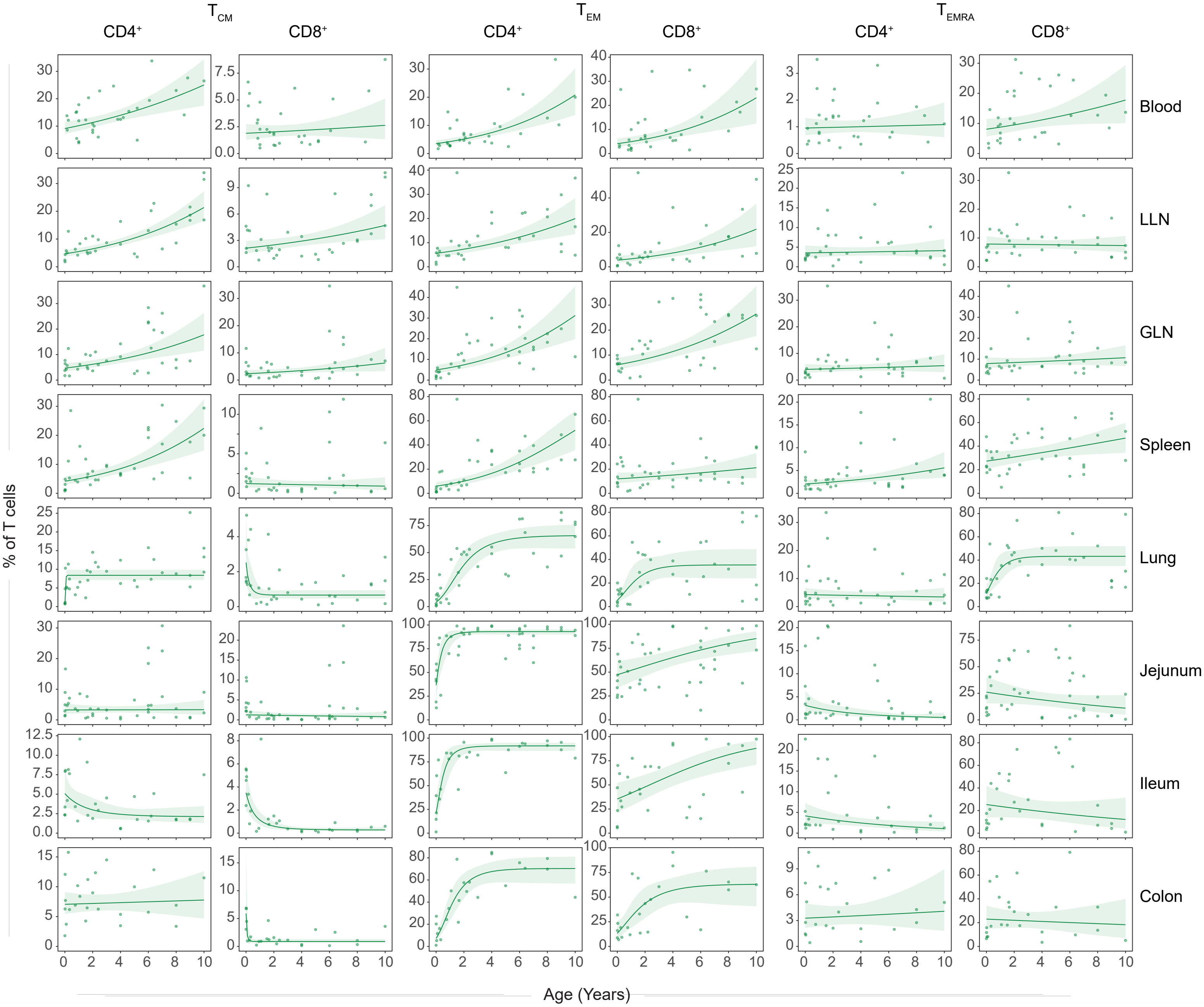

Figure S5: Dynamics of memory subset frequencies over age for each site. Related to Figure 2. Graphs show frequencies of T_{CM} (left), T_{EM} (center), and T_{EMRA} (right) cells for $CD4^+$ (left) and $CD8^+$ (right) lineages within indicated sites over infancy and childhood. blood, n= 31; LLN, n=33; GLN, n= 35; spleen, n= 36; lung, n= 36; jejunum, n= 41; ileum, n= 29; colon, n= 25. Lines represent best fit model (logit-linear vs nonlinear; see methods) with shading depicting 95% confidence interval.

