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Supplemental information

Tumor-specific CD4 T cells instruct monocyte fate in pancreatic ductal adenocarcinoma

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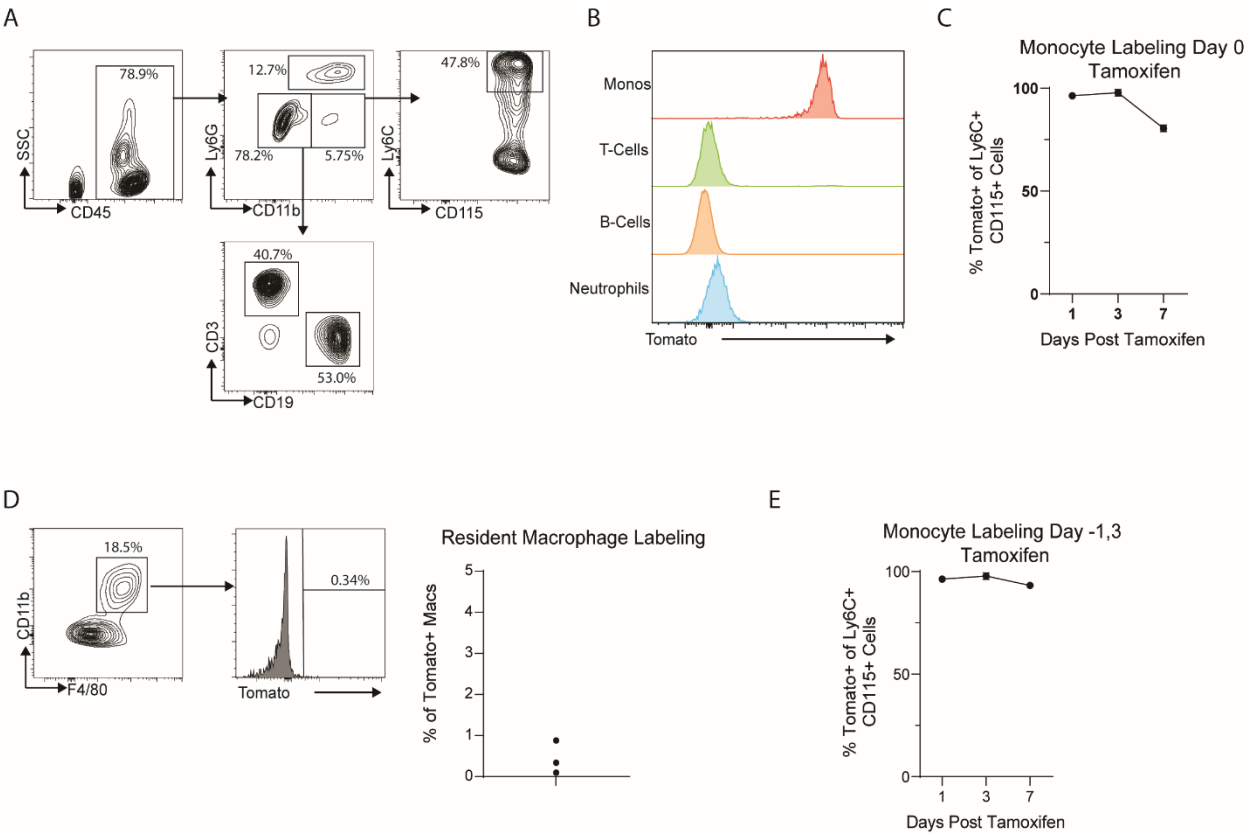
Supplemental Figure 1



Supplemental Figure 1. Labeling efficiency of monocytes in CCR2^{CreER} R26^{TdTomato} fate mapping mice. Related to Figure 1.

A) Gating strategy for analysis of circulating immune cells from tumor bearing CCR2^{CreER} R26^{TdTomato} mice 1 day post tamoxifen administration.

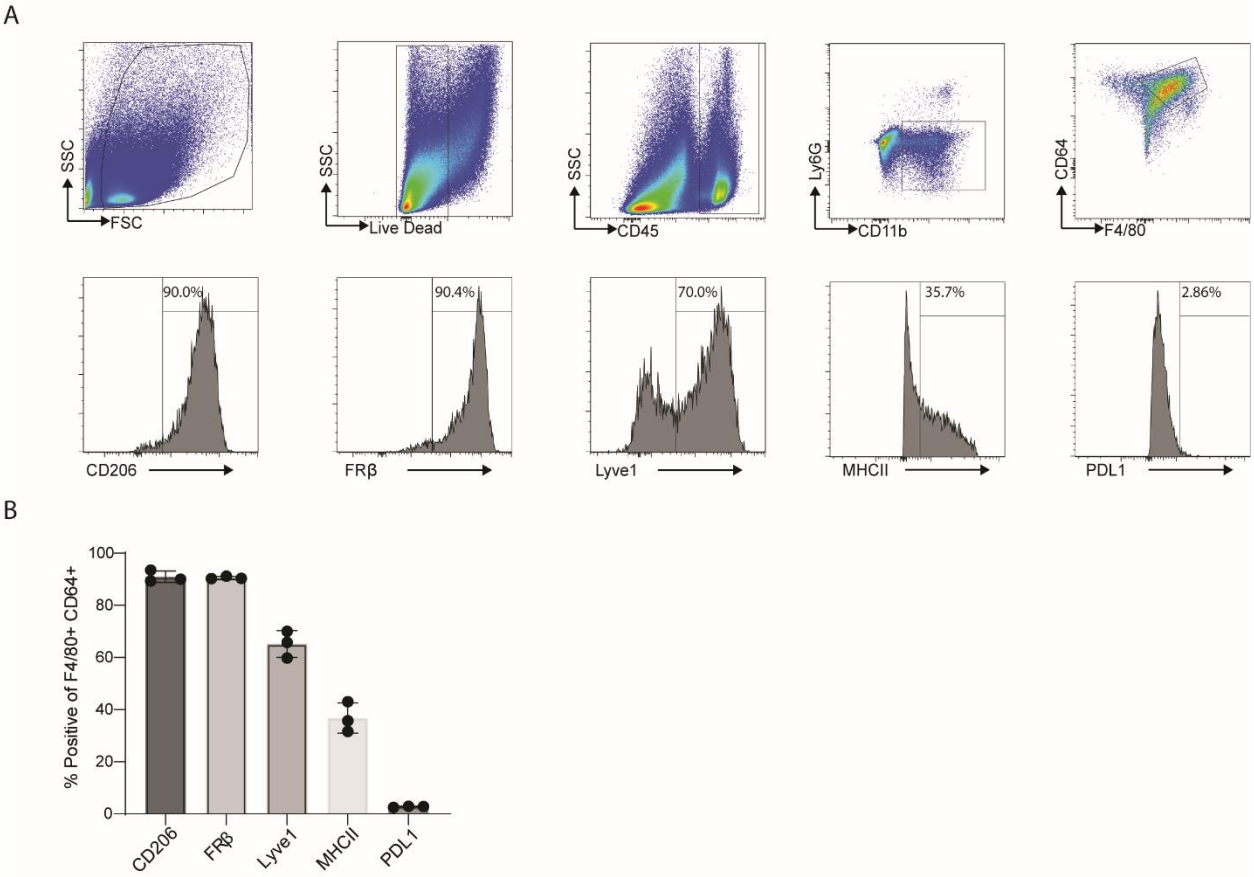
B) Tomato reporter expression by circulating immune subsets from tumor bearing CCR2^{CreER} R26^{TdTomato} mice 1 day post tamoxifen administration.

