Supplemental Table of Contents and Legends

Supplemental figure 1. CM aged and CM IR mice have similar cystic indices and cyst number at the time points chosen for scRNAseq. (A) Representative H&E images of the CM aged and cont aged mice used for scRNAseq. **(B)** Representative H&E images of CM IR, CM sham, cont IR mice used for scRNAseq. For both experimental groups, cilia loss was induced at ~8wks of age. **(C,D)** Quantification of **(C)** cystic index and **(D)** cyst number for CM aged and CM IR mice. Each dot represents an individual animal. The data shown in this graph come from the CM aged and CM IR *Rag1* control mice (as shown in Figure 6) and the mice used for scRNAseq. The red data points represent the animals that were used for scRNAseq experiments.

Supplemental figure 2. CAP digestion increases cell survival while reducing expression of stress response genes compared to standard 37C digest. (A) Schematic depicting the experimental design used for testing standard 37C digest and cold activated protease (CAP) 12C digest. **(B)** Quantification of CD45+ immune cell, LTA+ proximal tubule epithelial cell, and CD45-, LTA- cell viability using 37C and CAP digest as determined by flow cytometry. **(C)** qRT-PCR for stress response genes (*Fos, Jun, Egr1*) in cell populations isolated from undigested, 37C digested, and CAP digested tissue normalized to *Hprt*. N=3 mice per group. Significance was determined by Two-way ANOVA. *P <0.05, ***P <0.001.

Supplemental figure 3. Heatmap showing the top 5 DEGs in each cluster of cells from the whole kidney single cell atlas. The top 2 DEGs in each cluster of cells is labelled.

Supplemental figure 4. Heatmap showing the top 5 DEGs in each cluster of cells from the immune single cell atlas. The top 3 DEGs in each cluster of cells is labelled.

Supplemental figure 5. Heatmap showing the top 5 DEGs in each cluster of MNPs. The top 2 most DEGs in each cluster of cells is labelled.

Supplemental figure 6. CM aged mice have increased numbers of MNPs localized adjacent to cysts compared to CM IR mice. (A) Gating strategy and markers used to validate single cell RNA sequencing data. **(B)** Heatmap showing the quantification of MNPs as a percentage of live single cells as determined by flow cytometry. CM aged (N=2), cont aged (N=3), CM IR (N=6), CM sham (N= 3), cont IR (N=6). Statistical significance was determined as described in Figure 3. **(C)** Representative confocal microscopy images of CM aged (top) and CM IR (bottom) kidney sections stained with F4/80 (white), CD206 (green), and Hoechst.

Supplemental figure 7. Pathway analysis of MNP clusters. (A,B) List of the top enriched **(A)** pathways and **(B)** biological functions in *Mrc1*+ KRM as determined by Ingenuity Pathway Analysis (IPA) software. Pathways and functions were identified using all DEGs that were significantly enriched (adjusted P value <0.05) in each respective cluster. **(C,D)** List of the top enriched pathways in **(C)** CM aged and **(D)** CM IR MNP clusters as determined by Ingenuity Pathway Analysis (IPA) software. Pathways and functions were identified using all DEGs that were significantly enriched (adjusted P value <0.05) in each respective cluster from each experimental condition.

Supplemental figure 8. Heatmap showing the top 5 DEGs in each cluster of T-cells. The top 2 DEGs in each cluster of cells is labelled.

Supplemental figure 9. Naïve/Central memory T-cells express transcripts associated with naïve and antigen experienced T-cells. Violin plots showing expression of *Ccr7, Sell,* and *Cd69* in T-cell clusters shown in Figure 4.

Supplemental figure 10. CM aged mice have increased numbers of T-cells compared to all other groups. (A) Gating strategy and markers used to identify T-cell populations via flow cytometry. **(B)** Heatmap showing the quantification of T-cells as a percentage of live single cells as determined by flow cytometry. CM aged (N=2), cont aged (N=3), CM IR (N=6), CM sham (N= 3), cont IR (N=6). Statistical significance was determined as described in Figure 3.

Supplemental figure 11. Only a small fraction of CD4+ Tregs express *Foxp3* by single cell RNA sequencing. (A,B) Violin plots showing expression of **(A)** *Foxp3* and **(B)** *Ikzf2* in T-cell clusters. **(C)** A blended Featureplot showing expression of both *Foxp3* and *Ikzf2* in T-cell clusters.

Supplemental figure 12. Analysis of genes associated with T-cell activation or exhaustion. (A,B) Violin plots showing expression of genes associated with **(A)** T-cell activation or **(B)** T-cell exhaustion. **(C,D)** List of the top enriched pathways in **(C)** CM aged and **(D)** CM IR T-cell clusters as determined by Ingenuity Pathway Analysis (IPA) software. Pathways and functions were identified using all DEGs that were significantly enriched (adjusted P value <0.05) in each respective cluster from each experimental condition.

Supplemental figure 13. Heatmap showing the top 5 DEGs in each cluster of B-cells. The top 2 DEGs in each cluster of B-cells is labelled.

Supplemental figure 14. Integrated single cell data comparing immune cell clusters in cystic disease, AKI, and UUO. (A,B) Umap of integrated single cell data sets based on **(A)** cell clusters or **(B)** experimental condition. **(C)** Heatmap showing the quantification of cluster abundance as a percentage of CD45+ cells in each disease model. This quantification was generated by combining cells from all experimental groups in each disease model (i.e. both

controls and disease samples). * P< 0.05, ** P< 0.01, *** P<0.001 compared to all other experimental groups as determined by Two-way ANOVA. **(D)** Heatmap showing the top 2 DEGs in each cluster of cells.

Supplemental figure 15. Loss of adaptive immune cells does not affect cyst formation in C57BL/6 (slow) or BALB/c (rapid) *Pkd1*$^{RC/RC}$ mice. (A) H&E cross sections of *Pkd1*$^{RC/RC}$ *Rag1*+/+ or *Pkd1*$^{RC/RC}$ *Rag1*-/- mice at 3 months of age on the C57BL/6 (left) or BALB/c (right) background. Scale bar 1mm. Quantification of **(B)** percent kidney weight/body weight, **(C)** percent cystic area **(D)** average cyst size, and **(E)** average cyst number in each group. Red data points represent the H&E sections shown in **(A)**. ANOVA and Mann-Whitney test did not result in significance for any of the analyzed parameters when comparing age/strain matched *Rag1* wildtype versus *Rag1* -/- animals.

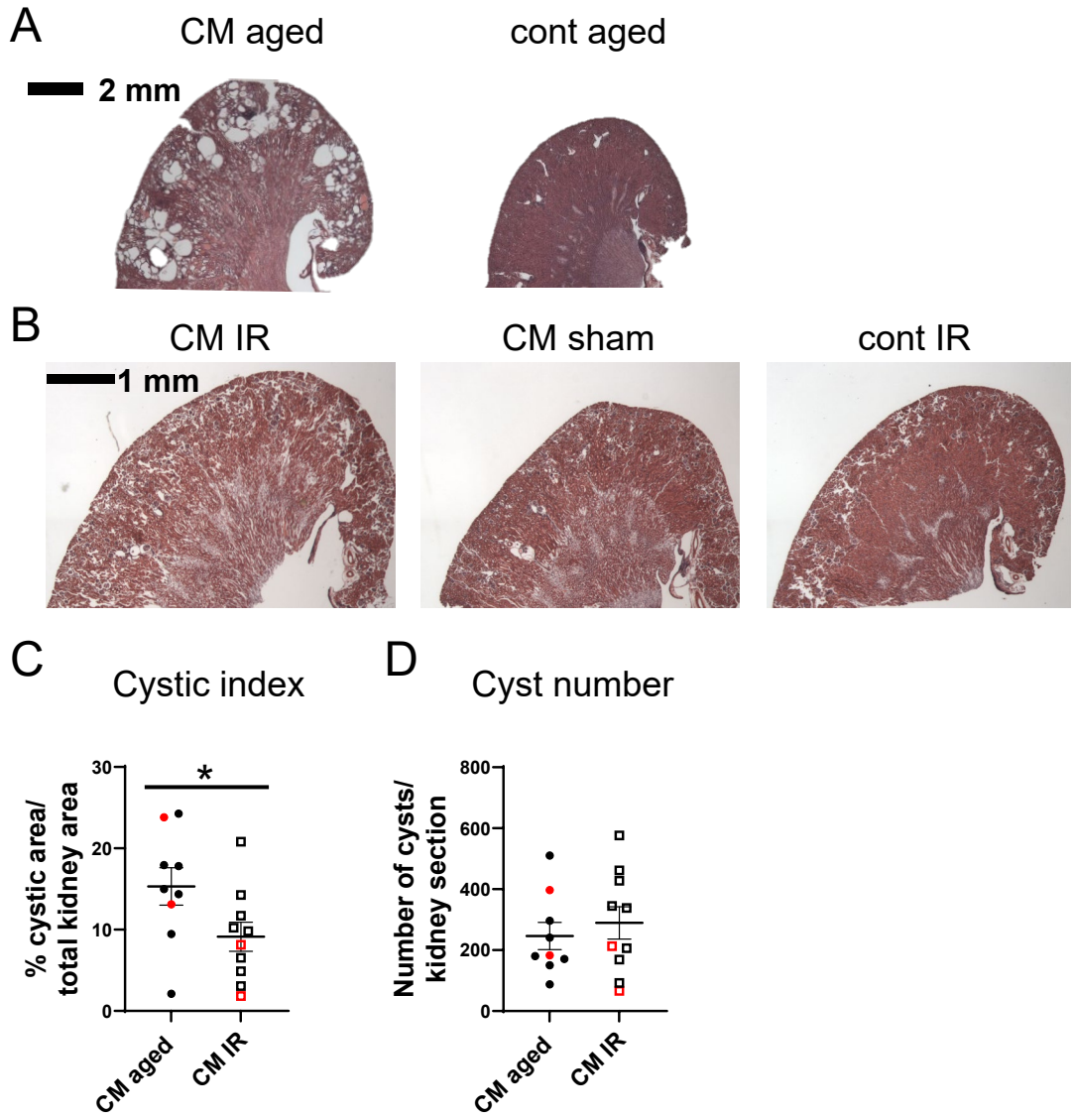
Supplemental figure 16. (A) Gating strategy used to identify Ifng expressing cells. **(B)** Representative FACS plots showing expression of Ifng in T-cell clusters 0-7. **(C)** Quantification of Ifng MFI in each experimental condition.

