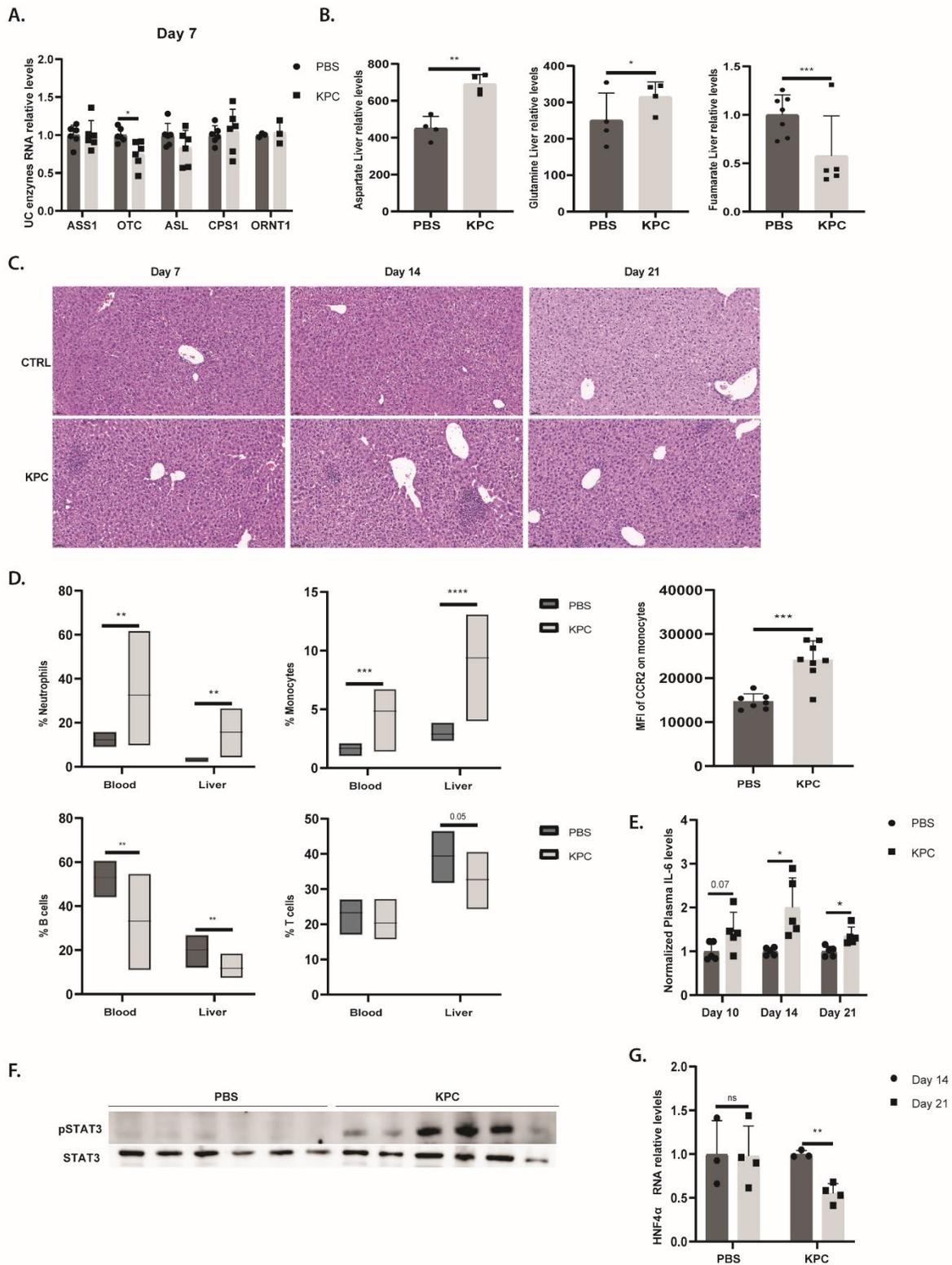
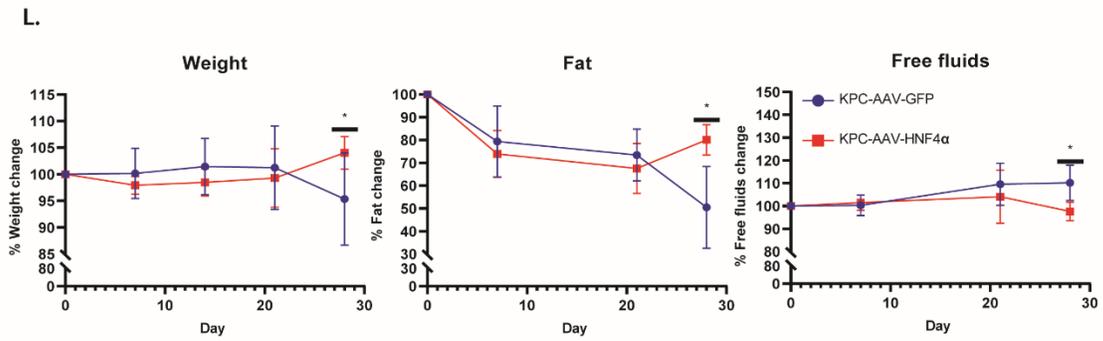
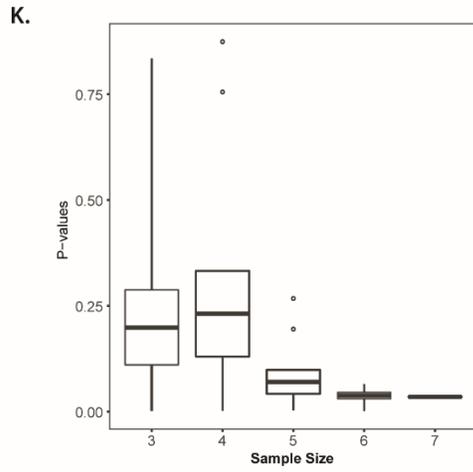
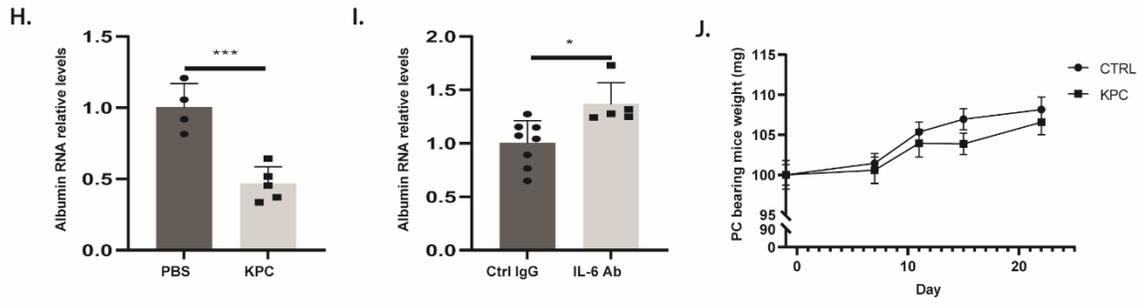


Supplementary Figure S4: Innate immune cells induce similar transcriptional and metabolic changes in the livers of KPC mice





**Supplementary Figure 4: Innate immune cells induce similar transcriptional and metabolic changes in the livers of KPC mice.** **A.** RT-PCR of livers from PC -bearing mice demonstrate a decreased RNA expression of OTC enzyme in the first week following cancer injection (n=6, 2-way ANOVA) P = 0.01. **B.** Elevated levels of aspartate and glutamate and decreased levels of fumarate in the livers of PC -bearing mice. UC intermediate levels were measured with GC-MS, and amino acids analyzer (AAA) (n = 4, glutamate - an outlier -IQR method. Fumarate - WT n = 5, KPC n = 7, student T-Test) P values: Asp = 0.001, Glu = 0.019, Fum = 0.0001. **C.** H&E staining of liver sections demonstrates increased immune cell infiltration following the injection of PC cells. Magnification 10X as detailed in the figure. **D.** Left panel- FACS analysis quantification of liver and blood CD45+ populations shows increased levels of innate immune cells and reduction of lymphocytes cells in the livers and blood of PC-bearing mice compared to WT-PBS injected mice (n=8, liver WT =7, student T-Test) P values: Blood: B cells = 0.003, monocytes = 0.0002, neutrophils = 0.006. Liver: B cells = 0.004, T cells = 0.05, monocytes < 0.0001, neutrophils = 0.004. Right panel- MFI = 0.0001. Live immune cells in the blood and livers of KPC PC-bearing mice were gated as CD45+, CD11b, CD3, CD19 Ly-6G, and, Ly-6C. Mean fluorescence intensity was done on Ly-6C high monocytes. **E.** ELISA assay demonstrates increased levels of IL-6 in PC- bearing mice (n = 5, student T-Test) P = 0.011, 0.01, respectively. **F.