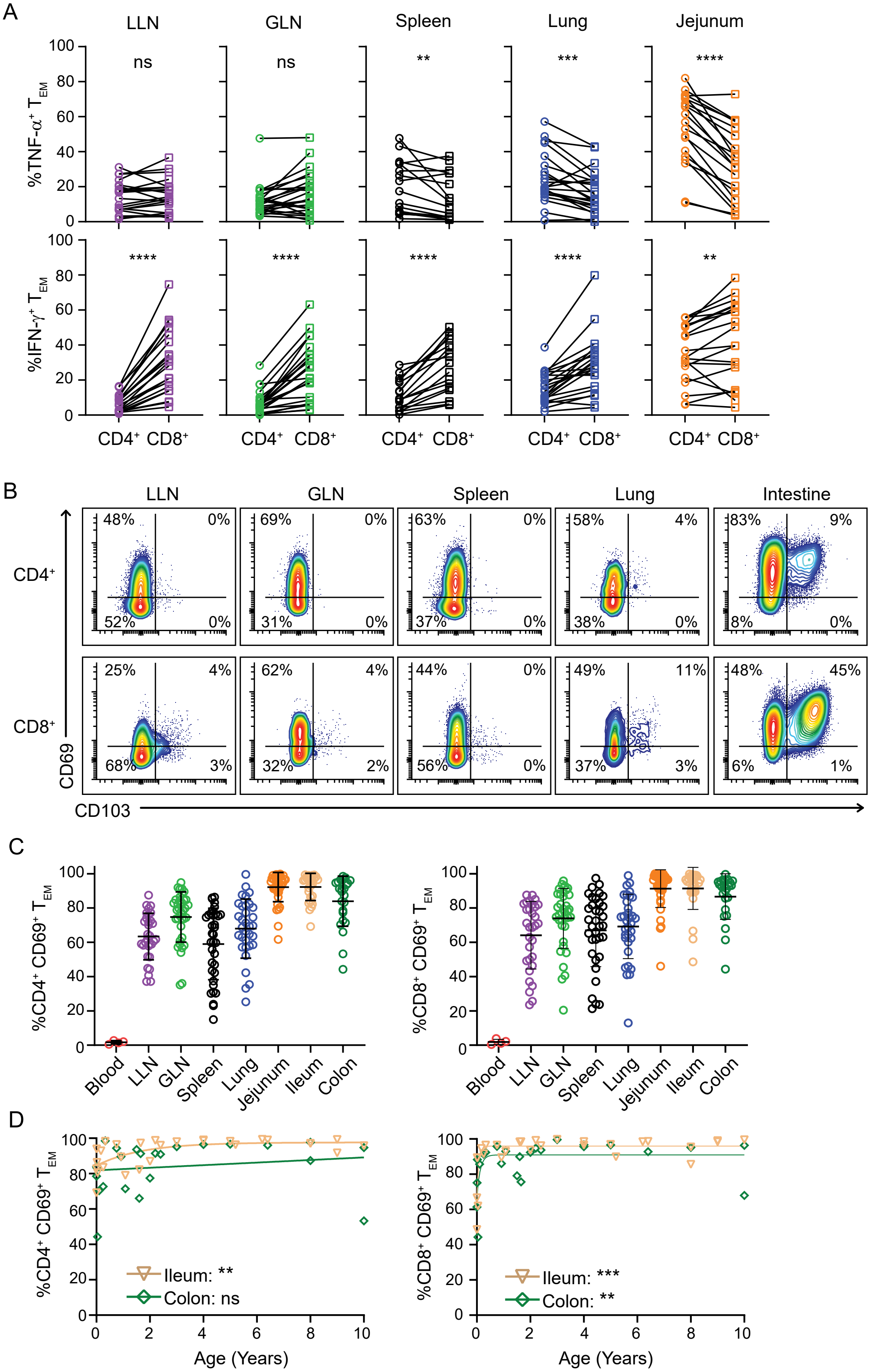

Figure S6: Differential function and CD69 expression by memory T cells in pediatric mucosal sites and lymphoid organs. Related to Figure 3 and 4. (A) Graphs show paired frequency of TNF-α (top) and IFN-γ (bottom) production by CD4$^+$ and CD8$^+$ T$_{EM}$ cells in each indicated site following stimulation with PMA/ionomycin. Statistical testing by paired t-test. (B) Representative flow cytometry plots showing coordinate expression of T$_{RM}$ markers CD69 and CD103 by CD4$^+$ (top) and CD8$^+$ (bottom) T$_{EM}$ cells from indicated tissues obtained from a 9-month-old donor, showing percent of T$_{EM}$ cells within each quadrant. (C) Comparison of CD69 expression by CD4$^+$ (left) and CD8$^+$ (right) T$_{EM}$ in different tissue sites from all donors. (D) CD69 expression by CD4$^+$ (left) and CD8$^+$ (right) T$_{EM}$ cells in the ileum and colon over infancy and childhood. Statistical testing via Pearson correlation with best fit linear or nonlinear model depicted. Number of samples: blood, n = 0-4; LLN, n = 21-31; GLN, n = 24-36; spleen, n = 17-35; lung, n = 22-33; jejunum, n = 20-39; ileum, n = 28; colon, n = 24. ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, **** = $p<0.0001$.

