Supplemental information

PD-L1 restrains PD-1$^+$Nrp1^{lo} Treg cells

to suppress inflammation-driven

colorectal tumorigenesis

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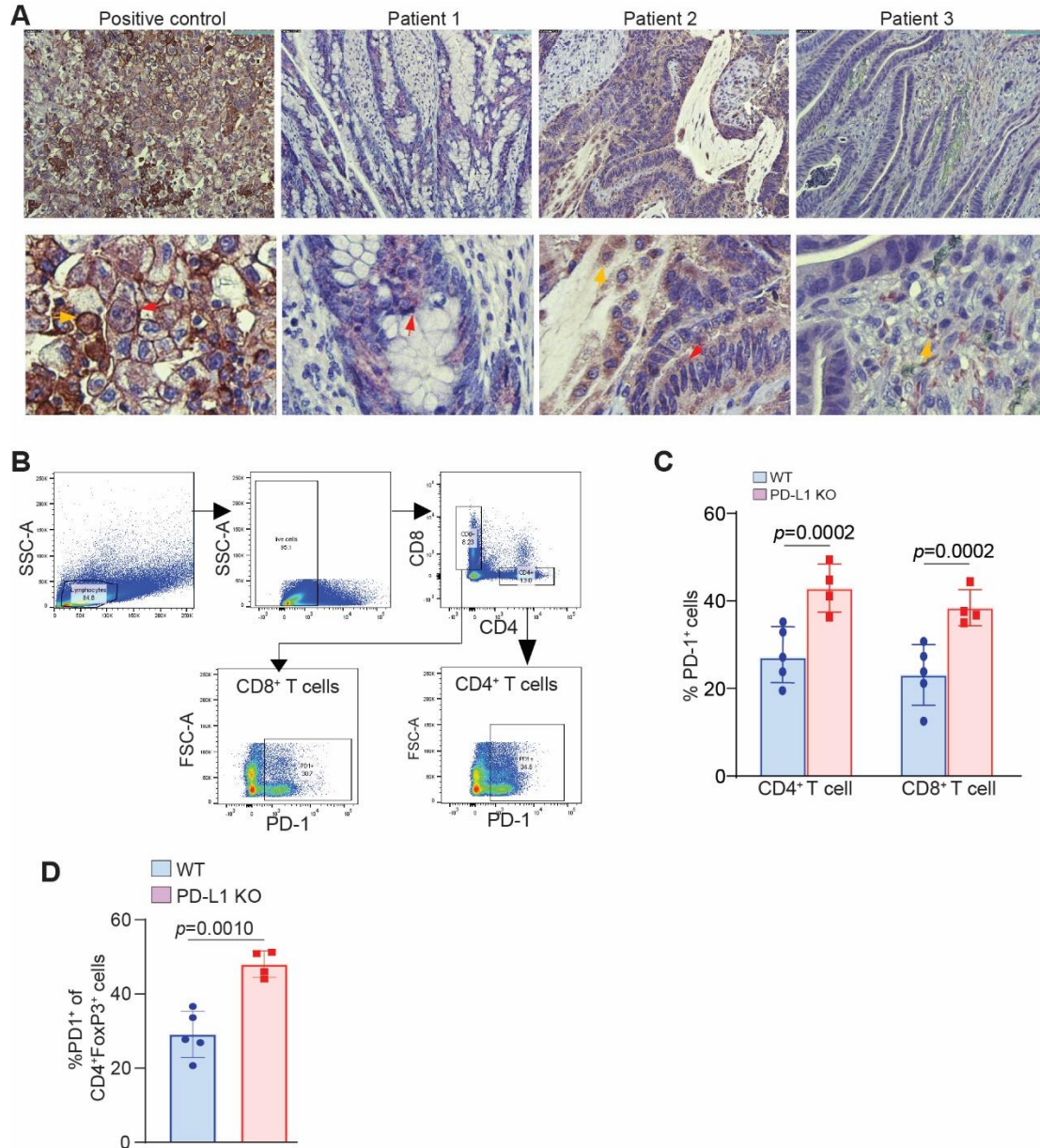
Figure S1. Loss of host PD-L1 results in PD-1$^+$ T cell expansion in colorectal tumor-bearing mice. **Related to Figure 2. A**. Immunohistochemical staining of human colon tumor tissues from three patients. Human adrenal tumor tissues were stained as PD-L1 positive control. Shown are low magnification (top, scale bar=100 μM) and zoomed (bottom) images. The images