** Western blots demonstrate increased protein expression levels of pSTAT3 in PC-bearing mice livers compared to WT-PBS injected mice (n = 6). **G.** RT-PCR of livers from pancreatic cancer-bearing mice demonstrates a significant decrease in HNF4 $\alpha$  RNA expression levels with tumor progression while remaining constant in WT-PBS injected mice (WT n = 3, KPC n = 4, student T-Test) P = 0.0012. **H.** RT-PCR of livers from PC -bearing mice demonstrate a significant decrease in albumin RNA levels (WT n = 4, KPC n = 5, student T-Test), P = 0.0009. **I.** Treatment of PC mice with IL-6 antibodies preserves plasma levels of albumin (Ctrl IgG = 8, IL-6 Ab = 5, student T-Test) P = 0.011. **J.** Weight measurements of PC mice along 22 days of tumor development show no weight loss in PC mice compared to WT control. **K.** Power analysis of the in vivo NMR experiment. Y-axis shows the Wilcoxon rank-sum P-value for the weight change differences between HNF4 $\alpha$  - injected vs. control GFP injected mice, and the X-axis shows the sample size per each condition in each experiment considered for the statistical test. In this experiment, we have two conditions (HNF4 $\alpha$ -injected vs. control GFP-injected) and 2 repeated experiments, so the total number of experiments is 4 times of the numbers shown in the X-axis, and the mean sample size is 7. For each sample size of less than 7, the mice were randomly selected for the statistical test, and each box shows the mean and standard deviation of the P-values, and the whisker shows the top and bottom 10 percentile of the P-values. **L.** Weight measurements and nuclear magnetic resonance (NMR) body composition analysis of PC AAV-HNF4 $\alpha$  injected mice demonstrate significantly less weight loss (Left panel), increased fat tissue (Middle panel), and decreased free fluids (Right panel) compared to AAV-GFP injected mice (relative to day 0); (n = 7)