C) Proportion of circulating Tomato+ monocytes from tumor bearing CCR2^{CreER} R26^{TdTomato} mice treated with tamoxifen on the day of tumor implantation (Day 0, n=4 mice per group). Data are mean ± S.E.M.

D) Labeling and quantification of pancreatic resident (Tomato-) macrophages from non-tumor bearing CCR2^{CreER} R26^{TdTomato} 1 day post tamoxifen administration. CD11b+F480+ macrophages were gated on live, CD45+Ly6G- cells.

E) Proportion of circulating monocytes that are Tomato+ from tumor bearing CCR2^{CreER} R26^{TdTomato} treated with tamoxifen 1 day prior to tumor implantation (Day -1) and on day 3 after tumor implantation (n=4 mice per timepoint).

Supplemental Figure 2

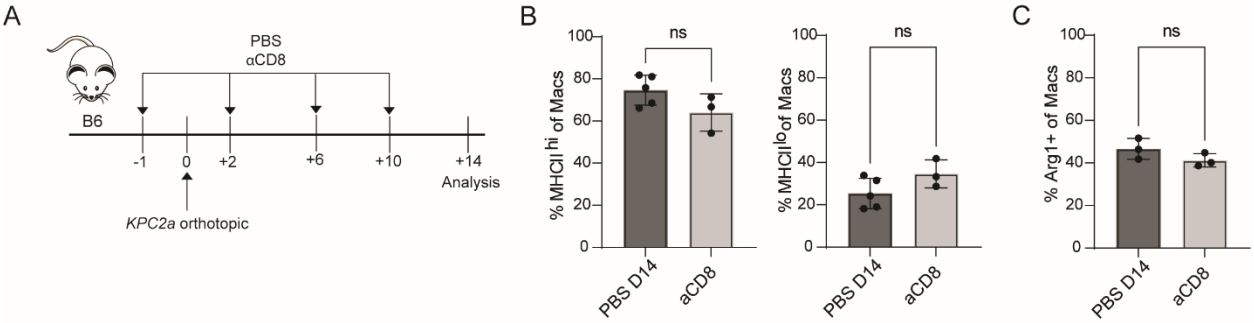


Supplemental Figure 2. Phenotyping of steady state pancreatic resident macrophages. Related to Figure 1M-O.

A) Gating strategy for analysis of pancreatic macrophages from untreated non-tumor bearing CCR2^{CreER} R26^{TdTomato} mice. Live cells are gated off single cells.

B) Proportion of CD64+F4/80+ pancreatic macrophages that express the indicated marker (n=3). Each dot is an independent mouse. Data are mean ± S.E.M.

Supplemental Figure 3



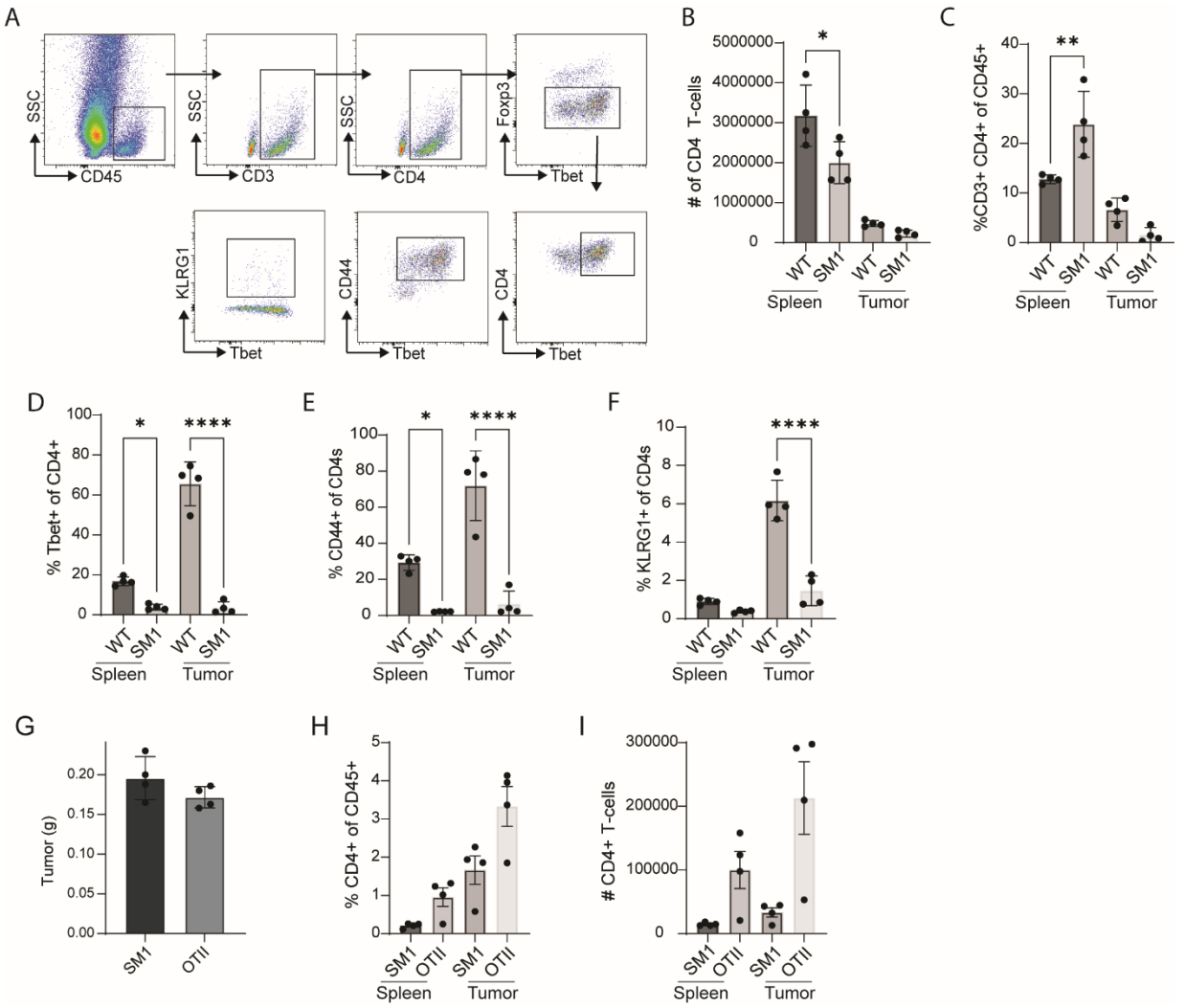
Supplemental Figure 3. Prolonged CD8 T cell depletion does not change macrophage phenotype. Related to Figure 2 A-K.