Supplemental figure 17. List of antibodies used for flow cytometry validation studies.

Supplemental Data 1.

Supplemental Data 2.

Supplemental figure 1



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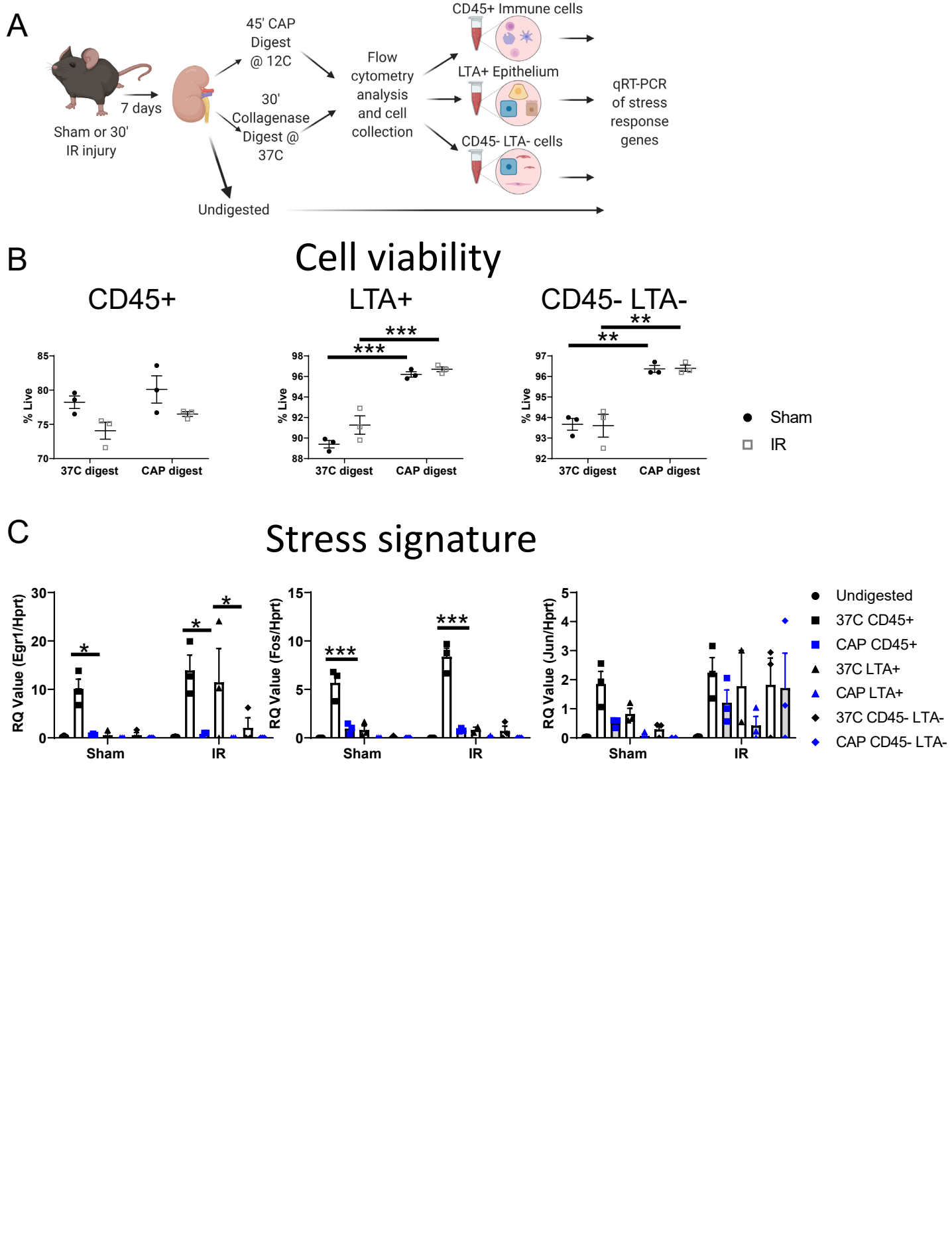

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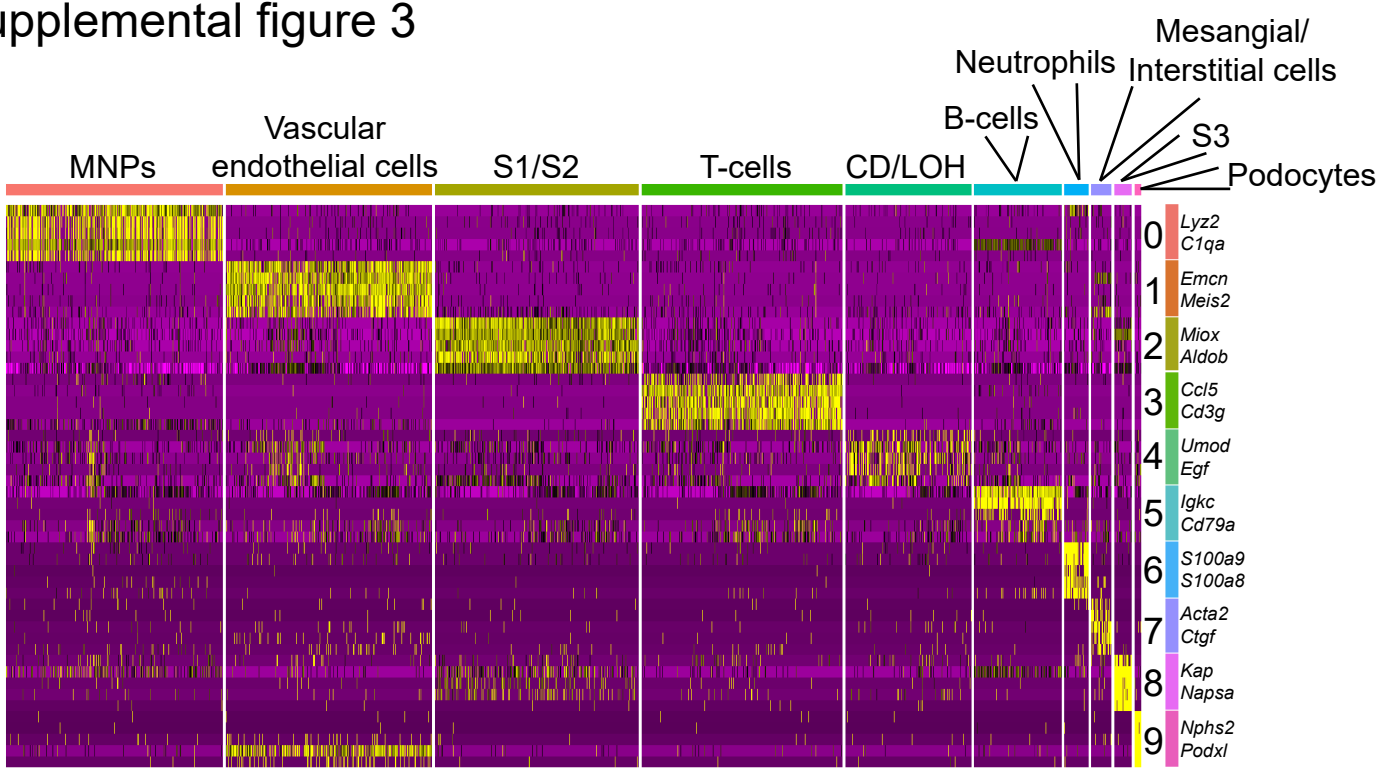

