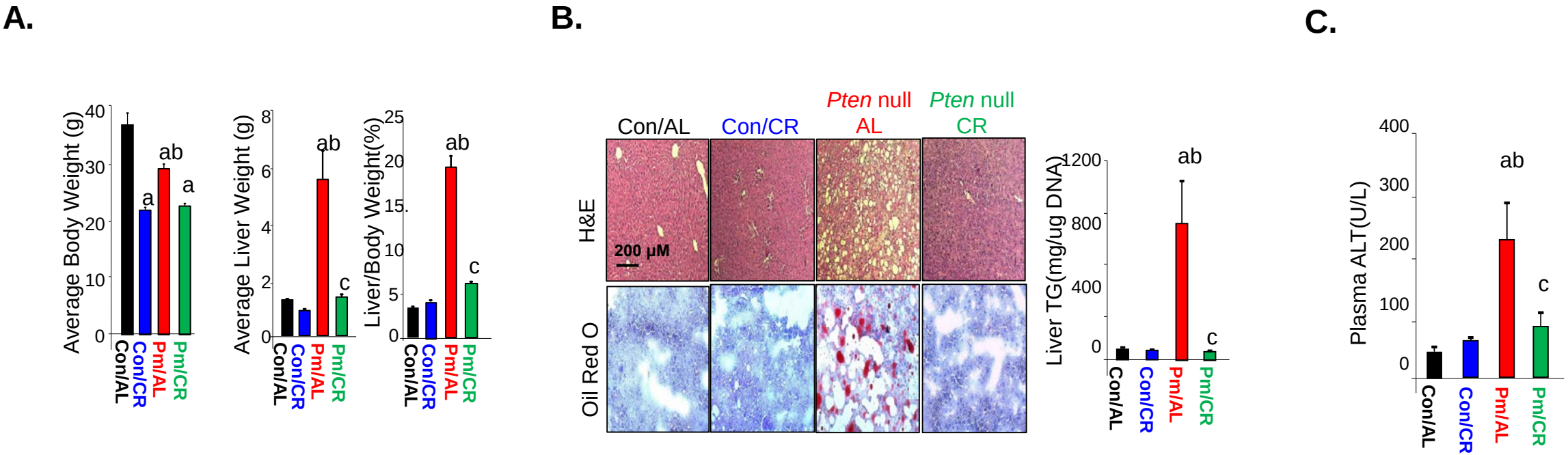


Supplemental Figure 1. Characteristics of mice on Caloric Restricted. Four months old mice were put on indicated diet for 7 months. **A**. Average body weight, liver weight and liver/body weight ratio at the end of the experiment. a, different from Con/AL; b, different from Con/CR, c, different from Pm/AL. P≤0.05. n=6-11. **B.** Left, liver tissue sections were collected from 13 month old control (Con) and *Pten* null (Pm) mice fed with *ad libitum* (AL) or Calorie restriction (CR) diet. H&E staining (top) and Oil Red O staining (bottom); Right, quantification of liver triglyceride (TG). **C**. Plasma ALT levels. All values are expressed as the mean ± SEM.

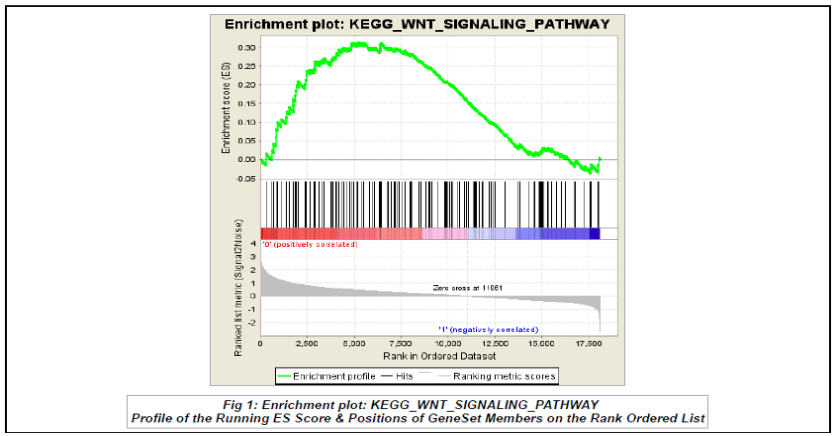


Fig 1: Enrichment plot: KEGG_WNT_SIGNALING_PATHWAY
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

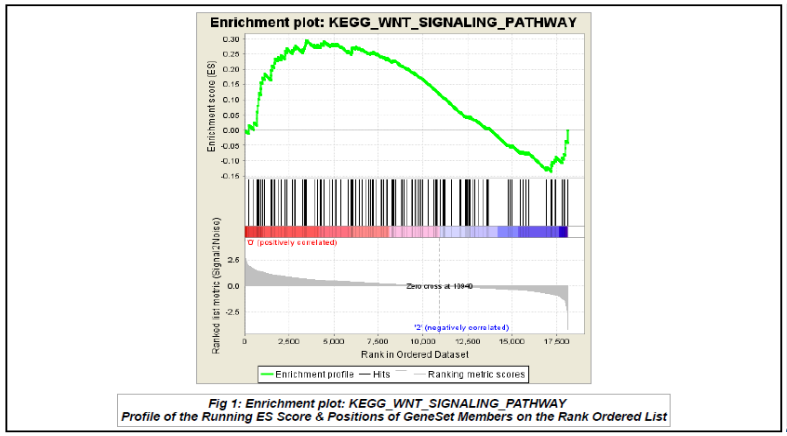


Fig 1: Enrichment plot: KEGG_WNT_SIGNALING_PATHWAY
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