A) Schematic for testing the impact of CD8 T cells on macrophage phenotype.

B) $MHCII^{hi}$ or $MHCII^{lo}$ macrophage frequency from tumors isolated from PBS or αCD8 treated mice at 14 days after tumor implantation (n=3-4 mice per group). Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

C) Arg1+ macrophage frequency from tumors isolated from PBS or αCD8 treated mice at 14 days after tumor implantation (n=3-4 mice per group). Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

Supplemental Figure 4



Supplemental Figure 4. Impact of tumor antigen specificity on intratumoral CD4 T cell quantity and phenotype. Related to Figure 2L-T.

A) Gating strategy for CD4 T cells on day 7 post orthotopic tumor implantation. Representative plots are from WT mouse at day 7 post tumor and gated on live, single cells.

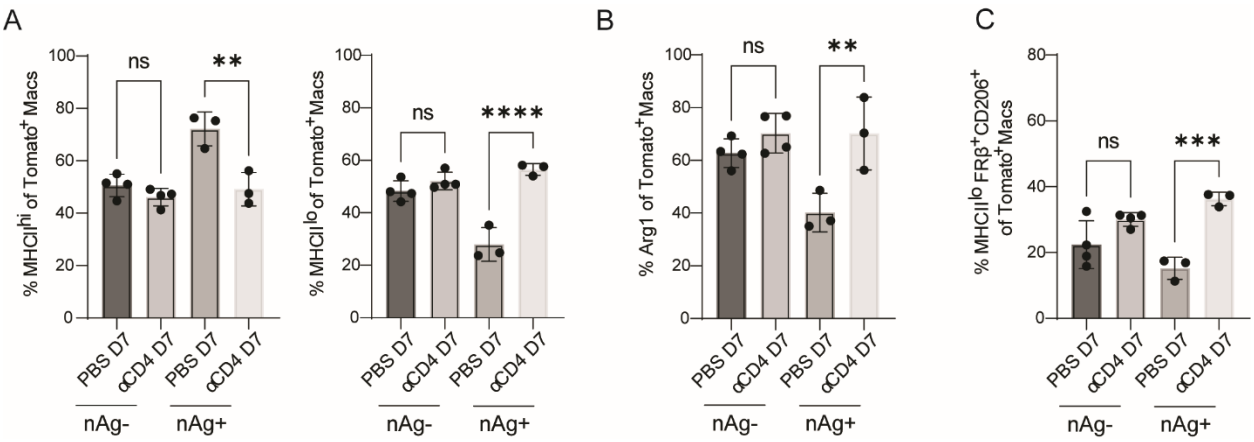
B-C) CD4+T cell number (B) or frequency (C) from WT or SM1 *Rag1-/-* mice at day 7 post tumor implantation (n=4 per group). Each dot is an independent mouse. Data are mean ± S.E.M. **p*<0.05, Student's t-test for each tissue.

D-F) Proportion of CD4+Foxp3- (Tcons) T cells that express Tbet (**D**), CD44 €, or KLRG1 (**F**) in WT or SM1 *Rag1-/-* mice at day 7 post tumor implantation (n=4 per group). Each dot is an independent mouse. Data are mean ± S.E.M. **p*<0.05, ***p*<0.005, *****p*<0.0001, Student's t-test for each tissue.

G) Tumor weight in grams from Figure 2Q. Data are mean ± S.E.M. n=4 mice per group.

H-I) CD4+T cell number or frequency from KPC-OVA tumor bearing recipients of SM1 *Rag1-/-* or OTII CD4 T cells at day 7 post tumor implantation (n=4 per group). Each dot is an independent mouse. Data are mean ± S.E.M. **p*<0.05, Student's t-test for each tissue.