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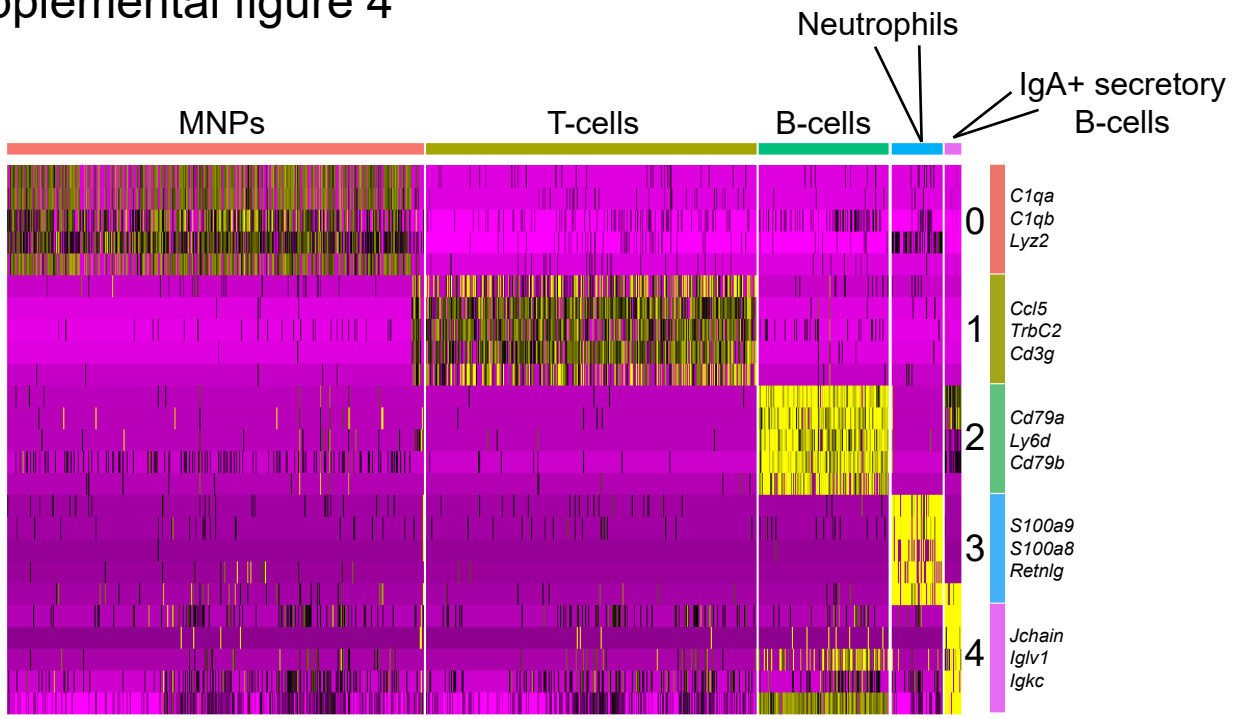

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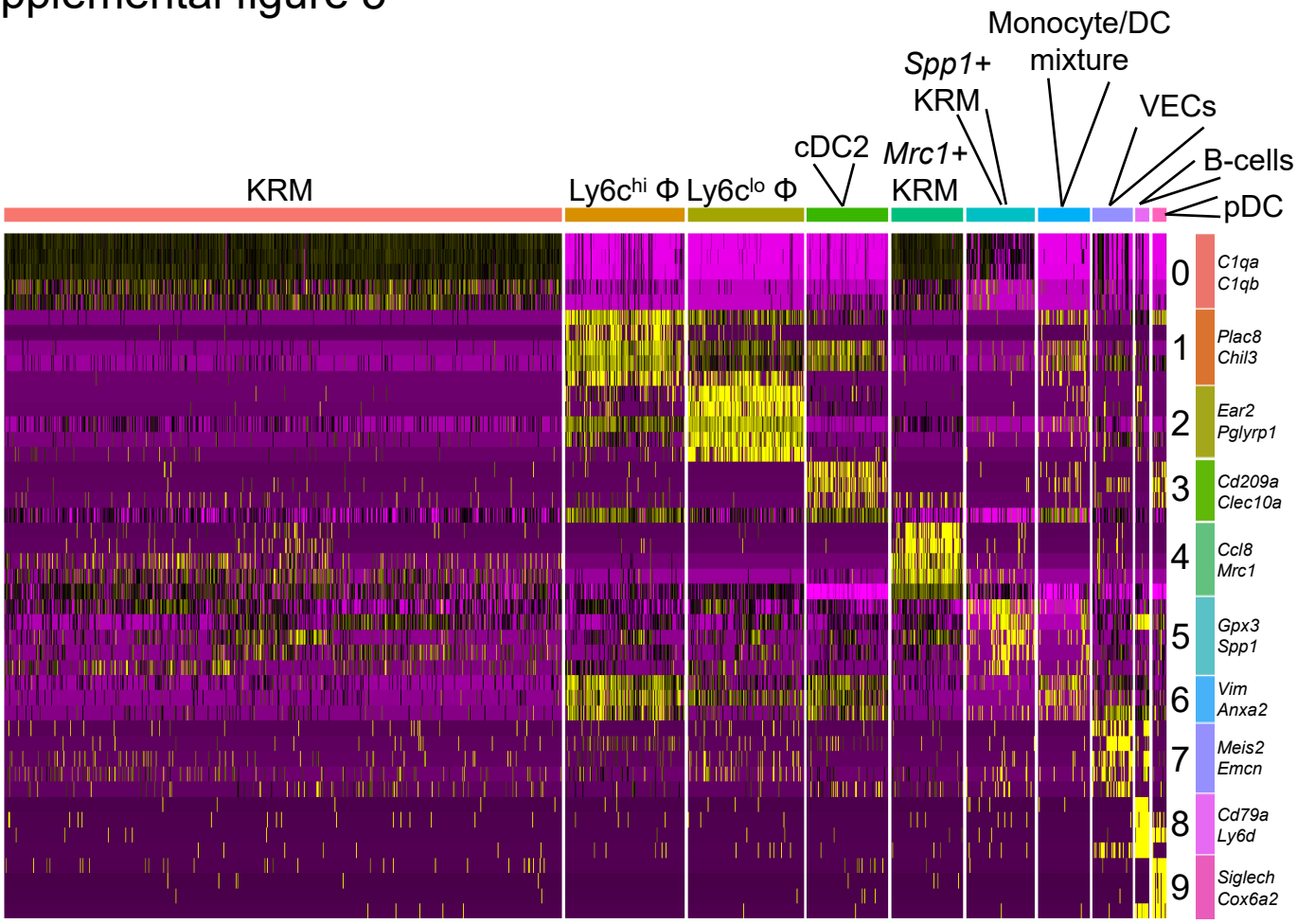

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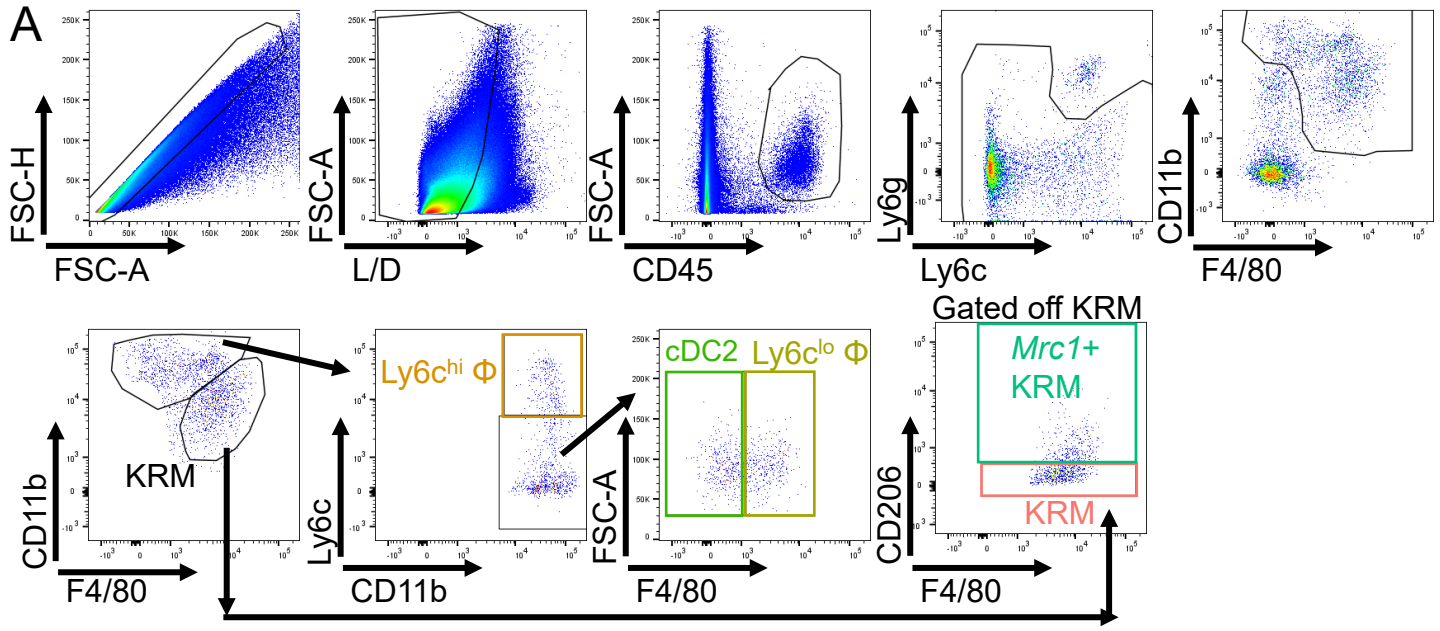
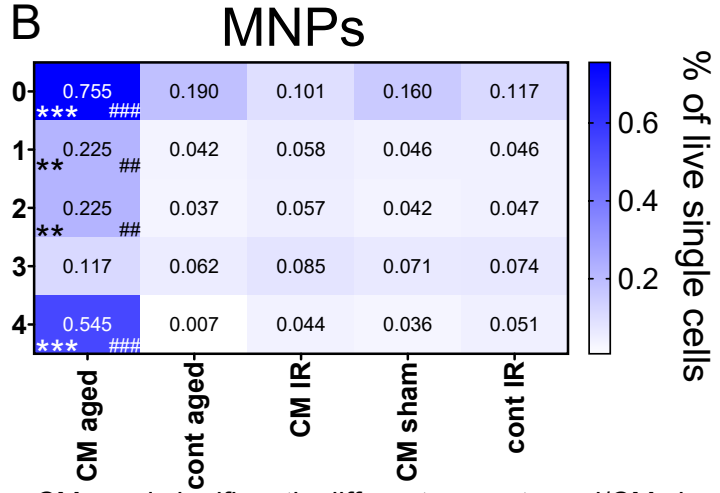
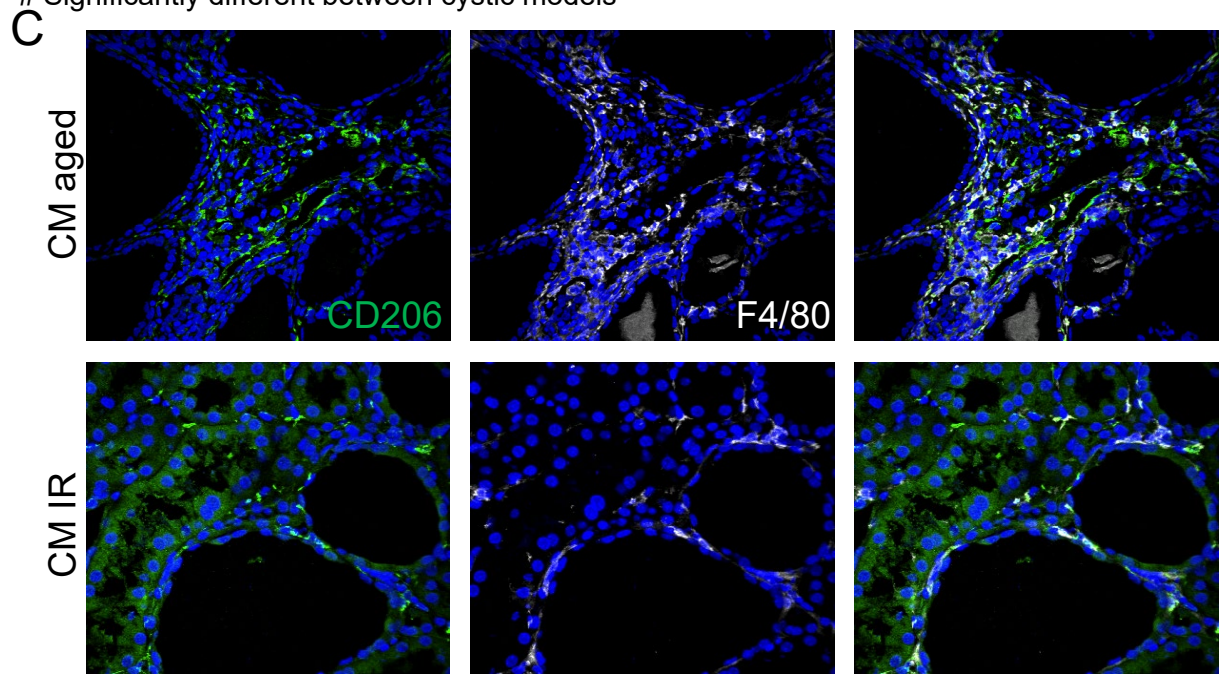