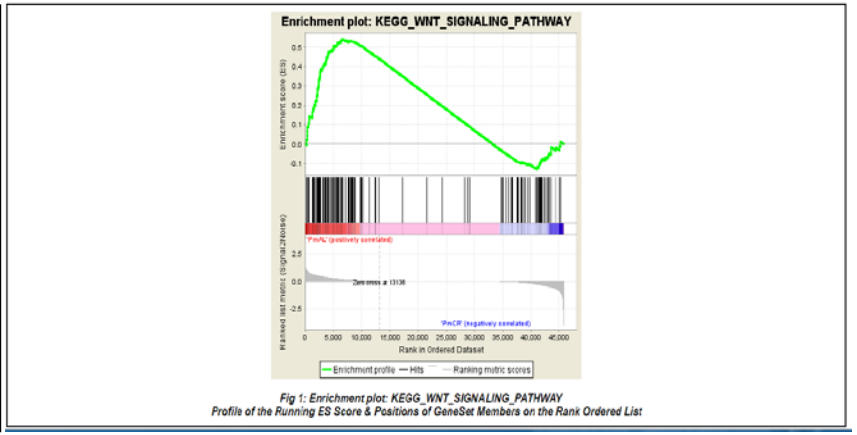
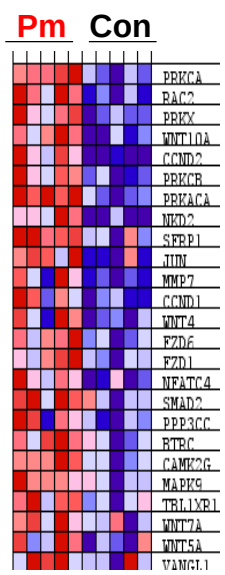
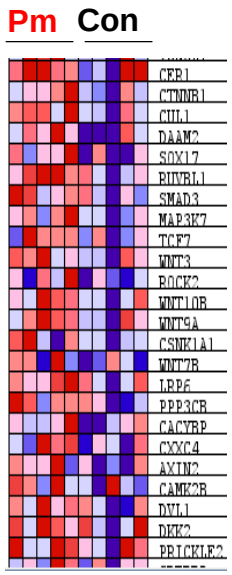
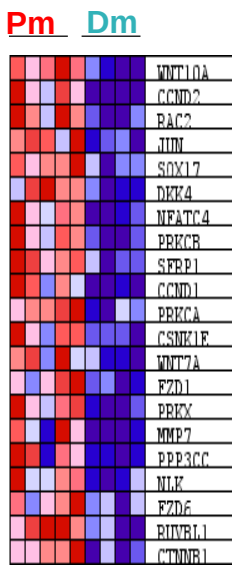
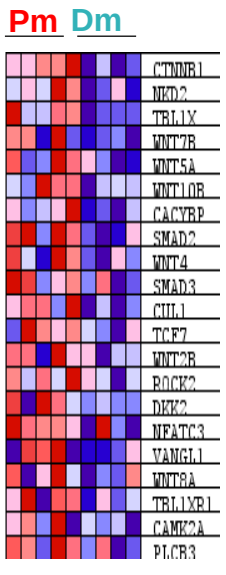


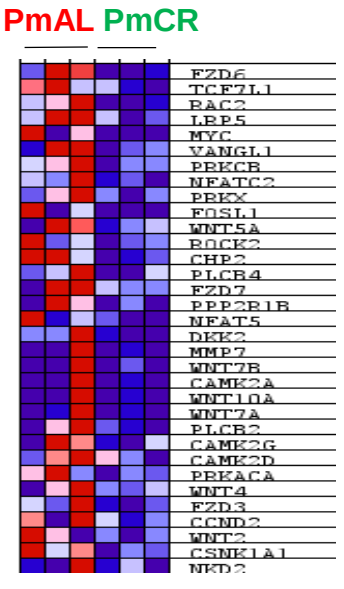
Fig 1: Enrichment plot: KEGG_WNT_SIGNALING_PATHWAY
Profile of the Running ES Score & Positions of GeneSet Members on the Rank Ordered List