Supplemental Figure 5



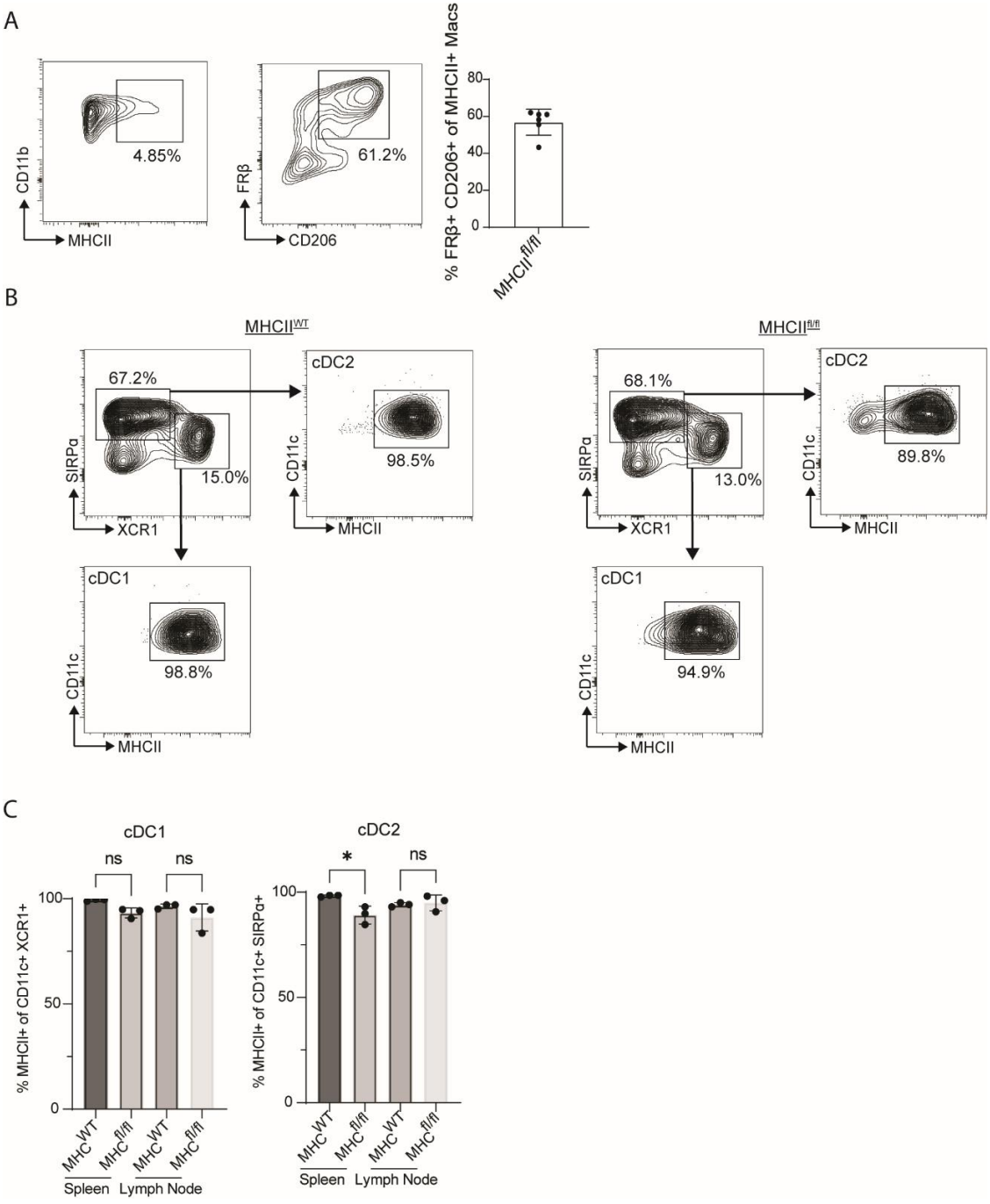
Supplemental Figure 5. Impact of tumor neoantigen expression on macrophage phenotype. Related to Figure 2.

A) Intratumoral MHCII^{hi} or MHCII^{lo} macrophage frequency from tamoxifen treated CCR2^{CreER} R26^{tdTomato} mice bearing CB neoantigen expressing (nAg+) or CB negative (nAg-) tumors 7 days after implantation (n=3-4 mice per group). CD4 T cell depletion was performed as in Figure 2. Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

B) Arg1+ macrophage frequency from tumors isolated from nAg+ and nAg- tumors 7 days after implantation (n=3-4 mice per group). Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

C) Intratumoral MHCII^{lo} CD206+ FRβ+ macrophage frequency from from mice in A. Populations are gated on CD64+ F4/80+ cells. Data are mean ± S.E.M.

Supplemental Figure 6



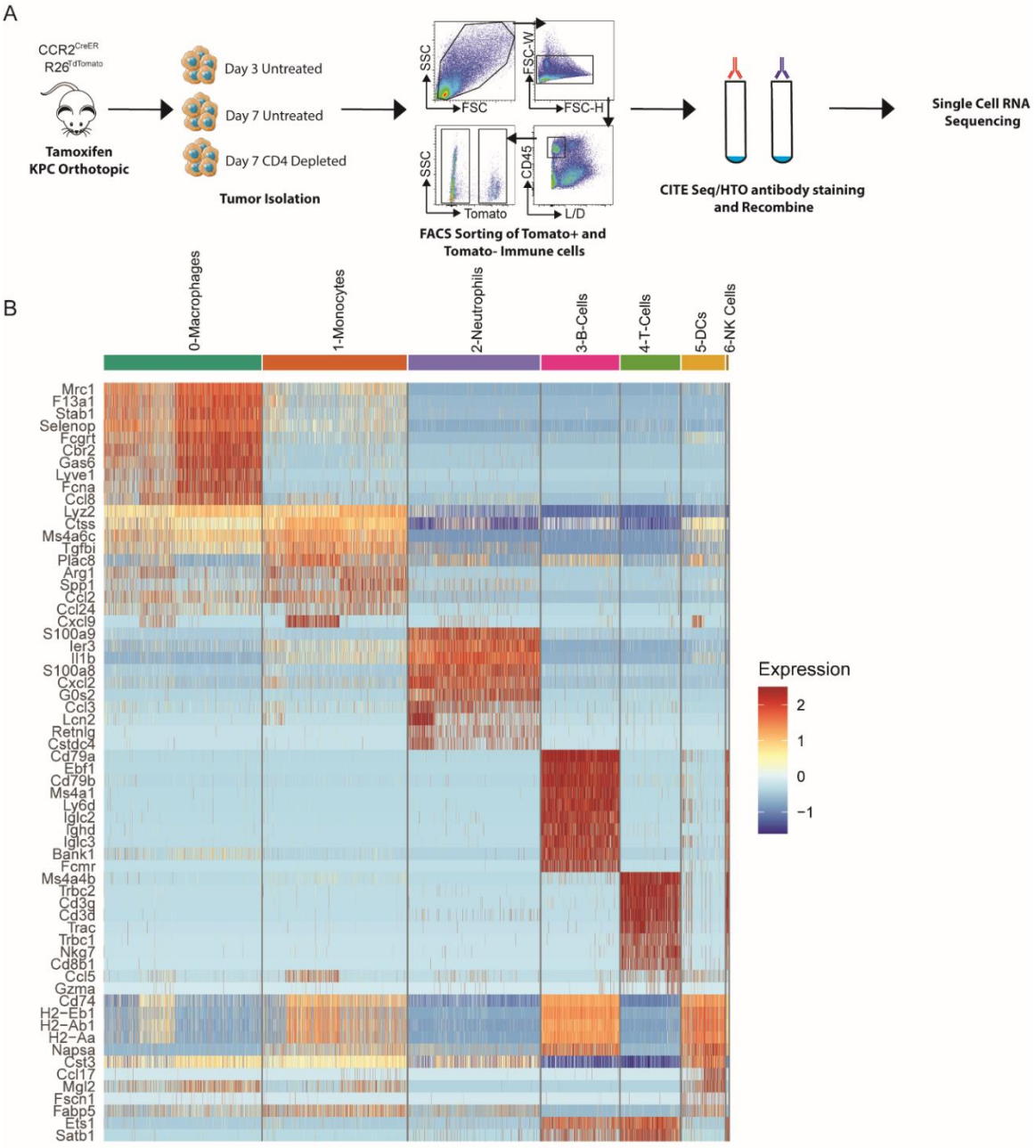
Supplemental Figure 6. MHCII is retained on tissue resident macrophages and dendritic cells from CCR2^{CreER} MHCII$^{flox/flox}$ tumor bearing mice. Related to Figure 3.