⁎CM aged significantly different vs cont aged/CM sham; $ CM IR significantly different vs CM sham/cont IR;
Significantly different between cystic models



Supplemental figure 7

A

Pathways

Cluster	Pathway	P value	# of genes expressed/# of genes in list
Mrc1+ KRM	Complement system	1.34E-09	8/33
	Phagosome formation	5.61E-08	11/121
	IL-10 signaling	4.65E-07	8/67

B

Functions

Cluster	Function	P value
Mrc1+ KRM	Hematological system development and function	7.47E-25
	Cellular movement	5.51E-22
	Immune cell trafficking	1.50E-10

C

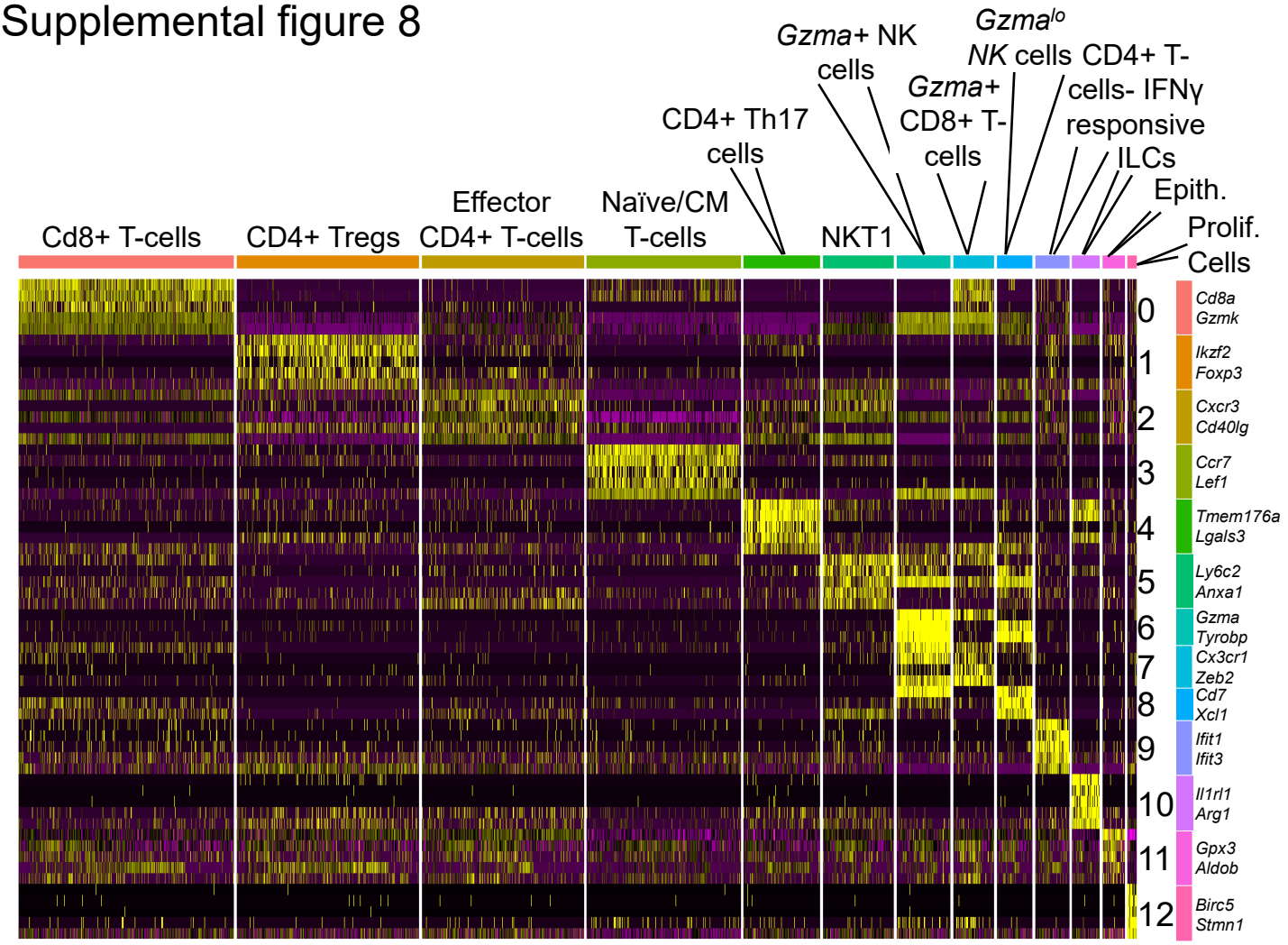
CM aged

Cluster	Pathway	Genes	10^LogP Value
0 (KRM)	Inflammatory response	*Lpl, Ccr5, Trem2*	-12.066
	Leukocyte chemotaxis	*Ccl12, Ccl2, Ccl8, Ccl9*	-9.477
	Phagocytosis	*Fcgr1, Trem2, Pycard*	-7.259
1 (Ly6chi ϕ)	Staphylococcus aureus infection	*H2-Dma, H2-Aa, Cd74*	-19.196
	Inflammatory response	*Apoe, Fcgr2b, Cx3cr1*	-12.847
	Leukocyte differentiation	*Cd74, Jun, Ccr1, Trem2*	-10.719
2 (Ly6clo ϕ)	Humoral immune response	*Igha, Igkc, Ccr2*	-5.684
	Tissue remodeling	*Camk2d, Ccr2, Spp1*	-3.725
	Cellular response to peptide	*Ptpn1, Nod1, Itga4*	-3.198
3 (cDC2)	Lipid localization	*Tspo, Fabp5, Spp1*	-5.233
	Endocytosis	*Ptpn1, Flot1, Grb2*	-4.657
	Negative regulation of mitochondrion organization	*Tspo, Ier3, Prelid1*	-4.410
4 (*Mrc1+* KRM)	Cell chemotaxis	*Ccl8, Ccl7, Pycard*	-8.202
	Leukocyte homeostasis	*Rps8, Rps25, Rpl35*	-7.828
	Adaptive immune response	*Igha, Igkc, Cd74*	-4.938
5 (*Spp1+* KRM)	Endocytosis	*Igkc, Clu, Igha*	-3.257

CM IR

Cluster	Pathway	Genes	10^LogP Value
0 (KRM)	Positive regulation of leukocyte adhesion	*Il1b, Cd74, Vcam1*	-7.735
	Regulation of ion transport	*Cxcr4, Egf, Lilra5*	-6.603
	Positive regulation of cell motility	*Tgfbr1, Il1b, Egf*	-5.944
1 (Ly6chi ϕ)	Neutophil degranulation	*Cd177, Fgr, Itgb2*	-8.347
	Leukocyte cell-cell adhesion	*Tgfb1, Cd44, Hes1*	-7.918
	Leukocyte migration	*Itgb2, Cd300a, Fgr*	-7.750
2 (Ly6clo ϕ)	Epithelial cell differentiation	*Pck1, Cxcr4, Klf2*	-6.345
	Sphingolipid metabolism	*Psap, Ugcg, Gm2a*	-5.059
	Transmembrane RTK signaling pathway	*Egf, Ddit4, Igfbp5*	-4.441
3 (cDC2)	Defense response to other organism	*Ddit4, Serinc3, Trf*	-3.22
4 (*Mrc1+* KRM)	Glycolysis and gluconeogenesis	*Pck1, Aldob, Got1*	-6.356
	Antigen processing and presentation	*H2-D1, Hspa8, Egf*	-3.697
	Positive regulation of cell adhesion	*Pck1, Cd83, Ccn1*	-3.692
5 (*Spp1+* KRM)	Antigen processing and presentation	*H2-Aa, H2-Ab1, Cd74*	-11.986
	Adaptive immune response	*Cd81, Cd74, Fcer1g*	-5.928
	Humoral immune response	*Fau, Cd81, C1qa*	-4.331