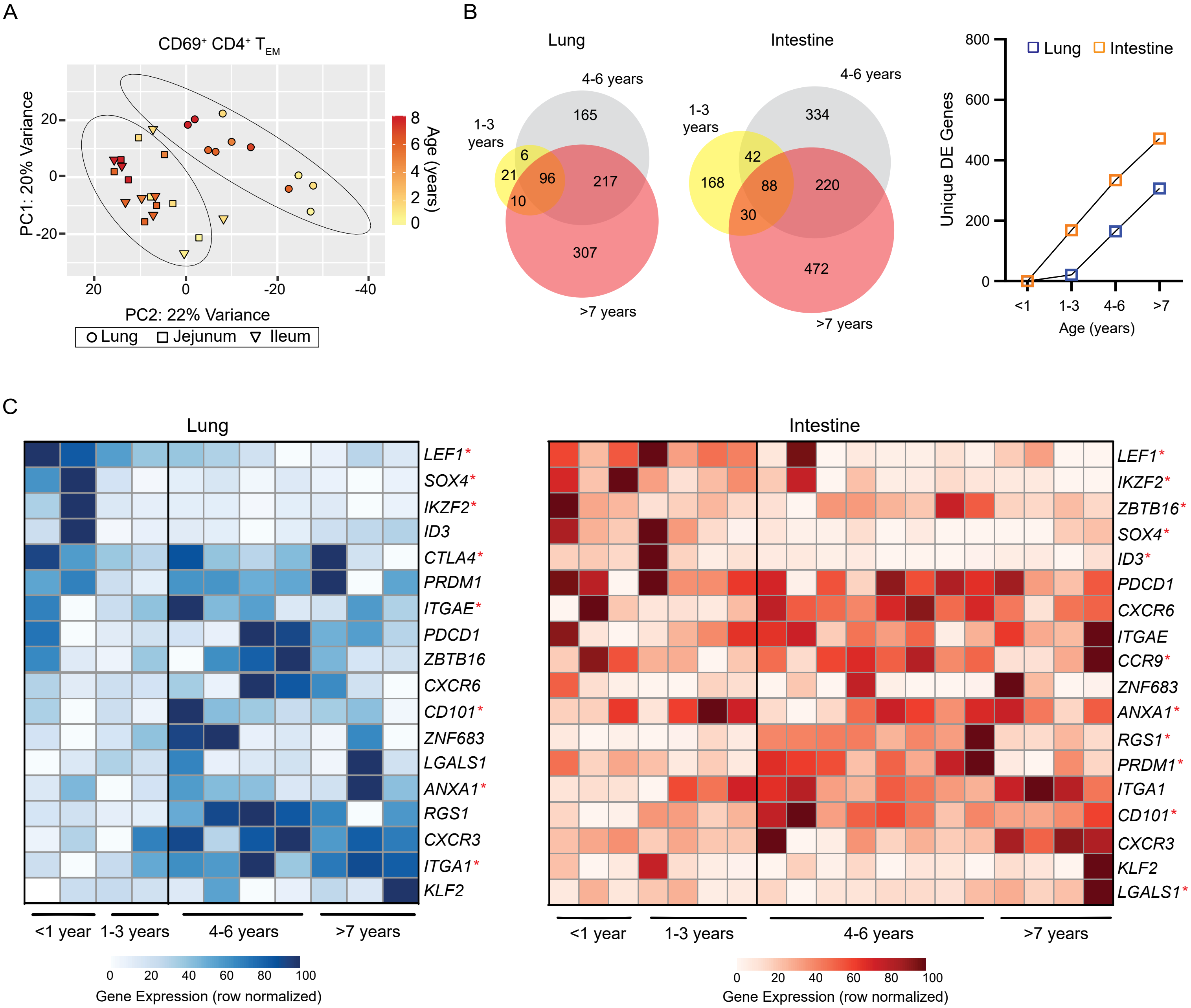

Figure S7: Transcriptional maturation of CD4^{+} resident memory T cells over infancy and childhood. Related to Figure 5. CD4^{+}CD69^{hi} T$_{EM}$ cells (CD4^{+}T$_{RM}$) were sorted from the lung and intestines of infant and pediatric organ donors (n=11, aged 3 months – 8 years) for whole transcriptome profiling by population RNAseq. (A) Principal component analysis of CD4^{+}CD69^{hi} T$_{EM}$ cells from lungs (circles; n = 11) and intestine (triangles; jejunum n=10, and ileum n=9). Samples colored based on age of donor. Circles represent 1 standard deviation based on tissue site (lung or intestine). (B) Differentially expressed (DE) genes comparing older organ donors (1-3 years; n=2, 4-6 years; n=4, >7 years; n = 3) to youngest donors (<1 years; n=2). Venn diagrams (left) depicting DE genes (log2fold > ±0.5, $p<0.05$) within and across donor groups compared to youngest donors. Line graph depicting unique DE genes in each age group compared to youngest donor group (lungs; blue squares and intestines; orange squares). (C) Heatmaps depict row normalized gene expression of differentially expressed genes in lungs (left) and intestines (right) in donors of increasing age. Statistical testing by Spearman or Pearson correlation analysis. * = $p<0.05$.

Table S1: Organ donor characteristics and tissue use, related to all Figures.