A) Phenotype of remaining intratumoral MHCII^{hi} macrophages from CCR2^{CreER} MHCII$^{flox/flox}$ tumor bearing mice treated with tamoxifen as in Figure 3 (n=6 mice). Data are mean ± S.E.M.

B) Gating of splenic and lymph node DC subsets from CCR2^{CreER} MHCII$^{flox/flox}$ and MHCII^{WT} mice treated with 2 doses of tamoxifen on days 0 and 4 and analyzed on day 5 (n=3 mice per group).

C) Proportion of cDC1 and cDC2 cells that express MHCII from mice in B. Data are mean ± S.E.M.

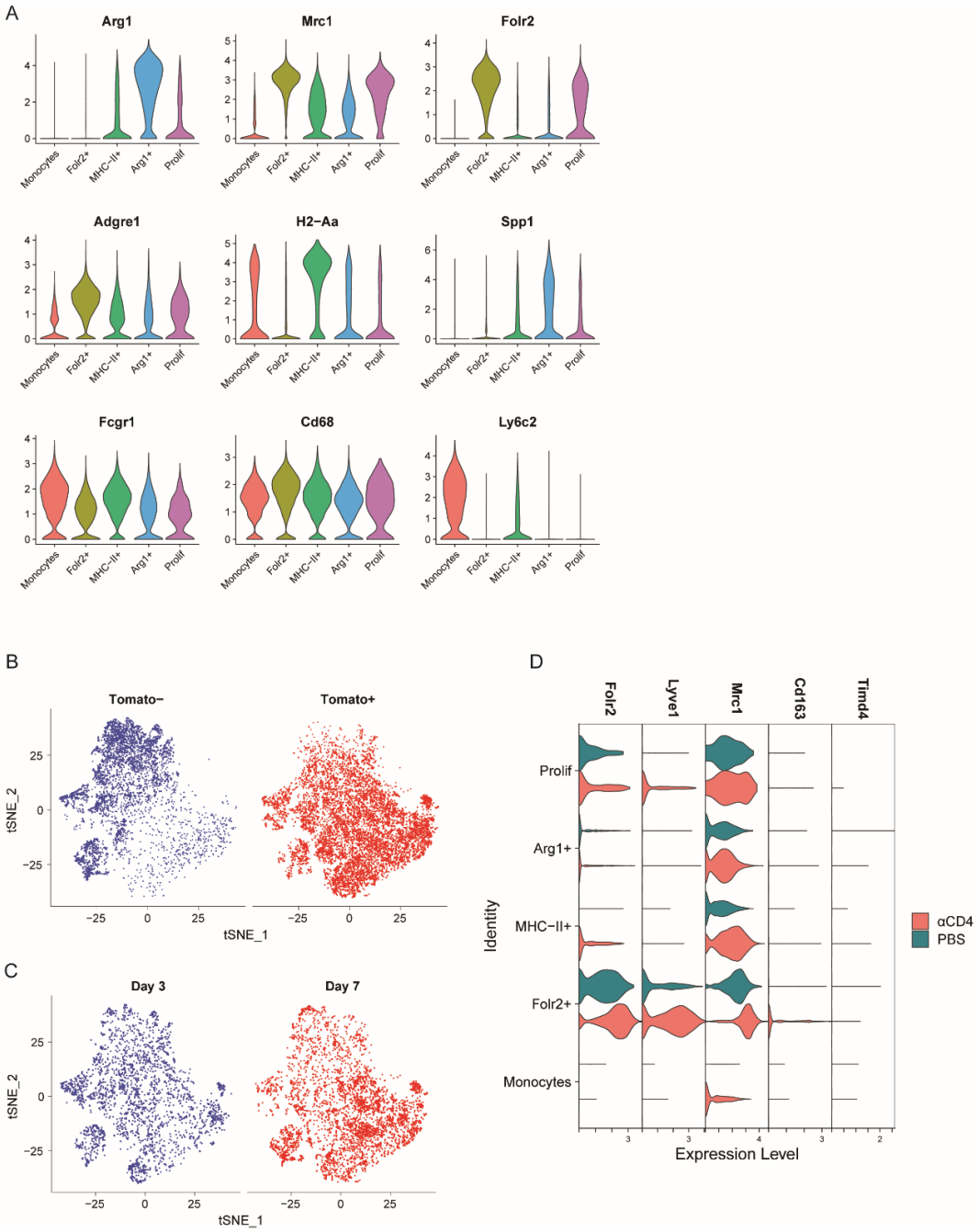
Supplemental Figure 7



Supplemental Figure 7. scRNAseq analysis of immune cells in monocyte-fate mapping tumor-bearing mice. Related to Figure 4.

A) Experimental approach for scRNAseq analysis. CCR2^{CreER} R26^{TdTomato} mice were orthotopically implanted with *KPC*2a tumors. One cohort was treated with anti-CD4 at day -1 and day +2. All cohorts were administered tamoxifen on the day of tumor implantation. Tumors were isolated from a total of 4 mice per cohort per timepoint. Tumors from mice treated with anti-CD4 were harvested on day 7. Intratumoral Tomato+ and Tomato- cells were FACS sorted, labeled with CITE-Seq antibodies, hash tagged and pooled at a 1:1 mixture for scRNAseq.
B) Heatmap showing top 5 differentially expressed genes for each cluster. singleR was used to name cell populations based on top differentially expressed genes.