Supplemental figure 8



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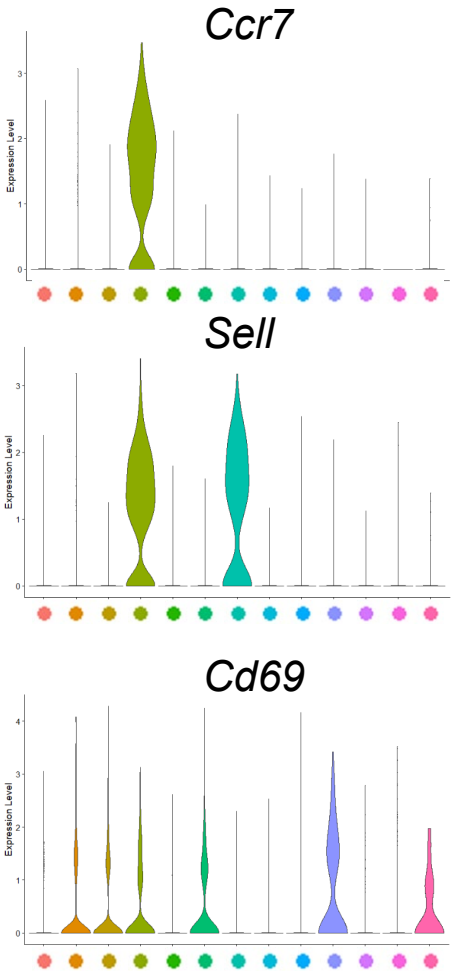

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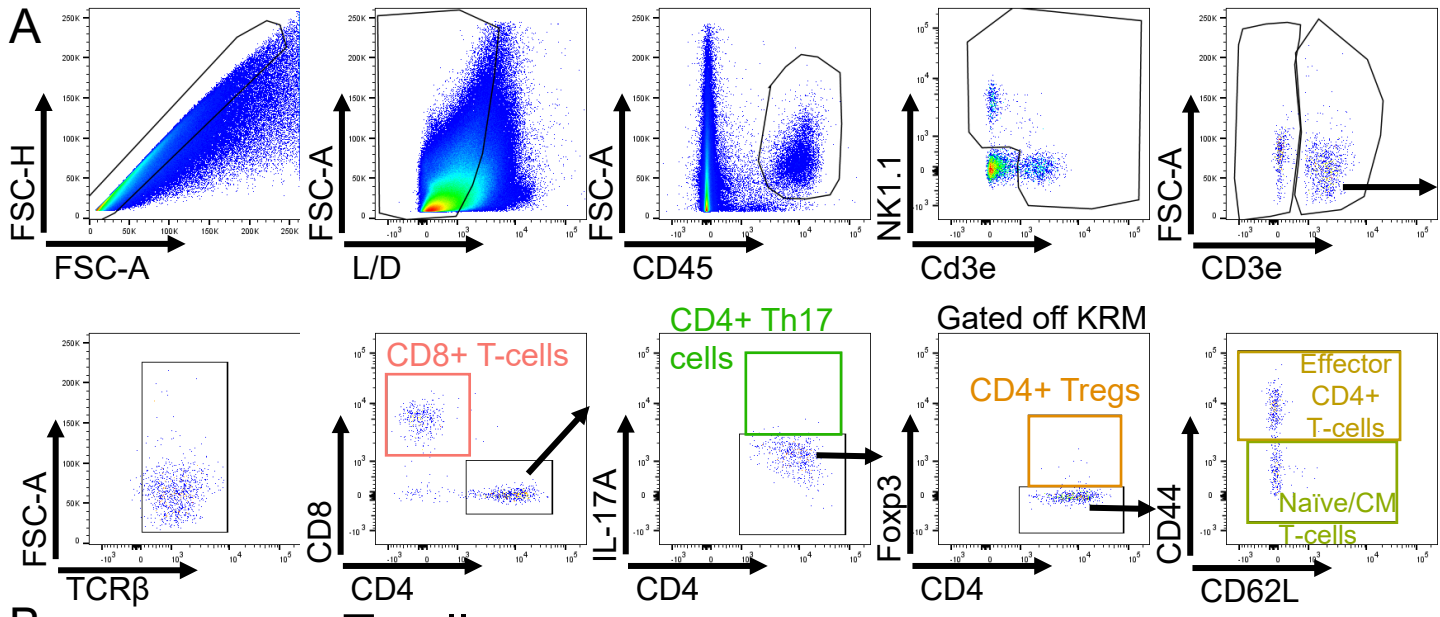

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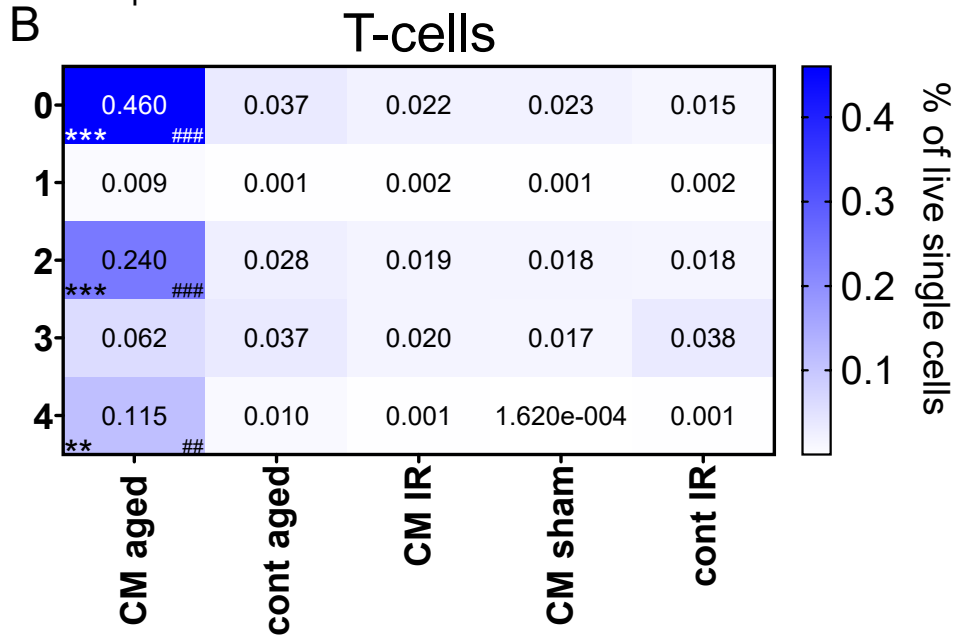
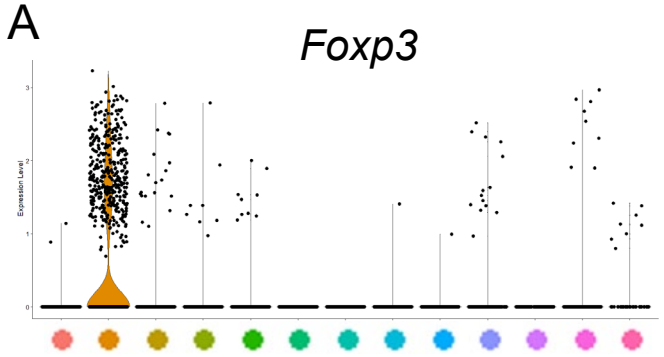


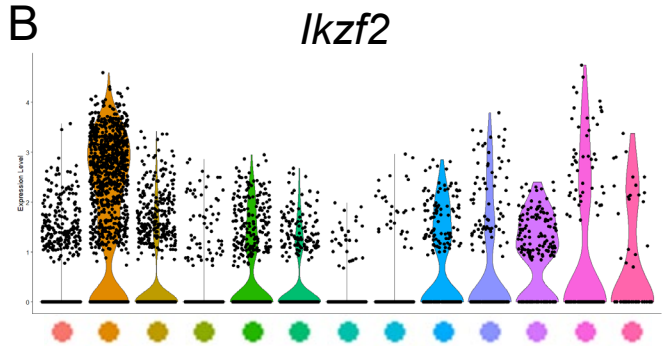
Supplemental figure 11

A



- 0 CD8+ T-cells
- 1 CD4+ Tregs
- 2 Effector CD4+ T-cells
- 3 Naïve/Central memory T-cells
- 4 CD4+ Th17 cells
- 5 NKT1
- 6 *Gzma*+ NK cells
- 7 *Gzma*+ CD8+ T-cells
- 8 *Gzma*^{lo} NK Cells
- 9 CD4+ T-cells- IFNγ responsive
- 10 ILCs
- 11 Epithelium
- 12 Proliferating cells