in the bottom panel are zoomed images of part of the respective images of in the top panel. The yellow arrow indicates leukocytes, and the red arrow indicates tumor cells. **B**. Spleen cells of WT and

PD-L1 KO tumor-bearing mice were analyzed by flow cytometry. Shown are gating strategies. **C**. Spleen cells of WT and PD-L1 KO tumor-bearing mice were analyzed for CD4$^+$ and CD8$^+$ T cells as shown in B. Shown is quantification of PD-1$^+$ CD4$^+$ and PD-1$^+$ CD8$^+$ T cells in the WT (n=5) and PD-L1 KO (n=4) tumor-bearing mouse spleens. Each dot represents data of one mouse. **D**. Quantification of PD-1+ Treg cells in WT (n=5) and PD-L1 KO (n=4) tumor-bearing mice.

Figure S2. Major cell clustering of AOM-DSS-induced colorectal cancer. Related to Figure 3. A. Heatmap of the cell cluster signature genes. **B**. Violin plots of the indicated genes in each cell cluster.
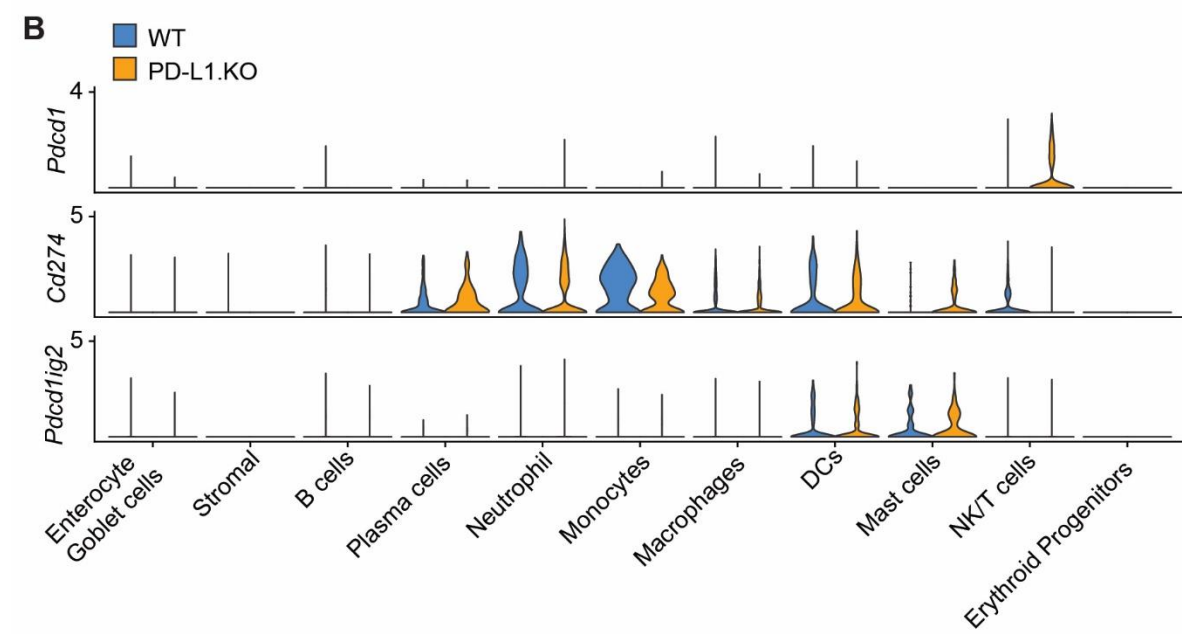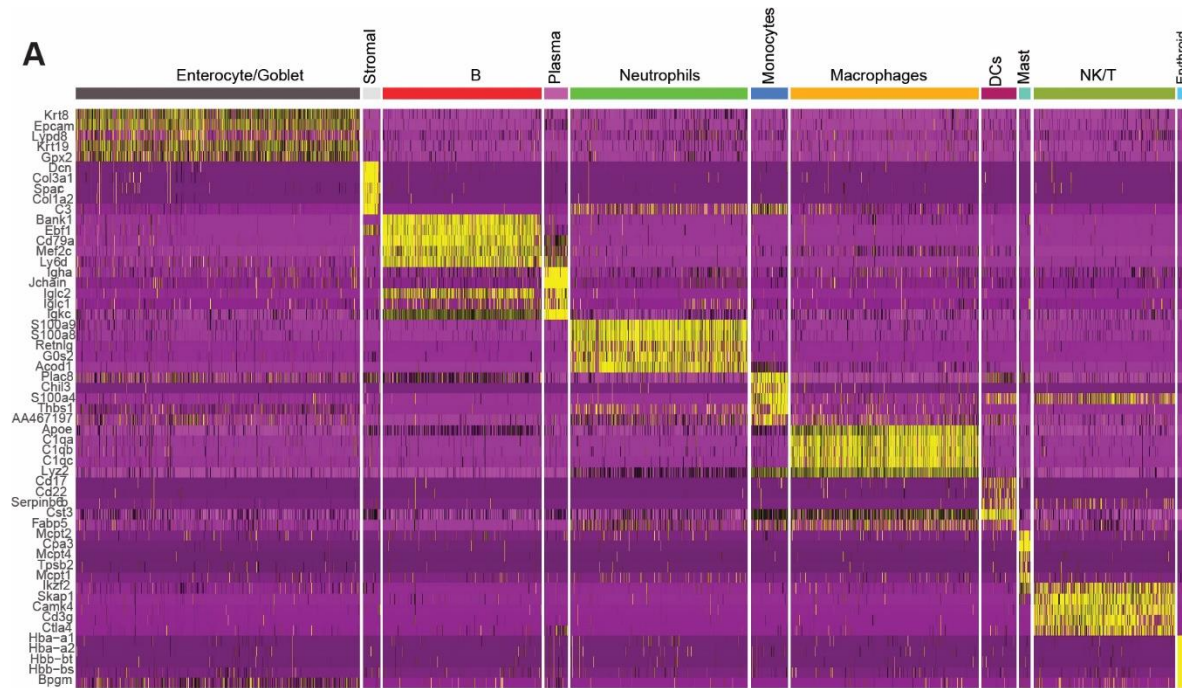
Figure S3. Immune cell clustering of AOM-DSS-induced colorectal tumor. Related to Figure 3. A. Immune cell clustering of AOM-DSS-induced colorectal tumor. **B.** Violin plots of the indicated genes in each cell cluster.