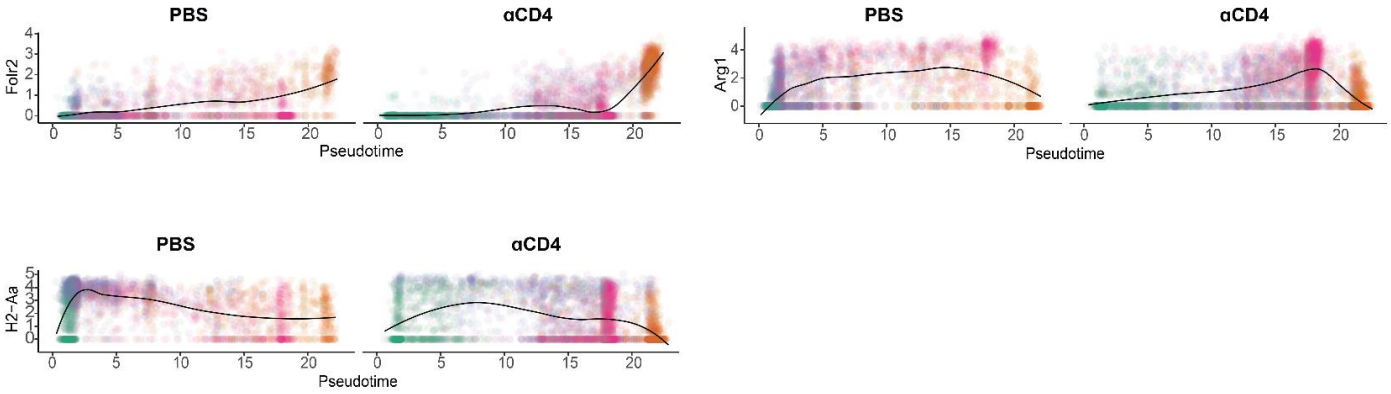
Supplemental Figure 8



Supplemental Figure 8. Monocyte/macrophage gene changes following CD4 T cell depletion. Related to Figure 4 and Figure 5.

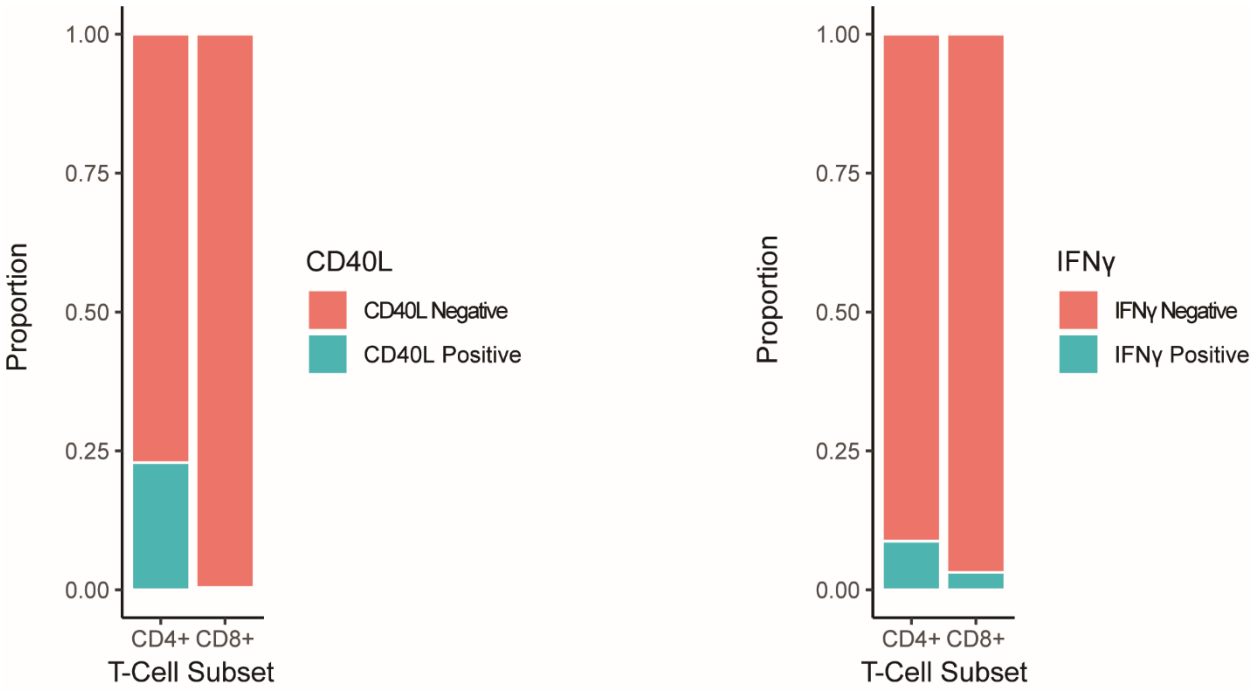
A) Violin plots of selected cluster defining genes from scRNAseq data.
B) tSNE plots of Tomato+ and Tomato- cells from PBS control mice merged from days 3 and 7.
C) tSNE plots of monocyte/macrophage clusters from PBS control mice merged from days 3 and 7.
D) Violin plots of selected genes associated with a tissue resident phenotype.

Supplemental Figure 9



Supplemental Figure 9. Kinetic analysis of cluster defining genes. Related to Figure 5. Kinetic analysis of cluster defining gene expression over pseudotime. Folr2 defines Folr2+ cluster, MHCII defines MHCII+ cluster and Arg1 defines Arg1+ cluster.