B



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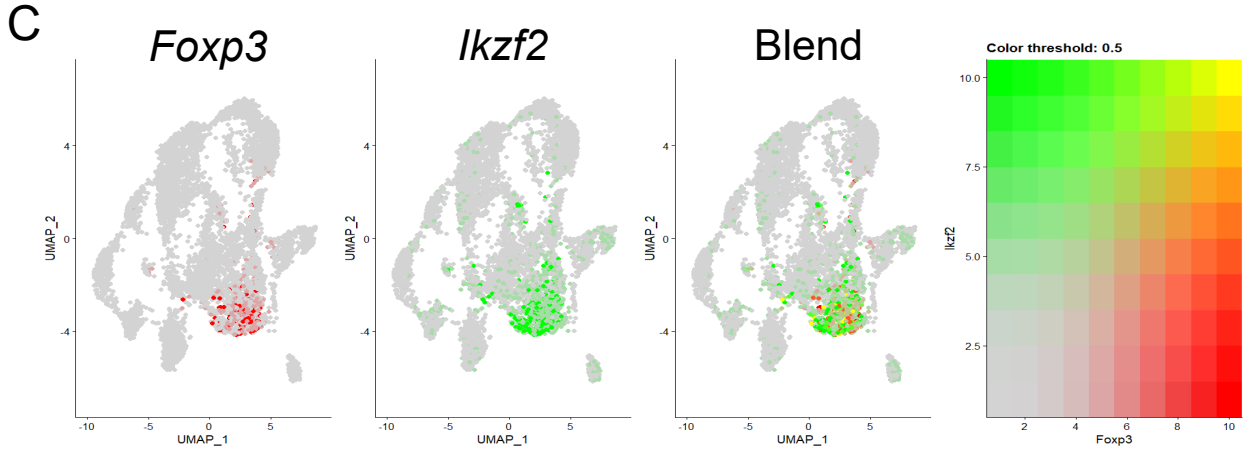
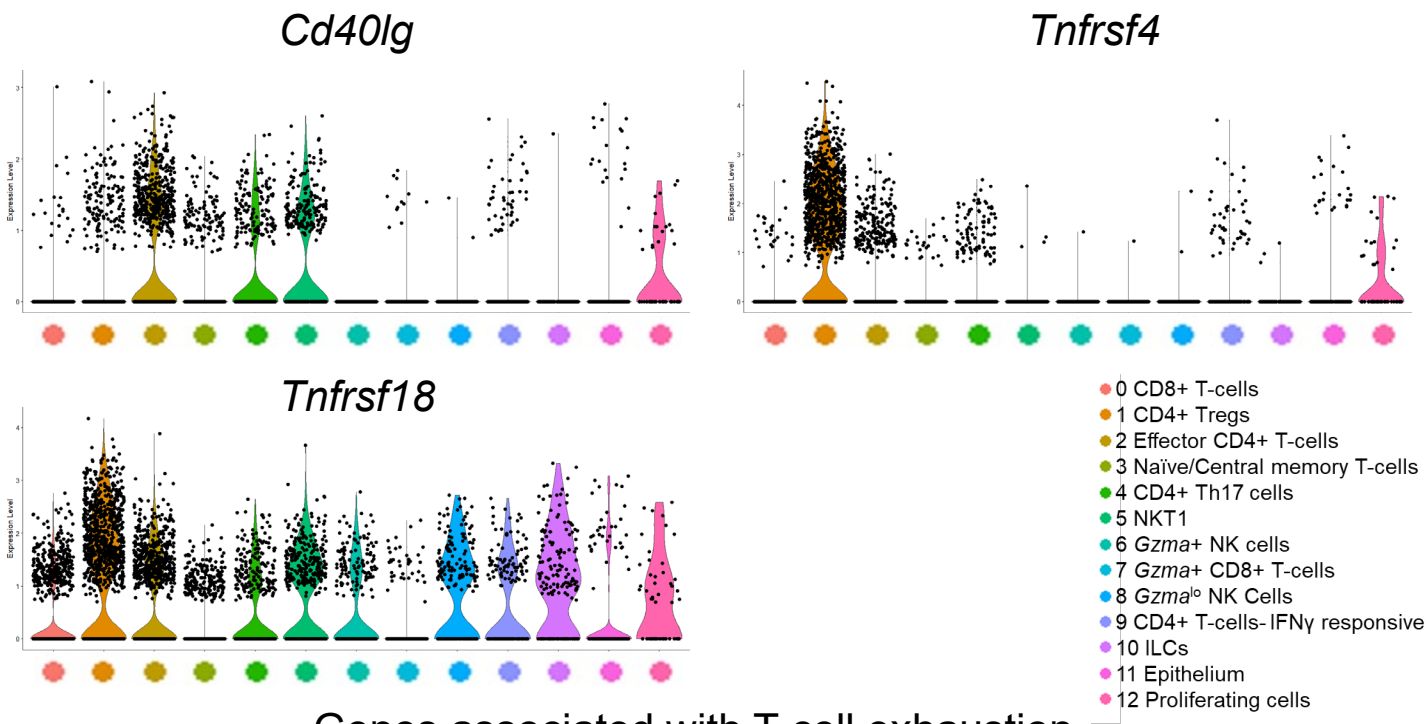


Supplemental figure 12

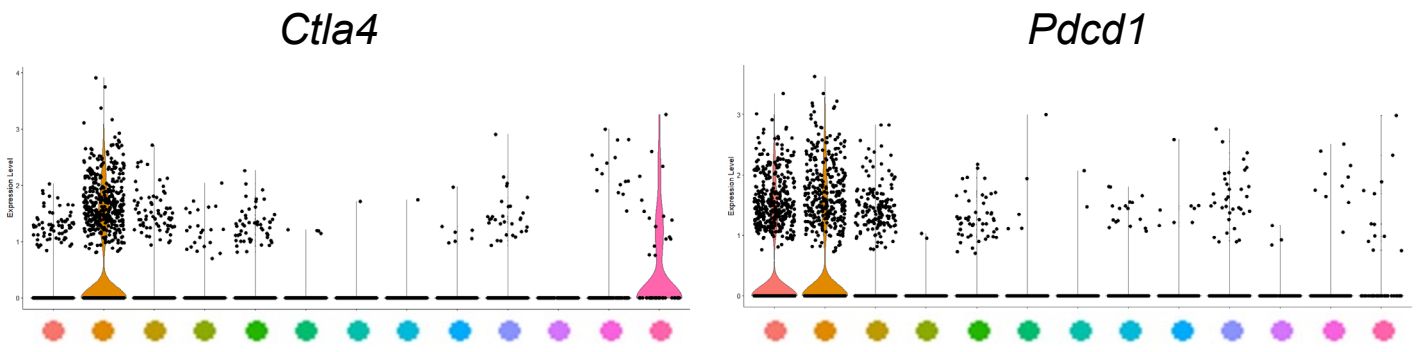
A

Genes associated with T-cell activation



B

Genes associated with T-cell exhaustion



C

CM aged

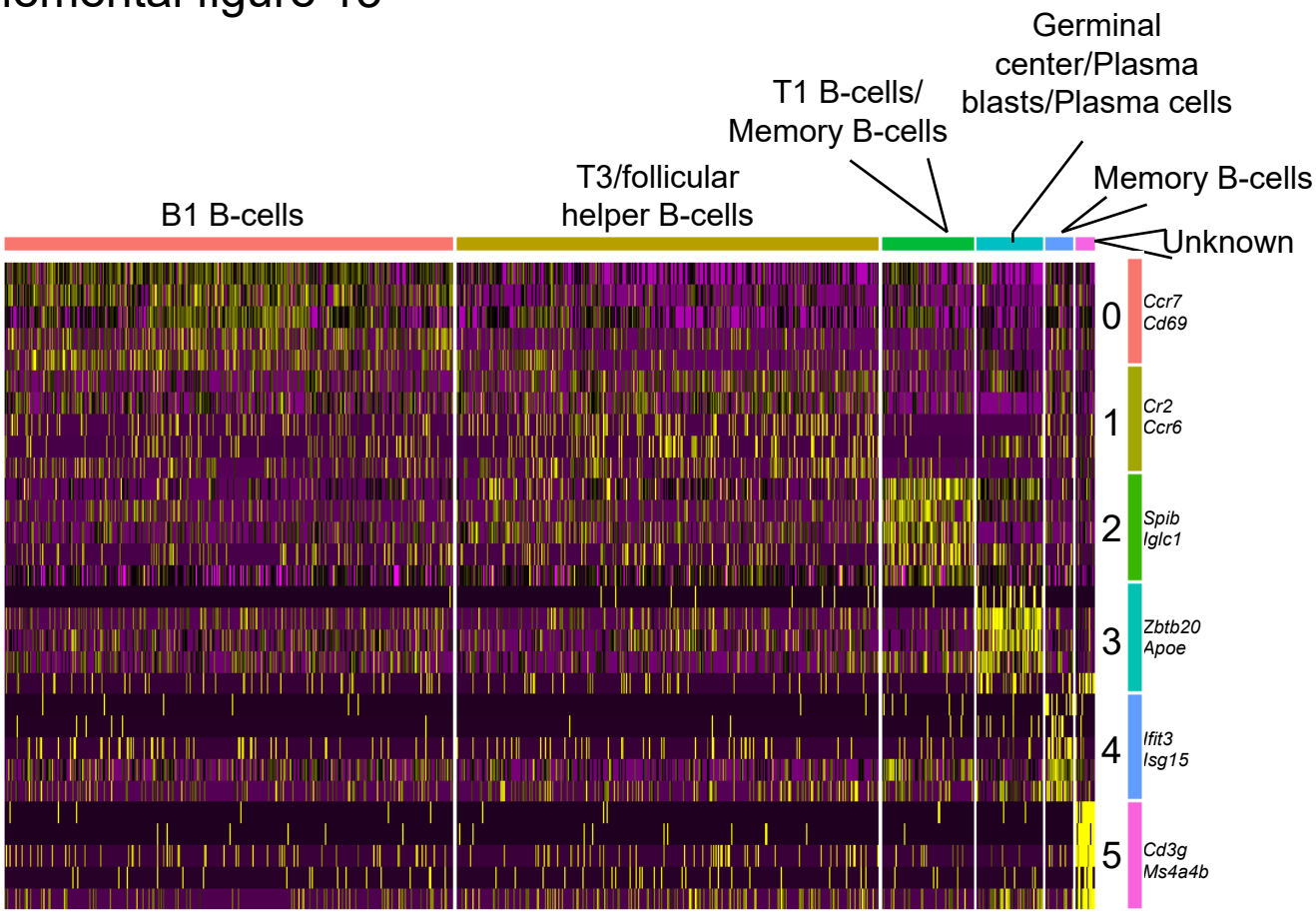
Cluster	Pathway	Genes	10^LogP Value
0 (CD8+ T-cells)	Translation	*Rps12, Rpl35, Rps23*	-18.528
	Ribonucleoprotein complex biogenesis	*Npm1, Rps25, Rps7*	-9.052
	Regulation of signal transduction by p53	*Npm1, Rpl23, Rps7*	-5.885
2 (Effector CD4+ T-cells)	Endocytosis	*Igha, Apoe, Actb*	-5.059
	Defense response to bacterium	*Igha, Rps19, Lcn2*	-4.524
	Organic hydroxy compound transport	*Actb, Clu, Apoe*	-3.789