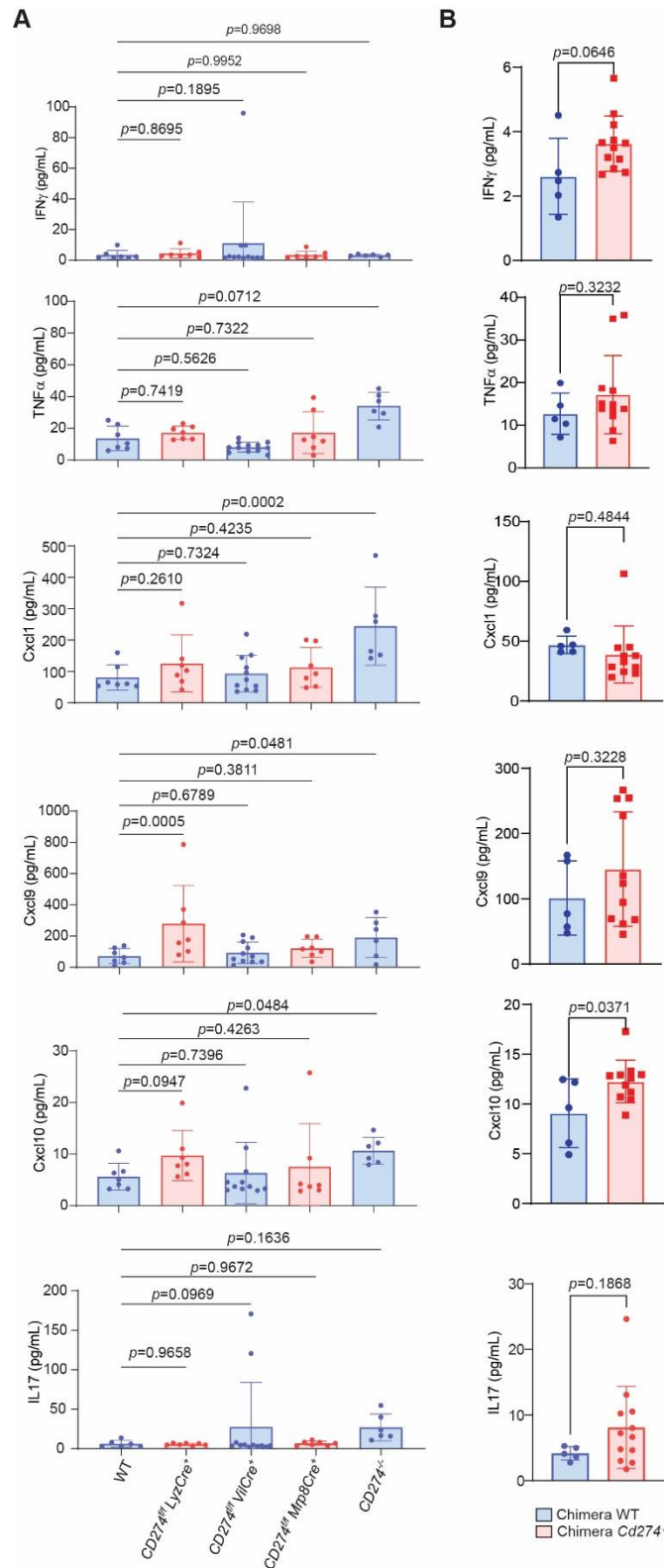
Figure S4. Cytokine profiles in WT and PD-L1 KO tumor-bearing mice. **Related to Figure 5.** Serum was collected from WT and PD-L1 KO tumor-bearing mice and analyzed for the indicated cytokines in WT and PD-L1 KO mice (A), and WT and PD-L1 KO chimera mice (B).