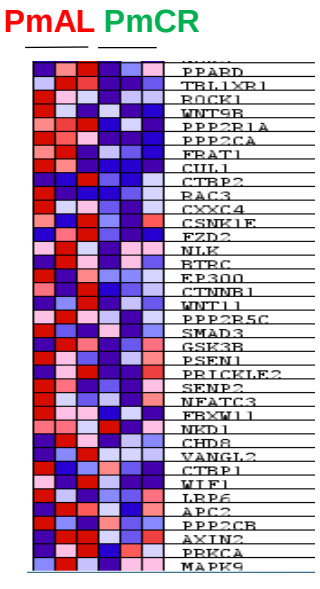




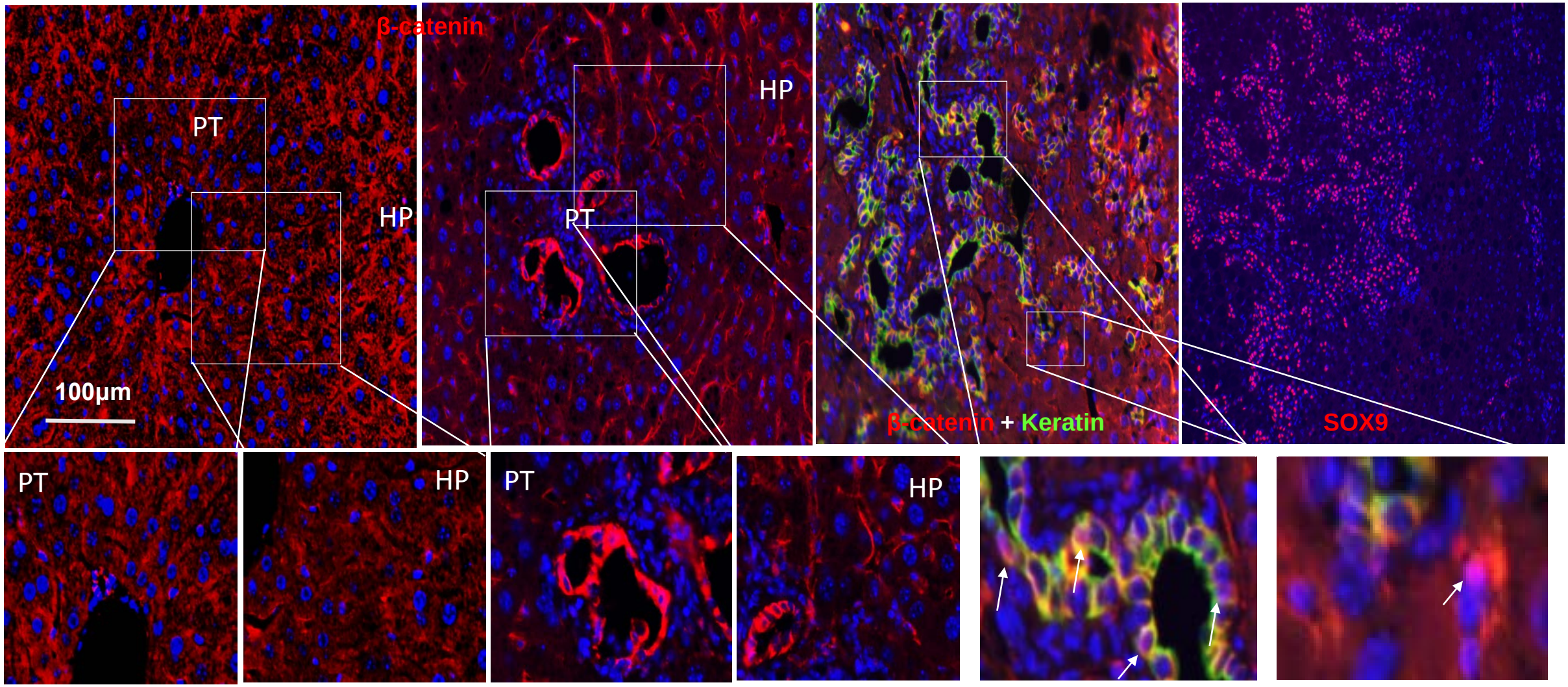




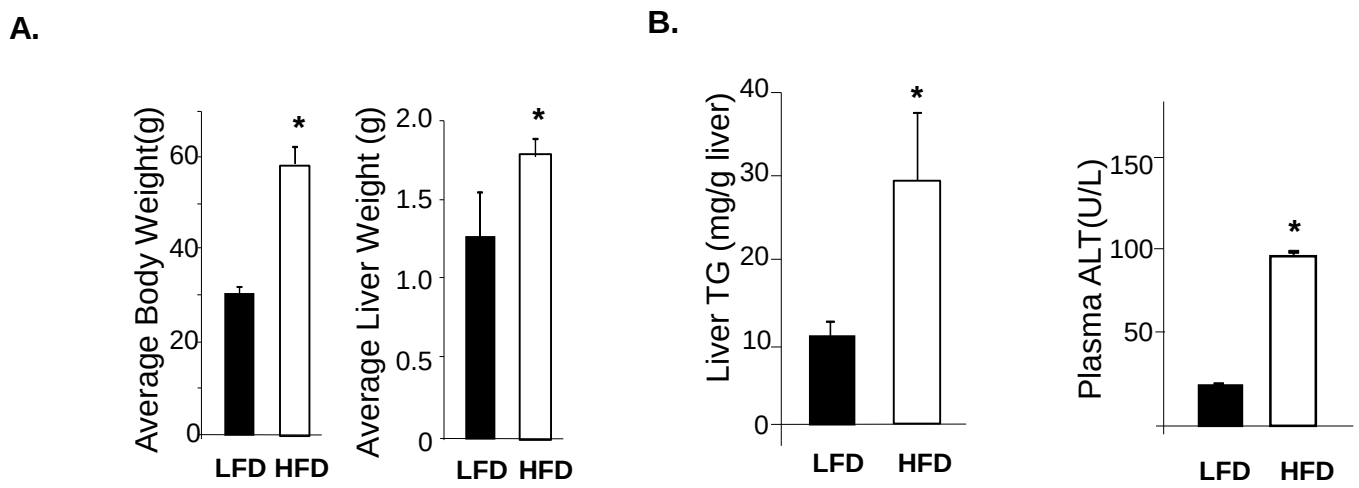




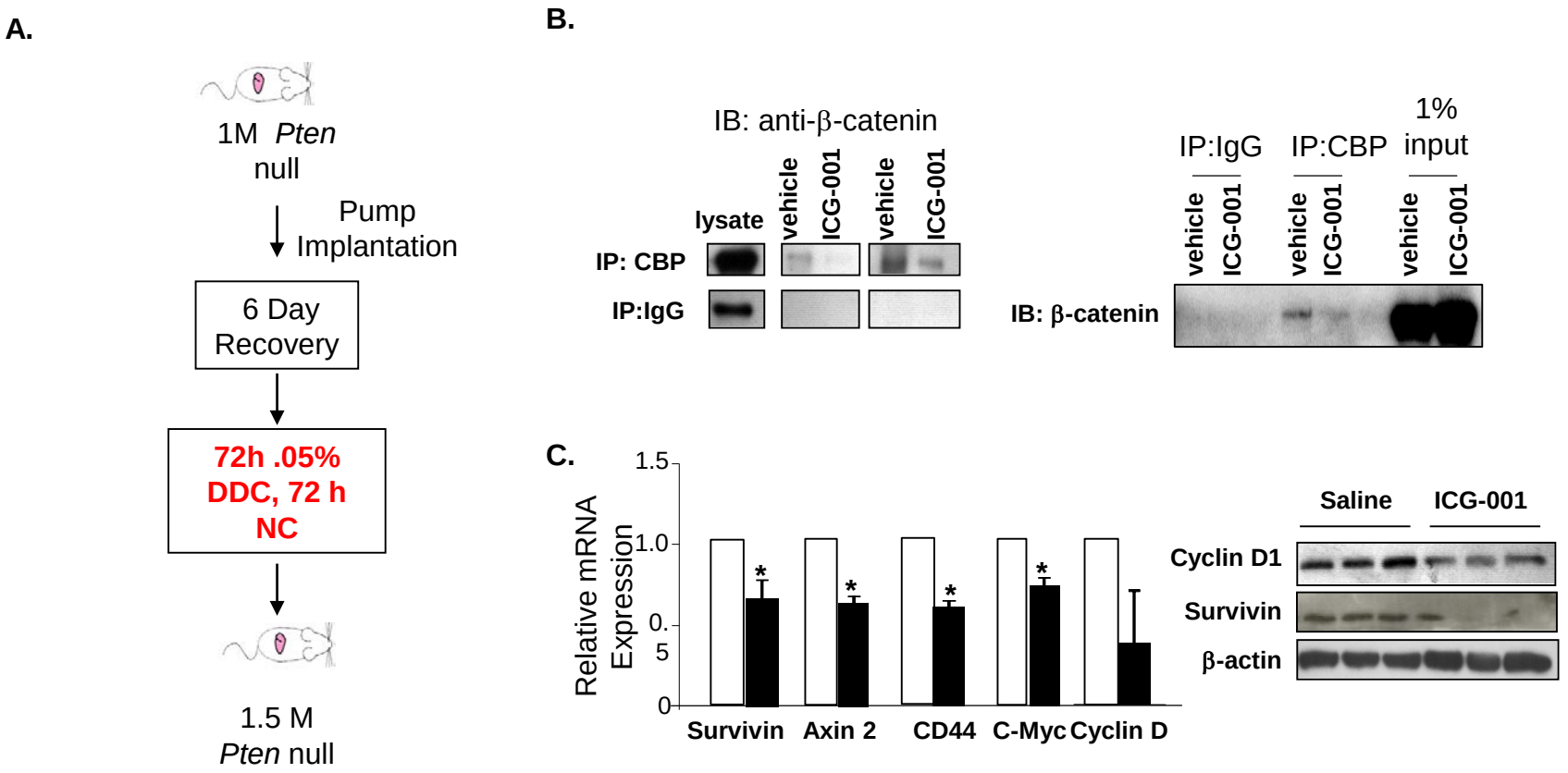
Supplemental Figure 2. Gene Enrichment Score of KEGG Wnt Signaling Pathway in Differentially Expressed Genes in *Pten* null vs. Control or *Pten/Akt2* Double Deleted Livers. Left, GSE score and plot for genes that are differentially expressed between *Pten* null liver vs. control samples. Gene analysis performed using Microarray. **Middle**, GSE score and plot for genes that are differentially expressed between Pten null liver vs. *Pten/Akt2* double deleted livers samples. Gene analysis performed using Microarray. **Right,** GSE score and plot for genes that are differentially expressed between Pten null livers from ad lib (AL) fed or caloric restricted (CR) mice. Gene analysis performed using RNA-seq. 11-12 months old mice.