D

CM IR

Cluster	Pathway	Genes	10^LogP Value
0 (CD8+ T-cells)	Endocrine and other factor-regulated calcium reabsorption	*Kl, Klk1, Fxyd2*	-5.537
	Sodium ion transmembrane transport	*Slc12a3, Slc12a1, Slc34a1*	-5.174
	Response to inorganic substance	*Gatm, Aldob, Cfl1*	-4.462
2 (Effector CD4+ T-cells)	Cellular response to steroid hormone stimulus	*Zfp36l2, Pck1, Ddx5*	-3.228
	PI3K activates AKT signaling	*Egf, Psmc4, Rac2*	-2.825
	Response to metal ion	*Aldob, Gatm, Egf*	-2.556

Supplemental figure 13



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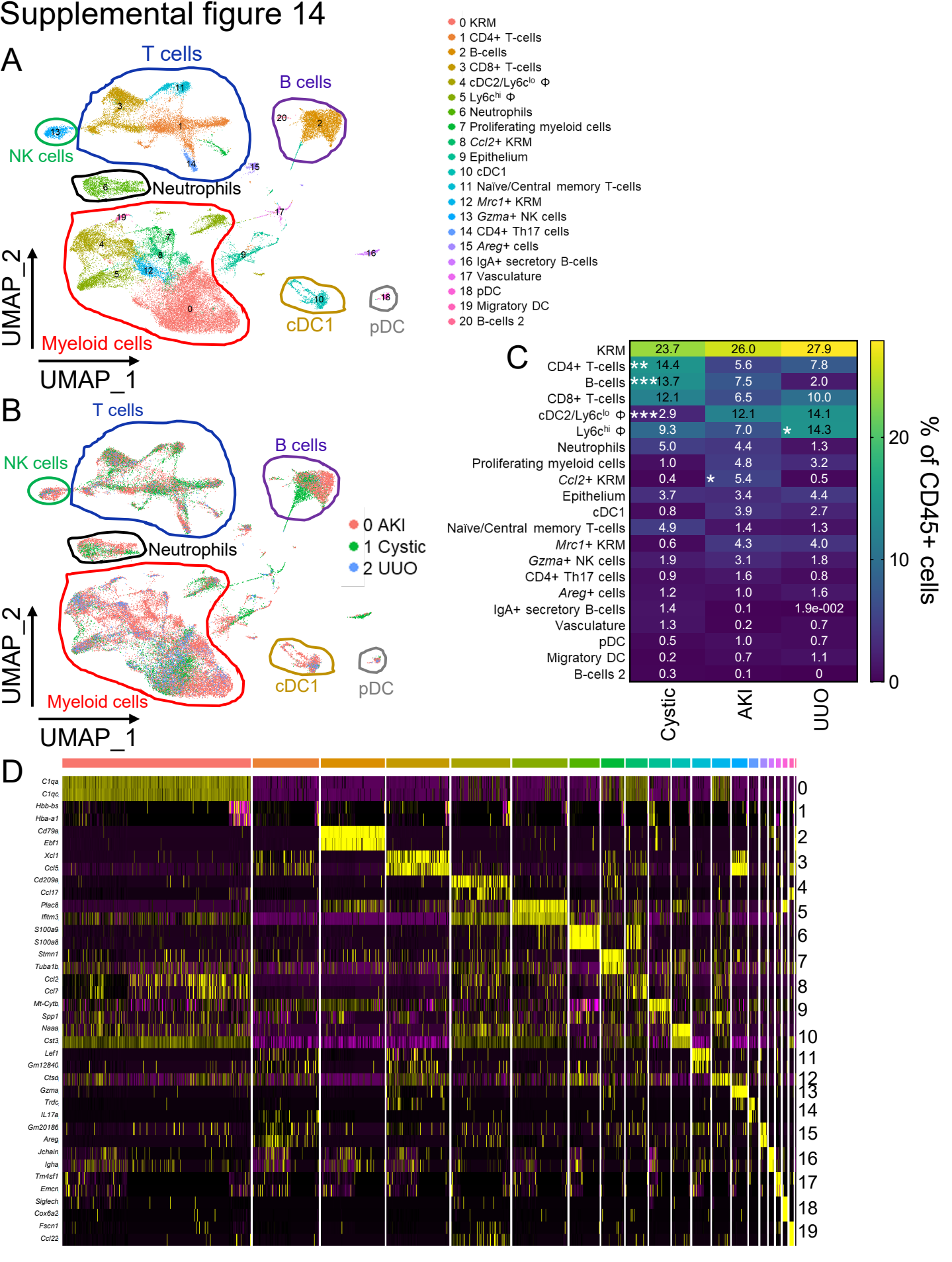

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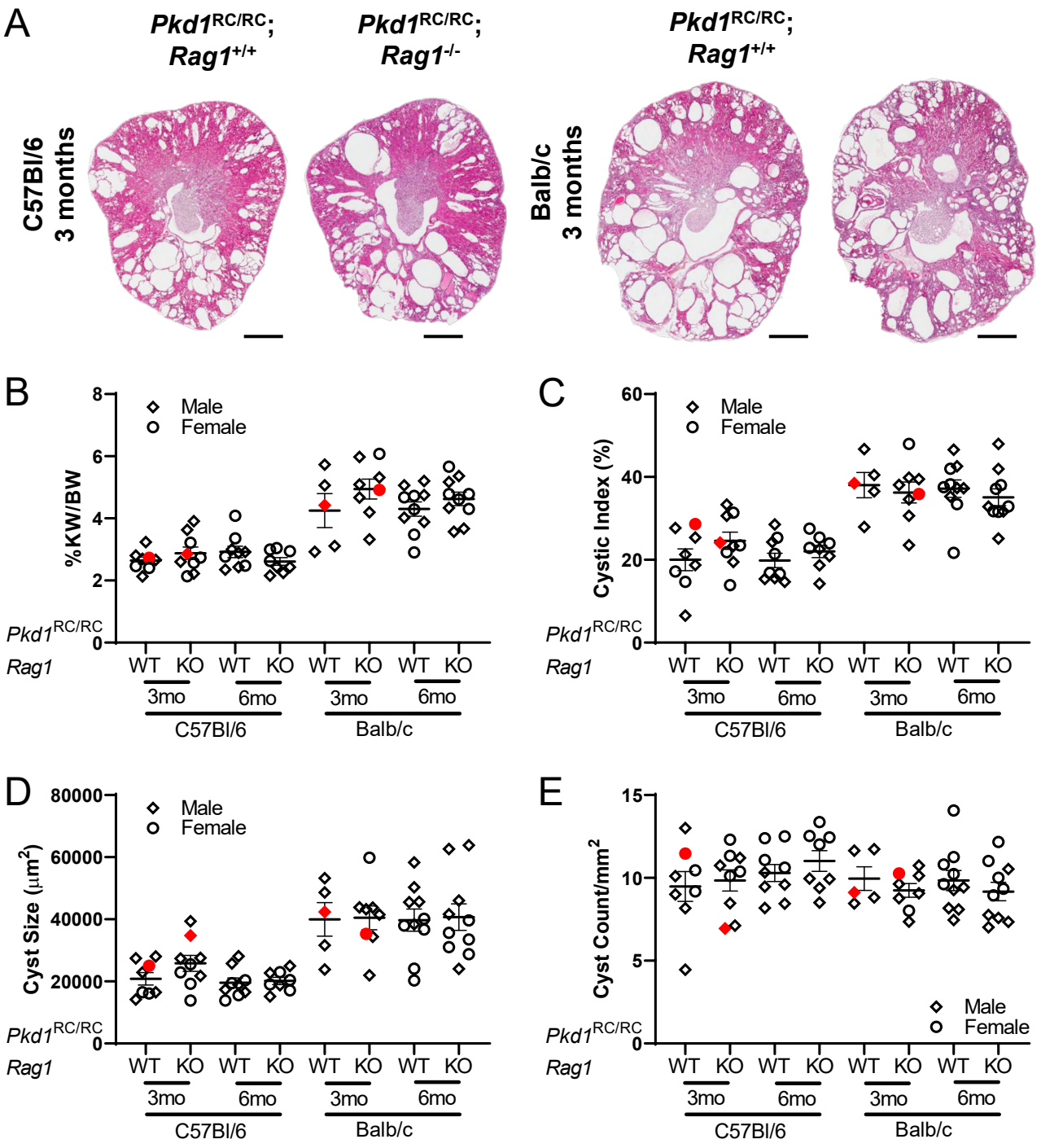