Supplemental Figure 10

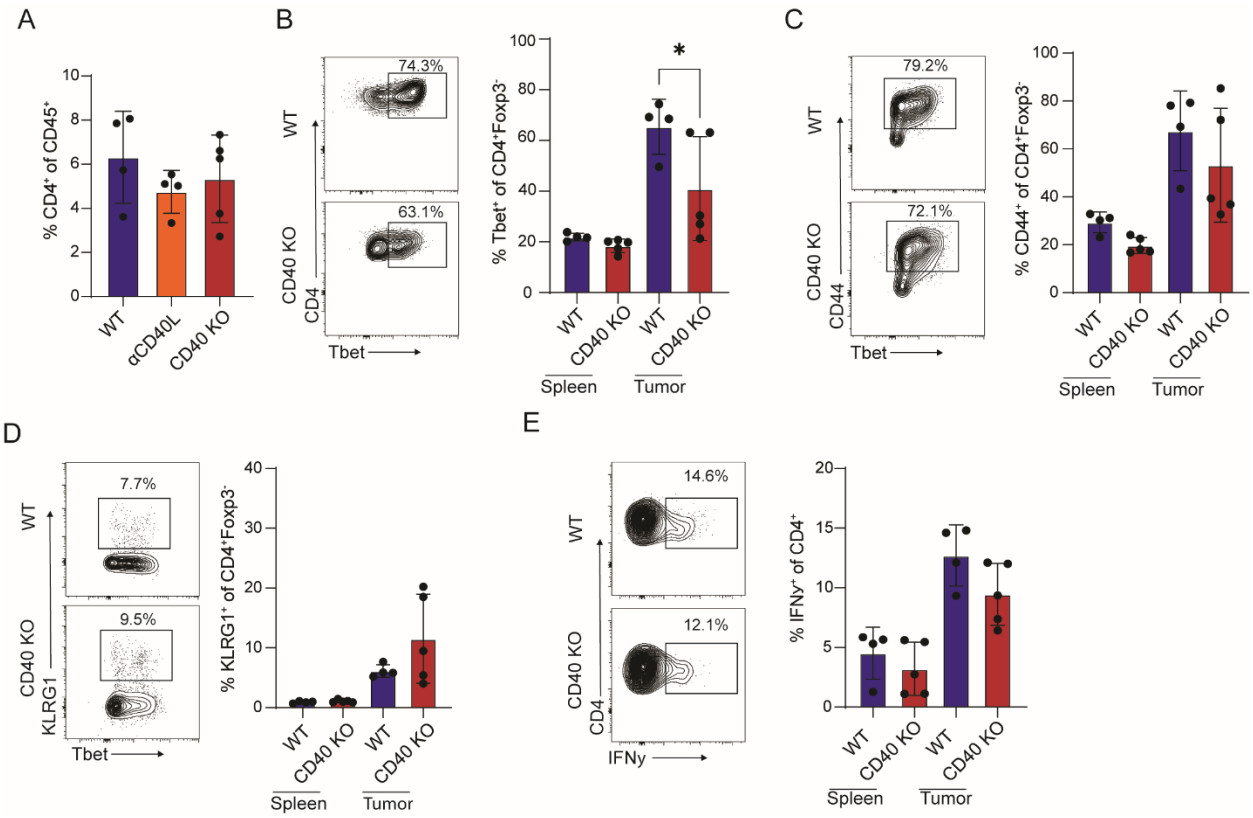


Supplemental Figure 10. Intratumoral CD4 and CD8 T cell *Ifng* or *Cd40l* **expression from Day 7 scRNAseq data. Related to Figure 6.** Proportion of CD4 and CD8 T cells that express *Ifng* or *Cd40l* was determined by scRNAseq analysis of intratumoral T cell clusters from day 7 tumors.

Supplemental Figure 11

Supplemental Figure 11. Bulk RNA sequencing of TAMs from *Ifngr1-/-* and WT mice. Related to Figure 6H-I. A) FACS sorting strategy for isolation of MHCII^{hi} and MHCII^{lo} TAMS from 4 pooled WT and 4 pooled IFNyR KO mice on 14 days post tumor implantation.
B) Heat map of top 50 differentially expressed genes for each macrophage population.

Supplemental Figure 12



Supplemental Figure 12. Impact of CD40 deletion or CD40L blockade on T cell phenotype. Related to Figure 6J-M.

A) Proportion of CD4+ T cells among intratumoral CD45+ immune cells from tumors of Day 7 WT, CD40 KO and CD40L treated mice. Each dot is an independent mouse. Data are mean ± S.E.M. n=4-5 mice per group.

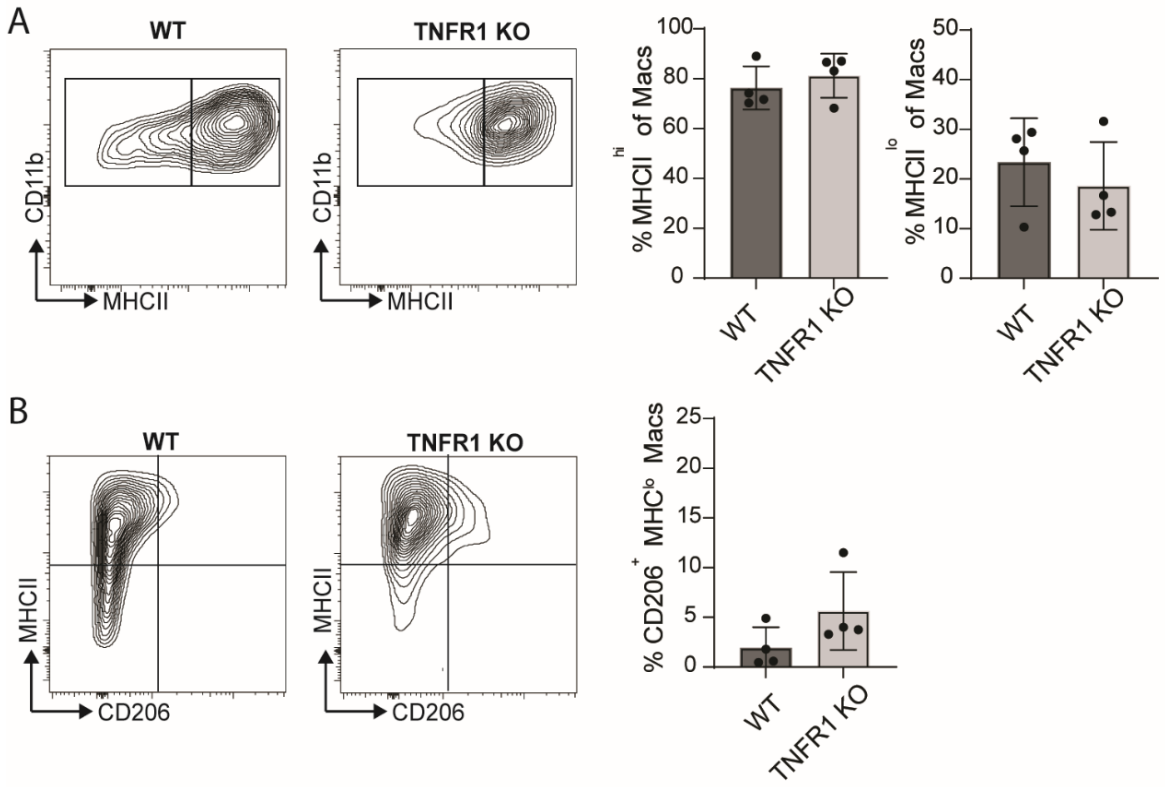
B) Representative plots and proportion of CD4+Foxp3- T cells that express T-bet from mice in A. Each dot is an independent mouse. Data are mean ± S.E.M. n=4-5 mice per group. *p<0.05, Student's t-test for each tissue.