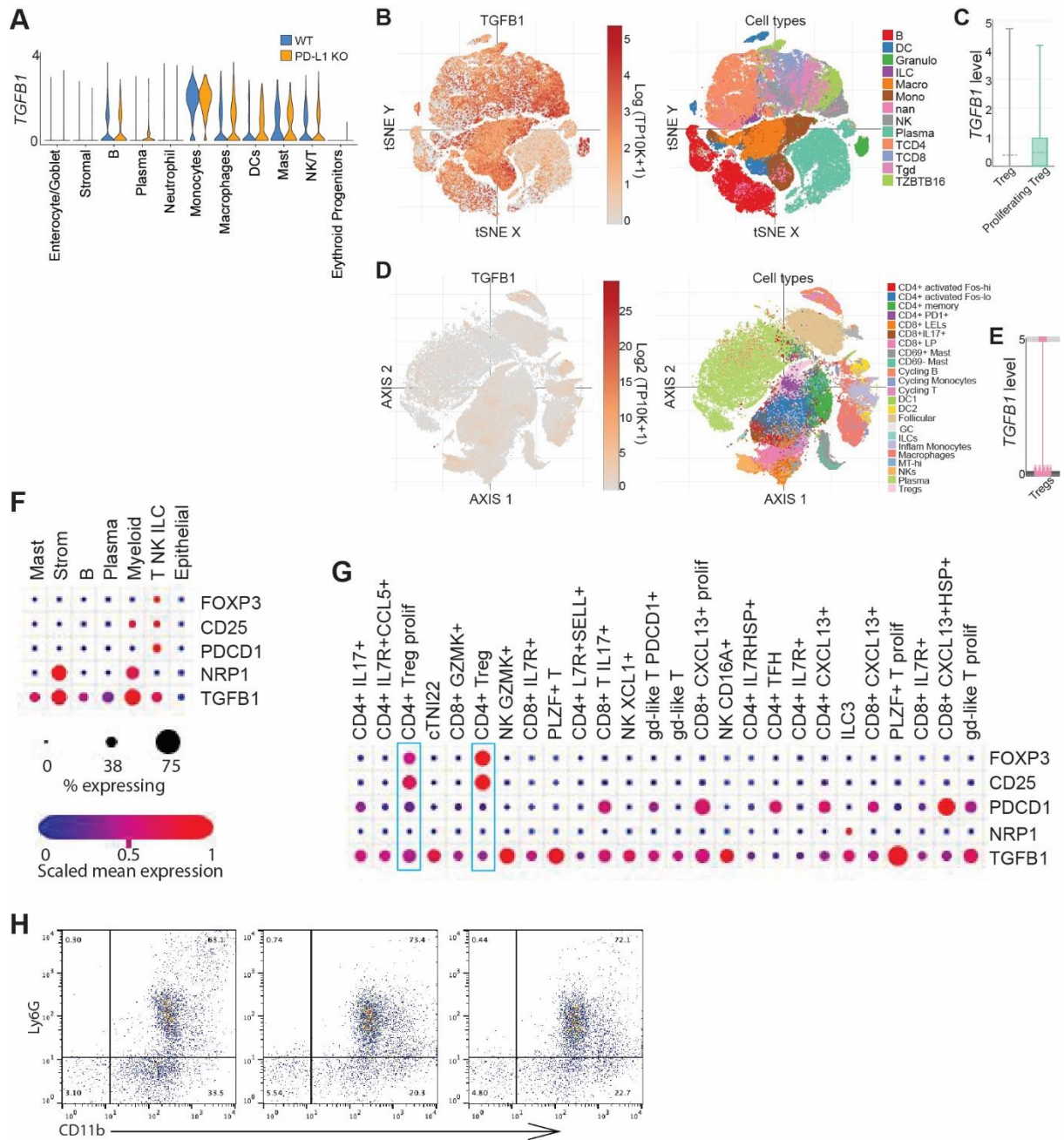
Figure S5. Human Treg cells express TGFβ. **Related to Figure 4.** **A**. Violin plot of *Tgfb1* transcripts in the indicated cell populations in colorectal tumor from WT and PD-L1 KO mice. **B**. UMAP plots of TGFB1 transcripts (left panel) and immune cells (right) of human colon tumor. Human colorectal cancer dataset were retrieved from the single cell portal (GEO:GSE178341). **C**. Box plot of *TGFB1* transcript level in the indicated cell subpopulation. **D**. UMAP projection of TGFB1 (left) and immune cell subpopulation (right) of human colon tumor. **E**. Box plot of TGFB1 transcript level in Treg cells. **F&G** Human colon tumor scRNA-seq datasets (GEO accession: GSE178341) were analyzed for expression of the indicated genes in major cell types (F) and T cell subpopulations (G). The correlations are shown in dot plots. **H**. Neutrophils were isolated from tumor tissues of tumor-bearing mice. Shown is purity of neutrophils from three tumor-bearing mice.