	Donor ID	Age (years)	Sex	Race/Ethnicity	Lungs	LLN	Intestines	GLN	Spleen	Blood
Pediatric Organ Donors	HDL034	0.02	Female	Alaskan Native	x	x	x	x	x	
	HDL035	2	Male	White	x	x	x	x	x	
	HDL040	0.33	Male	White	x		x	x	x	
	HDL042	1.6	Female	African American	x	x	x	x	x	
	HDL043	2	Male	White	x	x	I,C	x	x	
	HDL046	7	Female	White	x	x			x	
	HDL047	5	Male	White	x	x	x	x	x	
	HDL048	0	Not Reported	Unknown			x	x	x	
	HDL049	0.17	Female	White	x	x	J,I	x		x
	HDL050	0.01	Male	White	x	x	x	x		
	HDL052	9	Male	White	x	x	J,I		x	
	HDL053	2	Male	White	x	x	x	x	x	x
	HDL054	4	Male	White			x	x	x	
	HDL055	0.25	Male	White	x	x	x	x	x	
	HDL059	0	Not Reported	Unknown	x	x	J,I	x	x	
	HDL060	3	Male	White	x	x	x	x	x	
	HDL062	4	Female	White	x	x	x	x	x	
	HDL069	6	Male	Hispanic		x	x	x		
	HDL070	1.6	Female	White	x	x	x	x	x	
	HDL071	6	Male	Hispanic	x	x	x	x		
	HDL072	5	Male	White	x	x	J,I	x		x
	HDL073	0.92	Male	White	x	x	x	x	x	
	HDL074	6	Male	Native American	x	x	x	x	x	
	HDL078	1.83	Female	African American	x	x	x	x	x	
	HDL080	4	Male	White	x	x				
	HDL081	8	Male	White	x	x	x	x		x
	HDL082	0.75	Female	Hispanic	x	x	x		x	
	HDL085	0.02	Male	Hispanic	x		x		x	
	HDL086	1.5	Female	White	x	x	J,C	x	x	
	HDL090	8	Male	African American		x	x	x	x	
	HDL092	1.08	Male	Hispanic	x	x	x	x	x	
	HDL093	0.58	Female	Hispanic	x	x	J		x	
	HDL097	0.1	Female	White	x	x	C	x		
	HDL098	10	Male	Hispanic	x	x	x		x	
	HDL099	4	Male	White		x		x	x	
	HDL101	9	Male	Hispanic	x	x				
	HDL104	0.67	Female	Hispanic	x				x	
	HDL105	1.67	Male	African American	x	x		x	x	
	HDL106	4	Male	White	x	x		x		
	HDL110	0.42	Female	Hispanic					x	
	HDL111	3	Male	African American			J		x	
	HDL113	10	Male	Hispanic	x	x	J	x	x	
	HDL115	2	Female	Unknown		x	J	x		
	HDL116	6	Female	Hispanic	x	x	J	x		
	HDL118	0.17	Male	White	x		J	x	x	
	HDL120	0.03	Male	White				x		
	HDL124	0.02	Male	White	x		J		x	
	HDL125	4	Male	Hispanic	x		J	x	x	
	HDL129	2	Male	White			J			
	HDL130	7	Male	White	x		J	x	x	
	HDL132	6	Female	Hispanic			J	x	x	
	HDL134	0.33	Female	White	x					
	HDL136	6	Male	African American			J	x	x	
	HDL137	0.33	Male	White			J	x		
	HDL139	6	Male	African American			J	x	x	
	HDL141	2	Female	White				x	x	
	HDL142	1.33	Male	White			J	x		
	HDL143	9	Male	White	x	x			x	
	HDL144	0.02	Male	Hispanic			J	x	x	
	HDL147	10	Male	White	x	x			x	
	HDL150	7	Male	African American	x	x	J	x	x	
	HDL151	7	Male	White	x	x	J	x		
	HDL155	0.13	Female	White			J	x		
	LI002	5	Female	Hispanic			J	x		
	LI003	8	Male	White				x	x	
	nPOD6457	9	Female	White			J,I	x	x	
	D381	0.17	Male	Hispanic						x
	D460	0.58	Female	Unknown	x		J			
Adult Organ Donors	D198	23	Female	White			J			
	D314	35	Female	White			J			
	D338	49	Male	Hispanic			J			
	D344	44	Male	African American	x					
	D367	23	Not Reported	Unknown			J			
	D377	42	Female	White	x					
	D397	34	Male	White	x					
	D427	69	Male	Hispanic	x					

1. LLN = Lung Lymph Node
2. J = Jejunum, I = Ileum, C = Colon
3. X = donors where all three intestinal sites were used
4. GLN = Gut Lymph Node

Table S2: Cell counts for RNA sequencing per donor by site and subset, related to Figures 5-7.