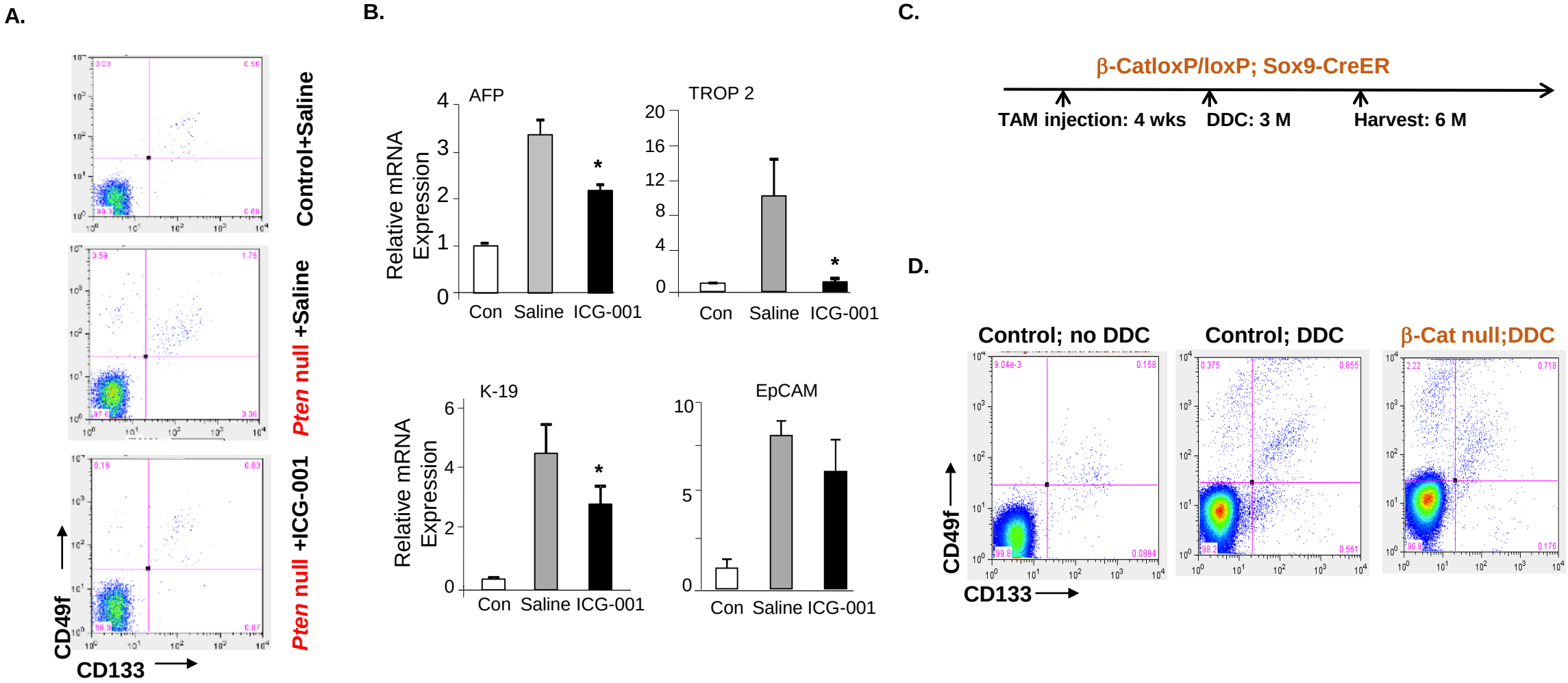
Supplemental Figure 3. β-catenin staining pattern in control and *Pten* null livers. β-catenin (red) and Dapi stained slides showing β-catenin staining primarily in hepatocytes in control liver sections and significantly intense β-catenin staining in ductal structure and portal triad in the *Pten* null livers sections. PT, portal triad; HP, hepatocytes. Top middle right panel, β-catenin (red) and keratin (green) staining to indicate β-catenin activation at the putative progenitor cell niche. Top right panel, staining for Sox9 (red) indicates tumors are positive for this progenitor marker. Arrows, nuclear β-catenin staining in periductal cells. 9 Months old mice.



