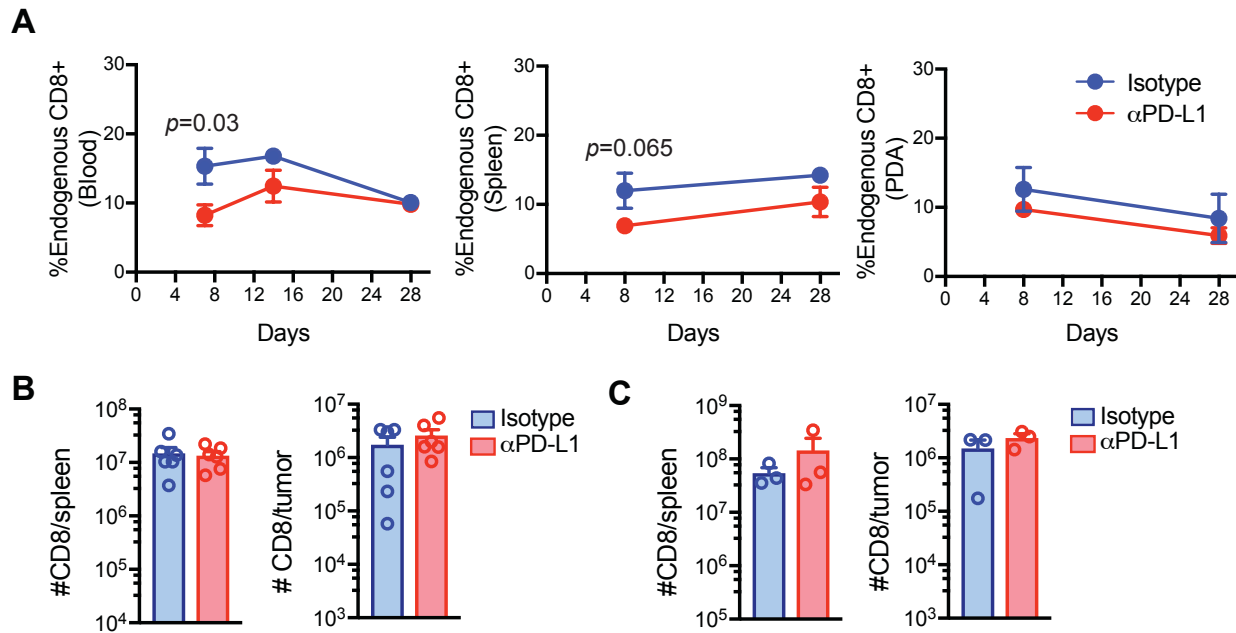


## Supplementary Figure 1



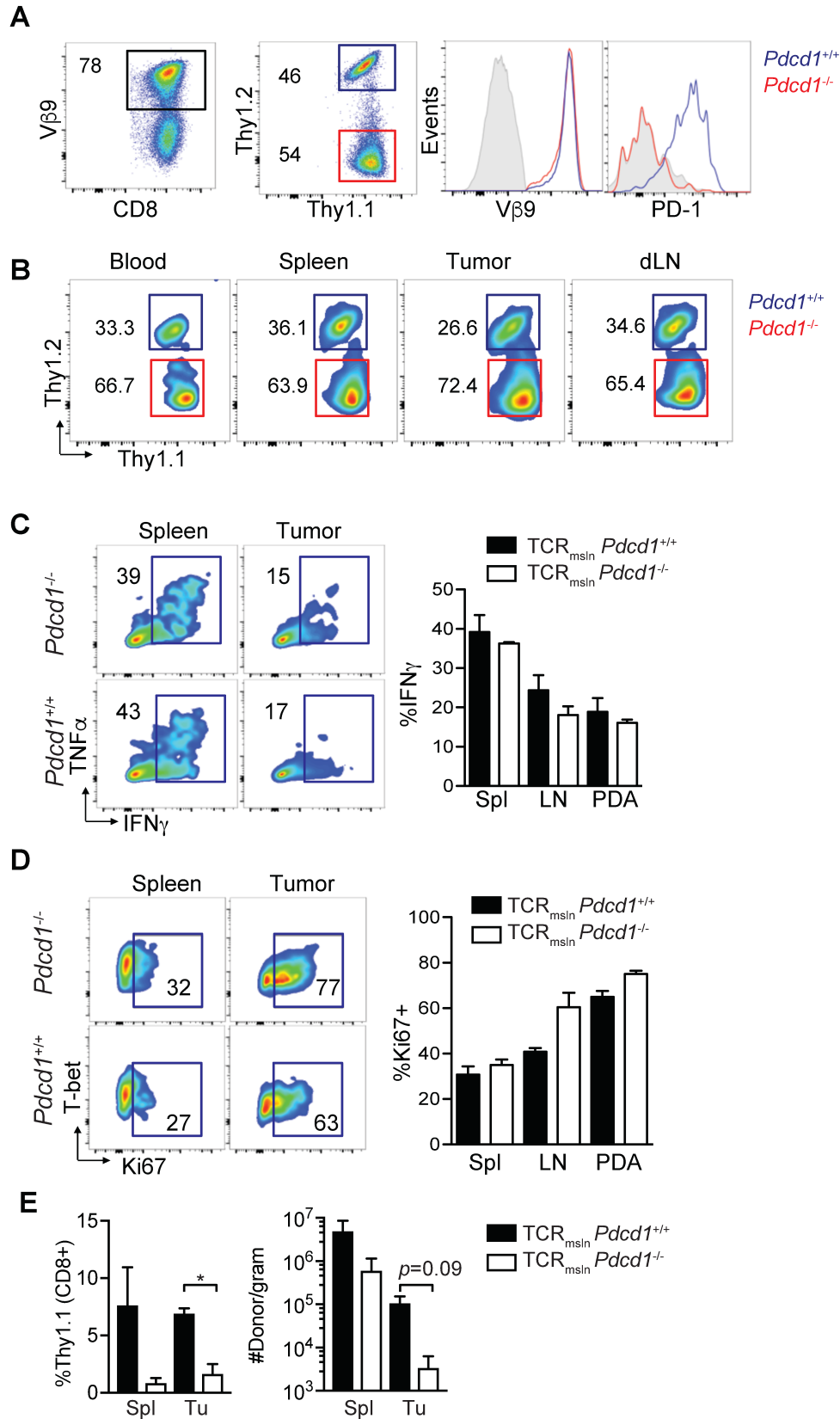
## Supplementary Figure 1. Impact of PD-L1 blockade on endogenous CD8+ T cells in KPC PDA.

**A)** Proportion of endogenous CD8+ T cells in blood following TCR<sub>Msln</sub> cell therapy and  $\pm$  PD-L1 blockade. Data are mean  $\pm$  SEM and reflect n=3-6 mice per group.

**B)** Total number of endogenous CD8+ T cells normalized to spleen or tumor gram at day 8. Data are mean  $\pm$  SEM and reflect n=3-6 mice per group.

**C)** Total number of endogenous CD8+ T cells normalized to spleen or tumor gram at day 28. Data are mean  $\pm$  SEM and reflect n=3-6 mice per group.