C) Representative plots and proportion of CD4+Foxp3- T cells that express CD44 from mice in A. Each dot is an independent mouse. Data are mean ± S.E.M. n=4-5 mice per group.

D) Representative plots and proportion of CD4+Foxp3- T cells that express Klrg1 from mice in A. Each dot is an independent mouse. Data are mean ± S.E.M. n=4-5 mice per group.

E) Representative plots and proportion of CD4+ T cells that are producing IFNy following PMA/Ionomycin treatment. Each dot is an independent mouse. Data are mean ± S.E.M. n=4-5 mice per group

Supplemental Figure 13

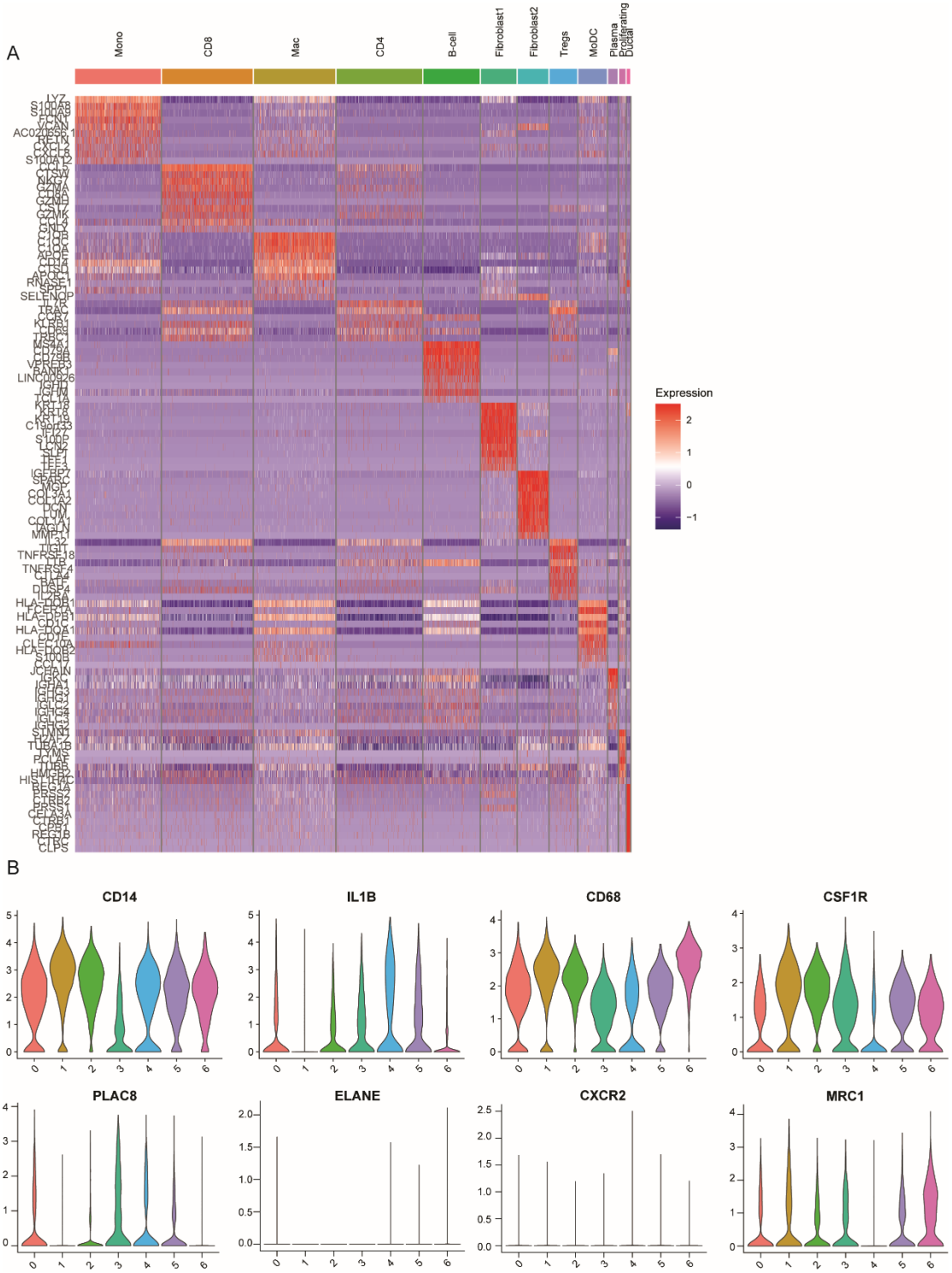


Supplemental Figure 13. Impact of host cell Tnfr1 deletion on macrophage phenotype. Related to Figure 6.

A) MHCII^{hi} and MHCII^{lo} macrophage frequency from tumors isolated from WT and *Tnfr1-/-* mice 7 days after implantation (n=4 mice per group). Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

B) MHCII^{lo} CD206+ macrophage frequency in tumors from WT and *Tnfr1-/-* mice in A. Populations are gated on CD64+ F4/80+ cells. Each dot is an independent mouse. Data are mean ± S.E.M.

Supplemental Figure 14



Supplemental Figure 14. Human PDA scRNAseq analysis. Related to Figure 7.
A) Heatmap of top differentially expressed genes from 6 resected human PDAs from Elyada *et al*. Cell populations were clustered in the UMAP space and named based off top differentially expressed genes
B) Violin plots of selected cluster defining genes from A.