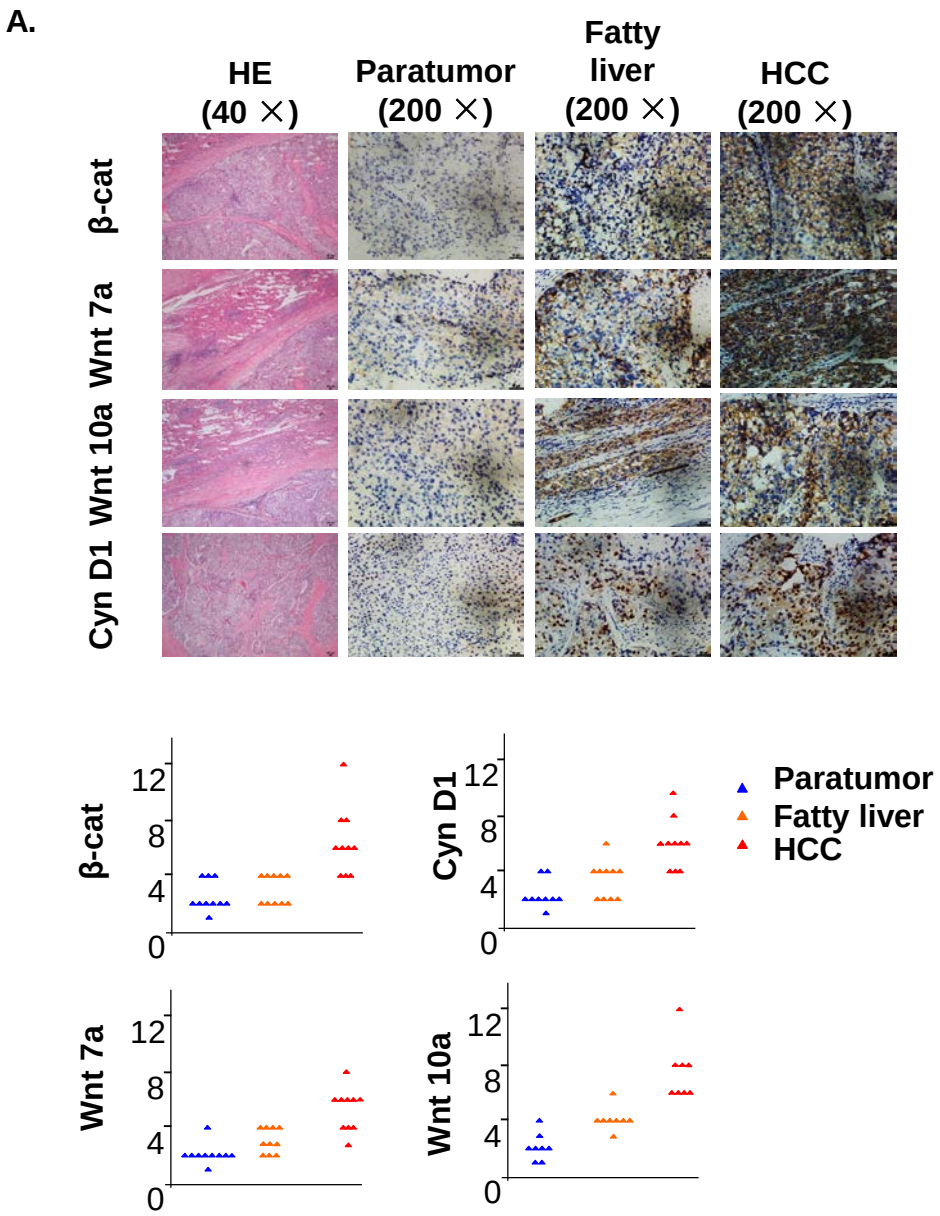
Supplemental Figure 4. Characteristics of mice on High Fat Diet. 3 months old mice were put on high fat diet (60kcal% fat diet) for 9 months. **A.** Final body and liver weight in LFD and HFD fed mice. **B.** Steatotic liver is developed in the HFD fed mice, leading to injury. Left, liver TG levels. Right, plasma ALT levels as indication of liver injury. n=6. All values are expressed as the mean ± SEM.



Supplemental Figure 5. Inhibition of β-catenin activity with ICG-001. A. Treatment regiment: 1 month old mice were implanted with Pump containing ICG-001. The mice were allowed recovery for 6 days before they were put on two rounds (72 hours each) of DDC containing diet (0.05% w/w). The mice were euthanized at the end of the treatment period for TIC analysis. **B.** Total liver (top panel) and nuclear (bottom) lysates from mice implanted with ICG-001 or saline pumps were immunoprecipitated with CBP and IgG antibodies and immunoblotted with β-catenin antibody. **C.** mRNA and protein expression of β-catenin regulated genes. n=3. n=3. * Significantly different from vehicle controls (Con) at $P \leq 0.05$.