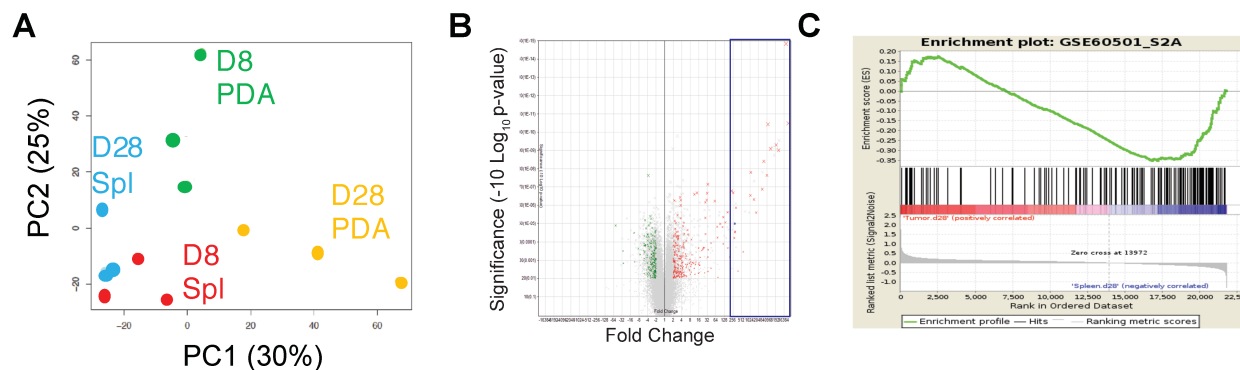
## Supplementary Figure 2



**Supplementary Figure 2. Impact of PD-1-deficiency on engineered T cells in pancreatic tumors.**

- A)** Representative flow cytometric staining of *Pdcd1*<sup>+/+</sup> and *Pdcd1*<sup>-/-</sup> TCR<sub>Msln</sub> T cells prior to transfer.
- B)** Proportion of *Pdcd1*<sup>+/+</sup> and *Pdcd1*<sup>-/-</sup> TCR<sub>Msln</sub> T cells at 8 days following infusion into KPC mice.
- C)** Proportion of *Pdcd1*<sup>+/+</sup> and *Pdcd1*<sup>-/-</sup> TCR<sub>Msln</sub> T cells producing cytokines at day 8 post infusion. Data are mean ± SEM and reflect n=3 mice per group.
- D)** Proportion of *Pdcd1*<sup>+/+</sup> and *Pdcd1*<sup>-/-</sup> TCR<sub>Msln</sub> T cells that express Tbet and Ki67 in spleen and tumor at day 8 post infusion. Data are mean ± SEM and reflect n=3 mice per group.
- E)** Proportion and number of TCR<sub>Msln</sub> engineered *Pdcd1*<sup>+/+</sup> and *Pdcd1*<sup>-/-</sup> CD8+ T cells at day 28 post infusion. Data are mean ± SEM and reflect n=3 mice per group. \*, *p*<0.05, unpaired student's T test.