Donor	Age (years)	Lung		Jejunum		Ileum	
		CD4	CD8	CD4	CD8	CD4	CD8
HDL040	0.33	60000	60000	30000	30000		18000
HDL073	0.92	21000	17000	124000	35000	154000	23000
HDL042	1.6	155000	114000	321000	350000	54000	90000
HDL078	1.83	17000	66000	212000	55000	152000	100000
HDL047	5	94000	92000	112000	364000	403000	445000
HDL069	6	50000	50000	50000	50000	50000	50000
HDL071	6	181000	157000	211000	274000	424000	417000
HDL074	6	198000	225000	525000	484000	384000	
HDL046	7	45195	16118				
HDL081	8	49000	57000	487000	139000	470000	231000
HDL090	8	38000		543000	881000	500000	500000

Table S4: Selected Gene Sets Enriched in Oldest (>7 years) Compared to Youngest (<1 year) Donors, related to Figure 5.

CD8 TRM Intestine	NES	nom p-val	Exact Source
GOBP_CELL_ADHESION	1.91	0.000	GO:0007155
GOBP_REGULATION_OF_CELL_DIFFERENTIATION	1.63	0.000	GO:0045595
GOBP_REGULATION_OF_INTRACELLULAR_SIGNAL_TRANSDUCTION	1.61	0.000	GO:1902531
GOBP_INFLAMMATORY_RESPONSE	1.61	0.003	GO:0006954
GOBP_NEGATIVE_REGULATION_OF_DEFENSE_RESPONSE	1.60	0.013	GO:0031348
GOBP_REGULATION_OF_CELL_ADHESION	1.57	0.014	GO:0030155
GOBP_REGULATION_INFLAMMATORY_RESPONSE	1.57	0.028	GO:0050727
GOBP_NEGATIVE_REGULATION_OF_IMMUNE_RESPONSE	1.51	0.047	go:0050777
CD8 TRM Lung	NES	nom p-val	Exact Source
GOBP_LEUKOCYTE_MEDIATED_IMMUNITY	1.61	0.012	GO:0002443
GOBP_LEUKOCYTE_CELL_CELL_ADHESION	1.60	0.016	GO:0007159
GOBP_DEFENSE_RESPONSE	1.56	0.003	GO:0006952
GOBP_CELL_ADHESION	1.45	0.017	GO:0007155
GOBP_IMMUNE_RESPONSE	1.43	0.014	GO:0006955
CD4 TRM Intestine	NES	nom p-val	Exact Source
GOBP_CELL_ADHESION	1.88	0.000	GO:0007155
GOBP_REGULATION_OF_CELL_ADHESION	1.72	0.000	GO:0030155
GOBP_REGULATION_OF_CELL_DIFFERENTIATION	1.69	0.000	GO:0045595
GOBP_INFLAMMATORY_RESPONSE	1.69	0.000	GO:0006954
CD4 TRM Lung	NES	nom p-val	Exact Source
GOBP_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	1.70	0.005	GO:0002682
GOBP_IMMUNE_EFFECTOR_PROCESS	1.66	0.022	GO:0002252

Table S5: Unique clonotypes on TCR analysis per donor by tissue and subset, related to Figure 7.

Donor	Age (years)	LLN		GLN		Lung		Jejunum		Ileum	
		CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8	CD4	CD8
HDL040	0.33		470	2391	5903	2272	2348	1976	2904		1855
HDL073	0.92	7424	2030	4284	766	145	1785	7055	3713	4097	2507
HDL042	1.6	12847	1666	7588	726	6153	4306	5904	3291	2958	2410
HDL078	1.83	353	558	3173	96	3417	4832	8106	3148	4090	3359
HDL047	5	13661	5685	12347	5277	7926	4085	9875	4784	5668	4190
HDL069	6	4797	3320	3177	2236	2814	1002	2252	602	1491	338
HDL071	6	5170	3319	4353	2533	3936	2511	3446	1149	3985	2521
HDL074	6	2339	1828	1013	799	2477	1106	1996	931	1685	68
HDL046	7	605	2168			3337	1477				
HDL081	8	10223	6760	15299	9713	5519	3176	7583	3352	7003	858
HDL090	8	4549	1566	7871	5070	539	165	5573	1557	3625	1346