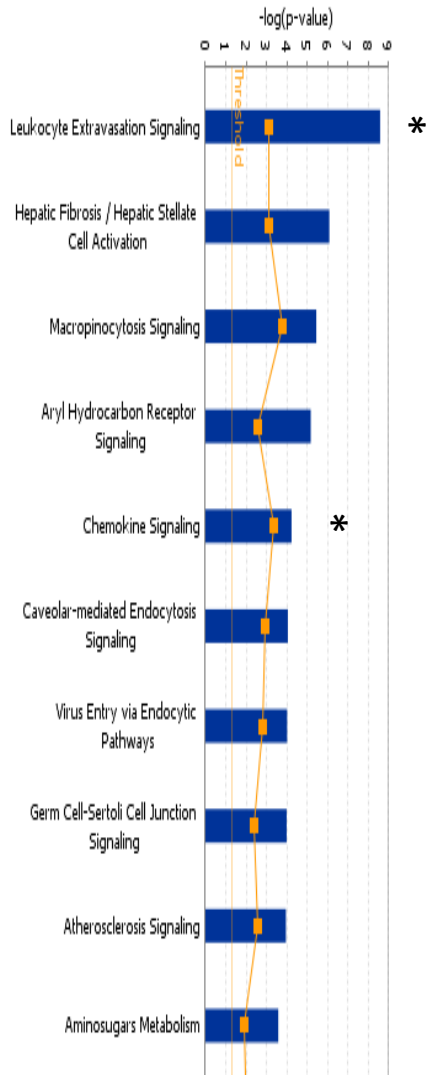


Supplemental Figure 6. Inhibition of β-catenin activity in liver progenitor cells lead to reduced TIC and TIC proliferation. A. Representative FACS plot of TICs in the control and Pten null mice treated with or without ICG-001 to block β-catenin activity. **B.** ICG-001 treatment reduces progenitor cell markers. AFP and keratin-19 (K-19) mRNA levels. n=3. **C.** Sox9-CreER; β-catenin+Tamoxifen (β-Cat null) and control mice were treated with DDC to induce TIC. TICs are quantified using CD133 and CD49f as cell survace markers. n=3. * Significantly different from vehicle controls (Con) at $P \leq 0.05$. **C.** Protocol for induced b-catenin deletion and TIC enrichment. **D.** Representative FACS plot of TICs in the control and β-Cat null mice treated with DDC.

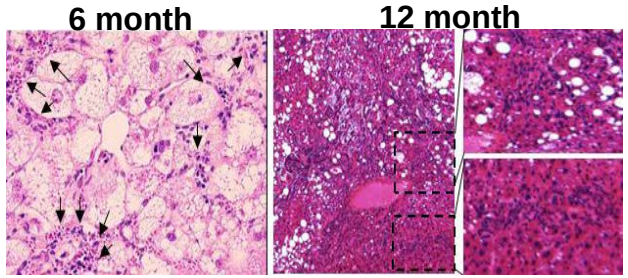


Supplemental Figure 7. Analysis of Wnt signaling molecules in human tumor vs. non-tumor tissues. **A**. Human liver sample specimen were stained with the indicated Wnt signaling molecules. Top, representative images; Bottom, histology scores for each patient sample. Each dot represent one patient sample. **B**. Immunohistochemical staining for Wnt signaling molecules in human liver specimen. Sample images from Human Protein Atlas website (http://www.proteinatlas.org)

A.

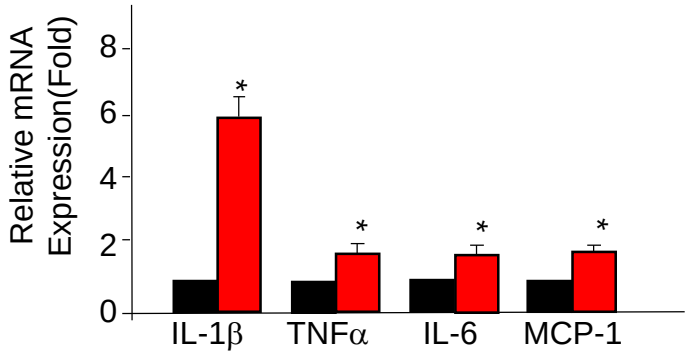


B.

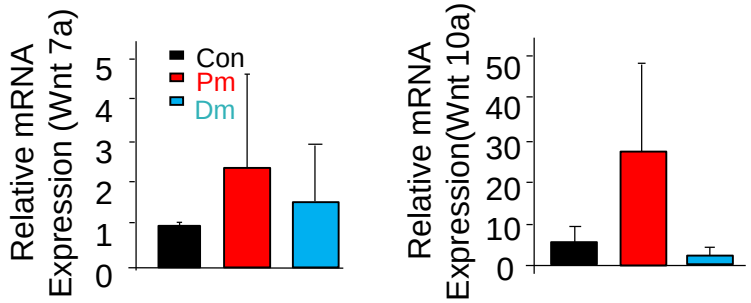


***Pten* null liver**

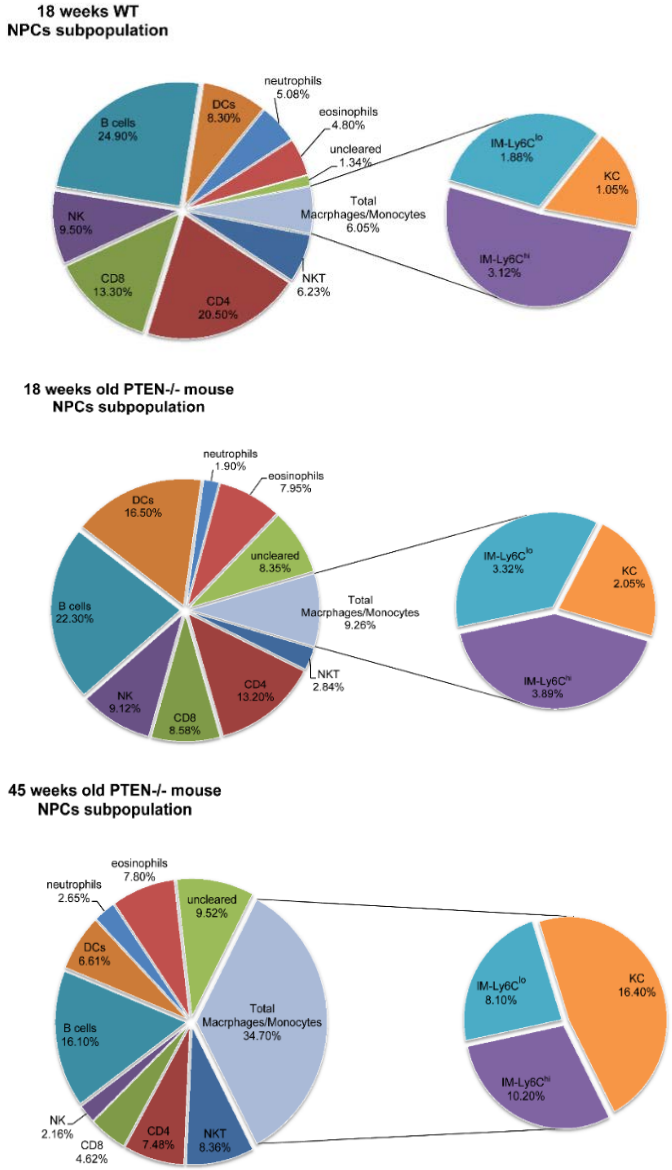
C.