## Supplementary Figure 3

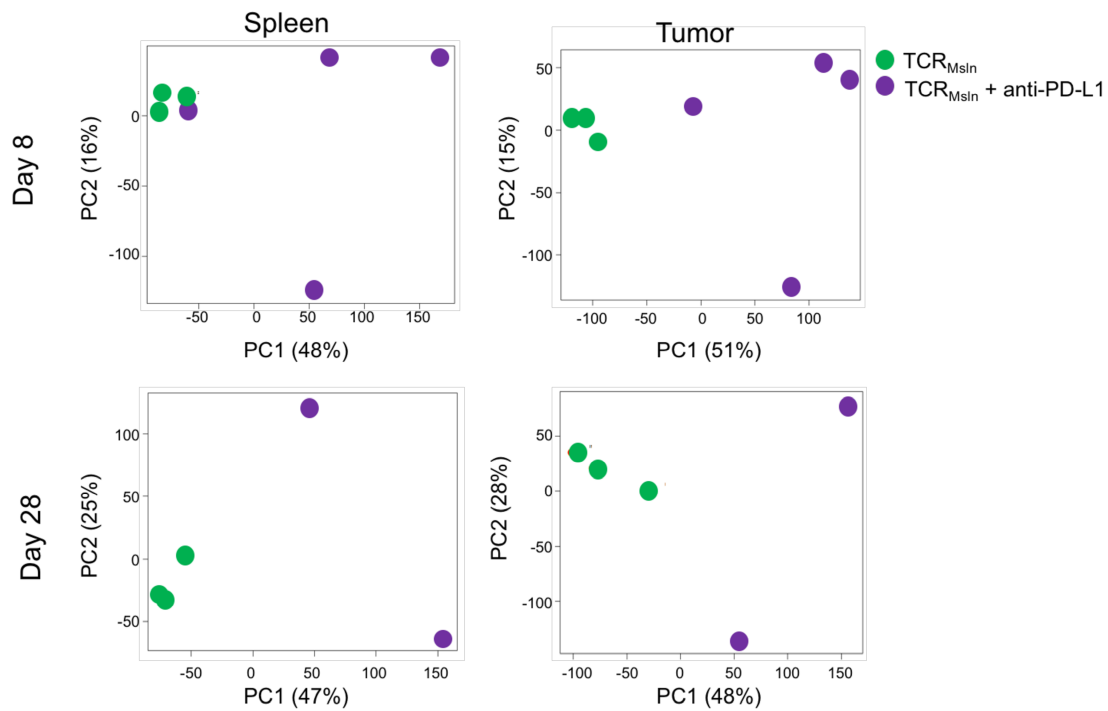


**Supplementary Figure 3. Gene expression of engineered T cells isolated from spleen and tumors of *KPC* mice and comparison to endogenously primed tumor-specific T cells.**

**A)** PCA plot of the indicated groups.

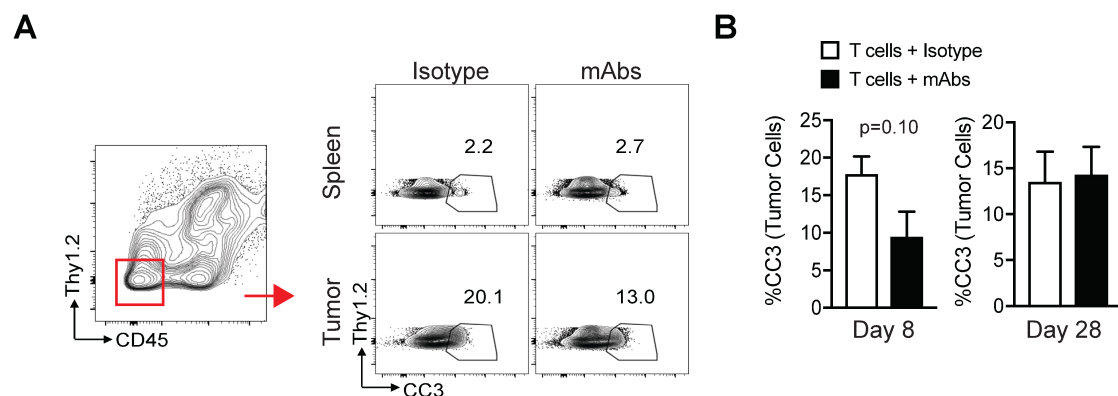
**B)** Fold change in gene expression in  $\text{TCR}_{\text{Mslin}}$  cells isolated from tumors vs. spleens. Box indicates highly upregulated genes in  $\text{TCR}_{\text{Mslin}}$  isolated from tumors that are pancreas specific.

**C)** GSEA comparison of DEGs overexpressed in intratumoral  $\text{TCR}_{\text{Mslin}}$  cells at day 28 to DEGs overexpressed in tumor-specific T cells that endogenously encountered a liver tumor antigen at day 30 (GSE60501). Normalized enrichment score, NES= -1.87.

**Supplementary Figure 4**

**Supplementary Figure 4. Impact of PD-L1 blockade on gene expression in TCR<sub>Msln</sub> cells.** PCA plots of TCR<sub>Msln</sub> cells isolated from control or anti-PD-L1 treated mice.

## Supplementary Figure 5



**Supplementary Figure 5. Impact of multiple coinhibitory receptor blockade with TCR engineered T cell therapy on tumor cell apoptosis.** *KPC* mice with 3-6 mm tumor mass received engineered T cell therapy +  $\alpha$ PD-1,  $\alpha$ Tim-3 and  $\alpha$ Lag3, or isotype, according to Figure 6A. On day 8 or 28 following T cell transfer, single cell suspensions from tumors were stained for CD45, Thy1.1 and intracellular cleaved caspase 3 (CC3). **A**) Representative gating strategy to assess apoptosis in tumor epithelial cells. Note that Thy1.2 is expressed on CD45- cancer-associated fibroblasts and thus by gating on CD45-Thy1.2- cells, we are enriching for tumor epithelial cells. **B**) Data are quantified at day 8 and day 28 post T cell therapy and are mean  $\pm$  S.E.M.  $n=3-6$  mice per group/per timepoint.