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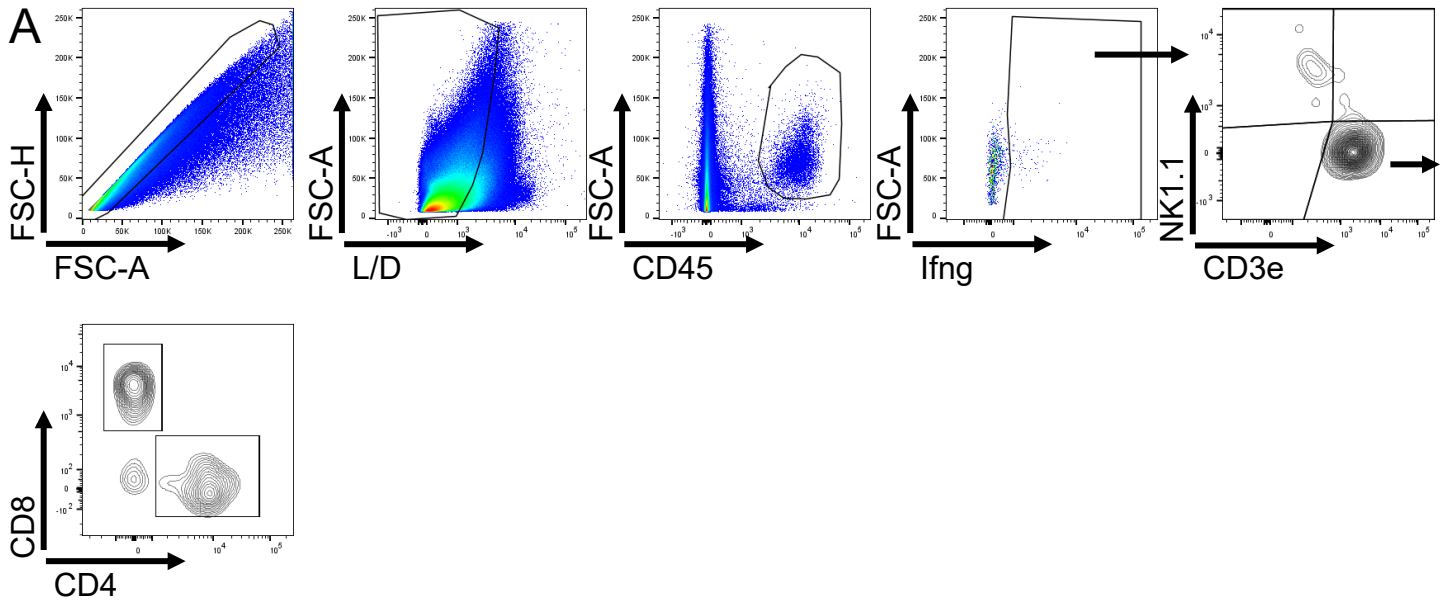

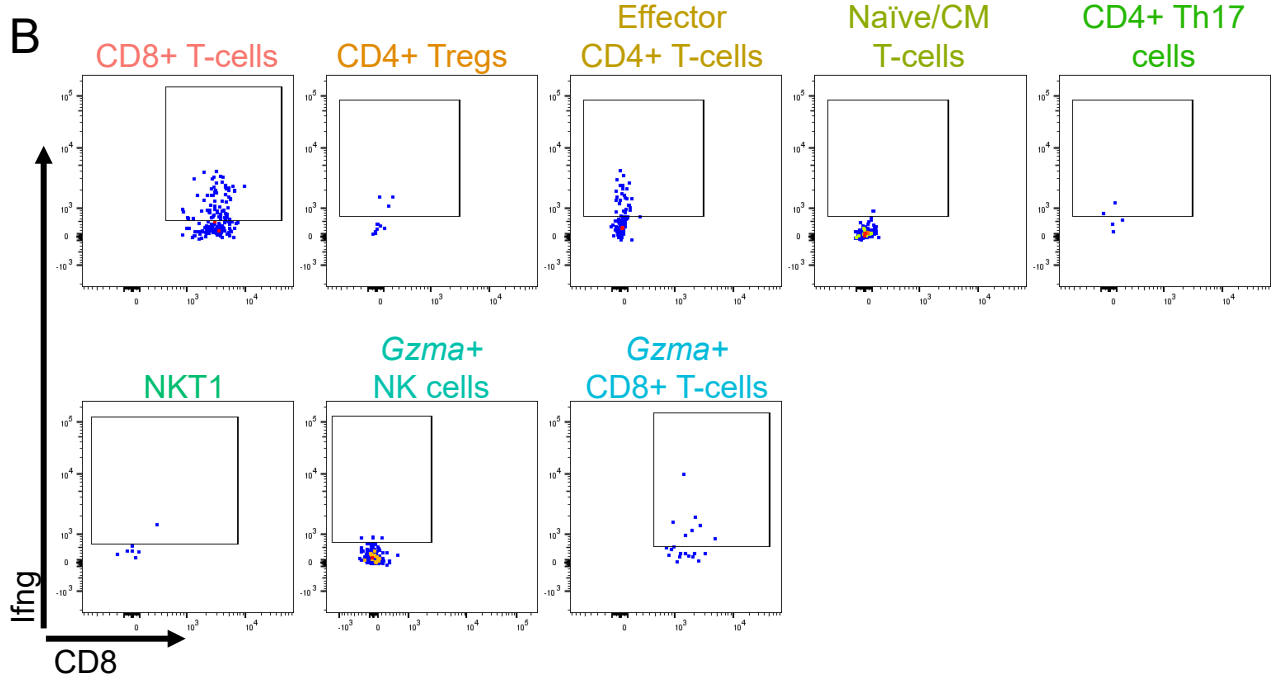

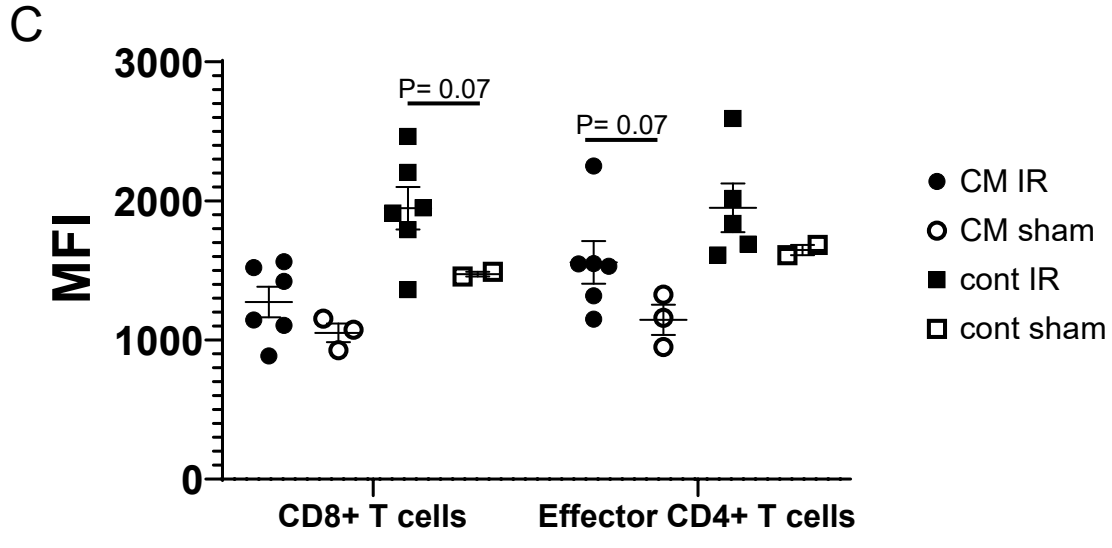

Supplemental figure 17

T-cell panel	Company	Catalog number	Clone	Dilution
CD45 BV650	Biolegend	103151	30-F11	1:200
CD3 Fitc	BD Biosciences	553062	145-2C11	1:100
CD4 PE-cy7	ThermoFisher	25-0041-82	GK1.5	1:300
CD8 Percpcy-5.5	ThermoFisher	45-0081-82	53-6.7	1:300
CD44 BV 786	BD Biosciences	563736	IM7	1:200
CD62L Pe-cy5	Biolegend	104410	MEL-14	1:200
Nk1.1 BV711	Biolegend	108745	PK136	1:100
TCRbeta BV605	Biolegend	109241	H57-597	1:200
IFNg 450	ThermoFisher	48-7311-82	XMG1.2	1:100
Foxp3 APC	ThermoFisher	17-5773-82	FJK-16s	1:100
IL-17a APC-Cy7	Biolegend	506940	TC11-18H10.1	1:100
Gzma PE	ThermoFisher	12-5831-82	GzA-3G8.5	1:100
Live/Dead	ThermoFisher	L34957		1:500

Macrophage panel	Company	Catalog number	Clone	Dilution
CD45 BV786	BD Biosciences	564225	30-F11	1:200
Ly6G Apc-cy7	Biolegend	127624	1A8	1:100
CD11b PE-cy7	ThermoFisher	25-0112-82	M1/70	1:200
CD11c Pe-cy5	Biolegend	117316	N418	1:200
F480 450	ThermoFisher	48-4801-82	BM8	1:100
Ly6C BV711	Biolegend	128037	HK1.4	1:200
CD206 PE	Biolegend	141704	C068C2	1:200