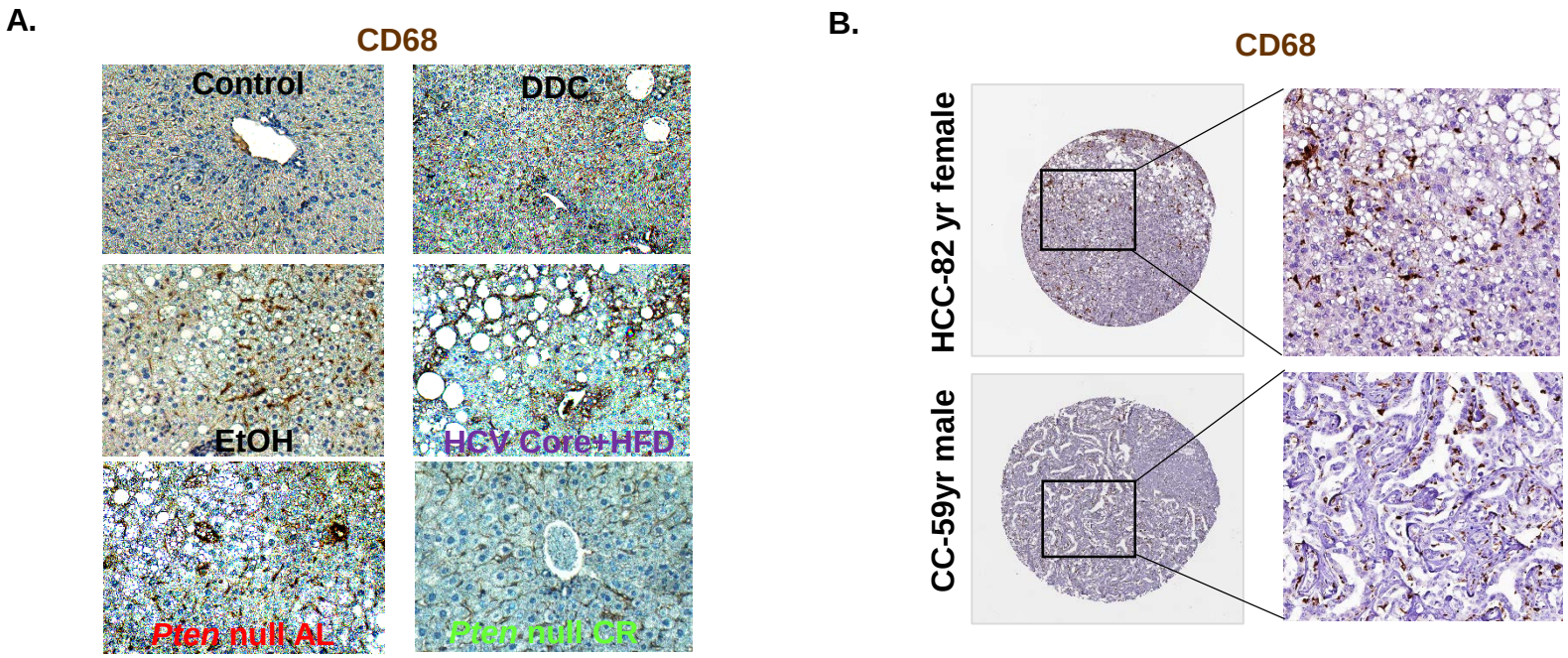
D.



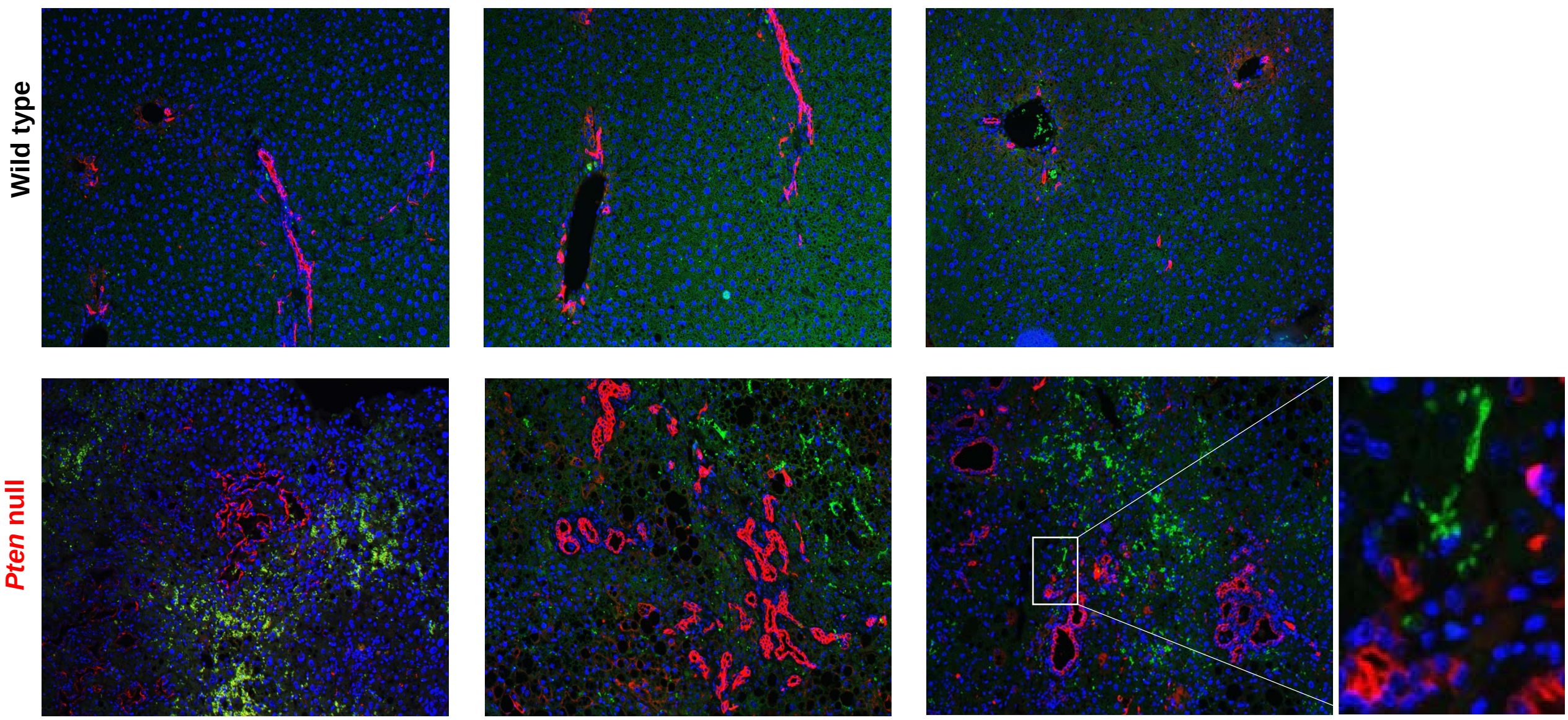
E.



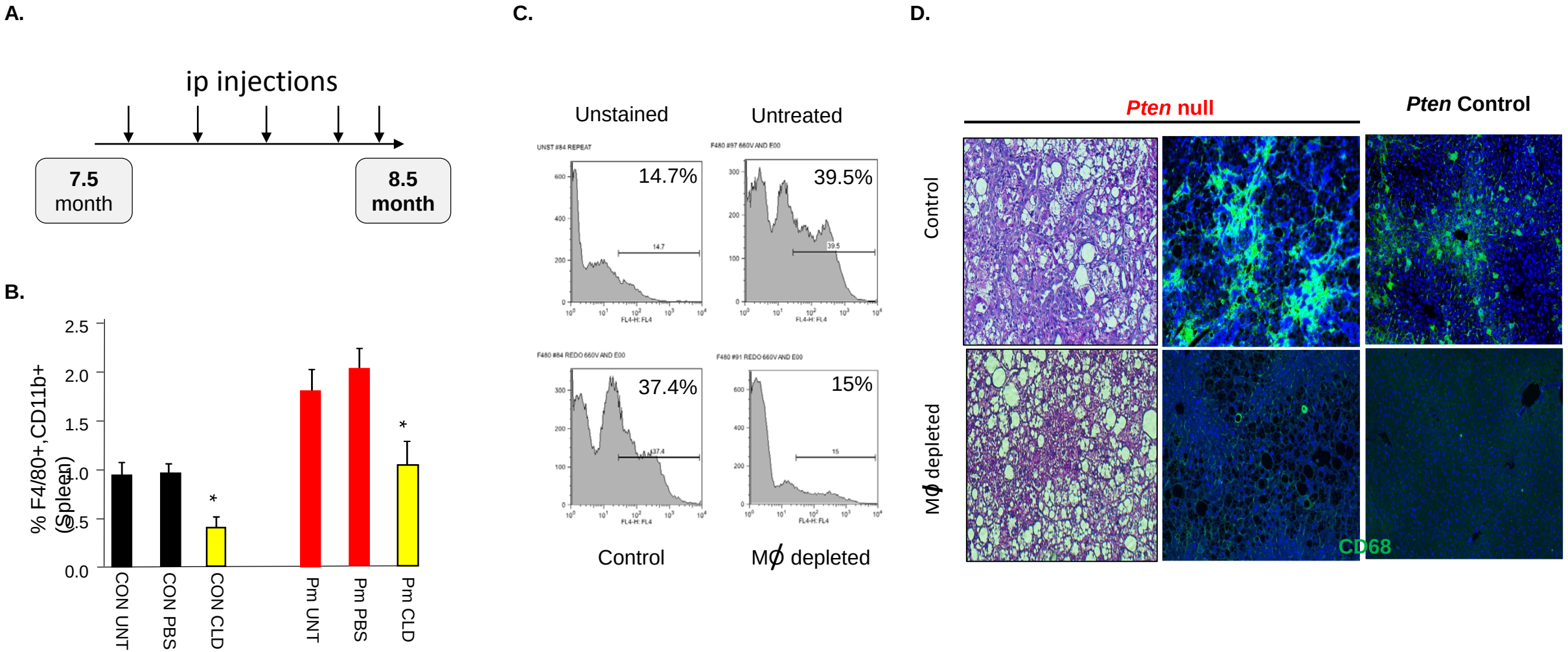
Supplemental Figure 8. Accumulation of inflammatory cells may lead to production of Wnt. **A.** Ingenuity Pathway Analysis of genes significantly over expressed in *Pten* null vs. control livers. Top 10 most significantly altered pathways shown. *Pathways involved in the inflammatory process. **B.** Inflammatory cell infiltration is a common feature in *Pten* null induced fatty liver and liver cancer. **C.** Expression of cytokines in the *Pten* null vs. control livers. n=3. **D.** Isolated inflammatory cells from 5 months old *Pten* null liver produces Wnt 7a and 10a whereas *Akt2* deletion reduces their production. **E**. Flowcytometry analysis of immune cells in the CD45+ nonparenchymal cells isolated from the control (WT) and *Pten* null livers.



Supplemental Figure 9. Macrophage (MO) accumulation is common in liver injury models and persist in cancers. A. Macrophage accumulation in the various mouse models indicated as CD68 staining. DDC, liver injury induced by feeding of 0.05% DDC for 2 months. EtOH, liver injury induced by feeding alcohol. HCV+HFD, liver injury induced in HCV transgenic mice by feeding of high fat diet (HFD). **B.** human liver cancer specimens. Sampless are human liver HCC (hepatocellular carcinoma) from a 82 year old female and CC (Cholangiocarcinoma) from a 59 year old male patient. CD68 stained images of human samples are sample images from Human Protein Atlas website (http://www.proteinatlas.org).



Supplemental Figure 10. Localization of liver macrophage and ductal cell. Macrophages stained with CD68 (green) are typically found at the periductal region that stains positive for keratin (red), particularly in the *Pten* null livers.



Supplemental Figure 11. Macrophage (MO) accumulation is common in liver injury models and persist in cancers. A. Liposome-Choldrolate or liposome-PBS injection protocol. **B.** Flowcytometry analysis of macrophages in the spleen. n=4-7. *significantly different from control at P≤0.05. F4.80 and cd11b are used as markers to define macrophage populations. **C.** CD68 staining for macrophages in CLD or PBS treated livers. **D**. Flow cytometry sorting demonstrating depletion of macrophages.