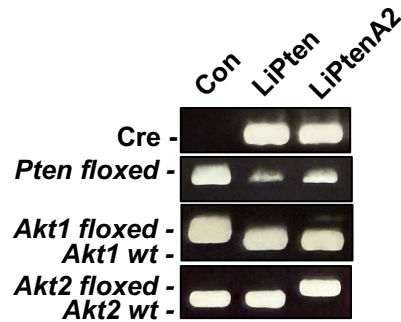
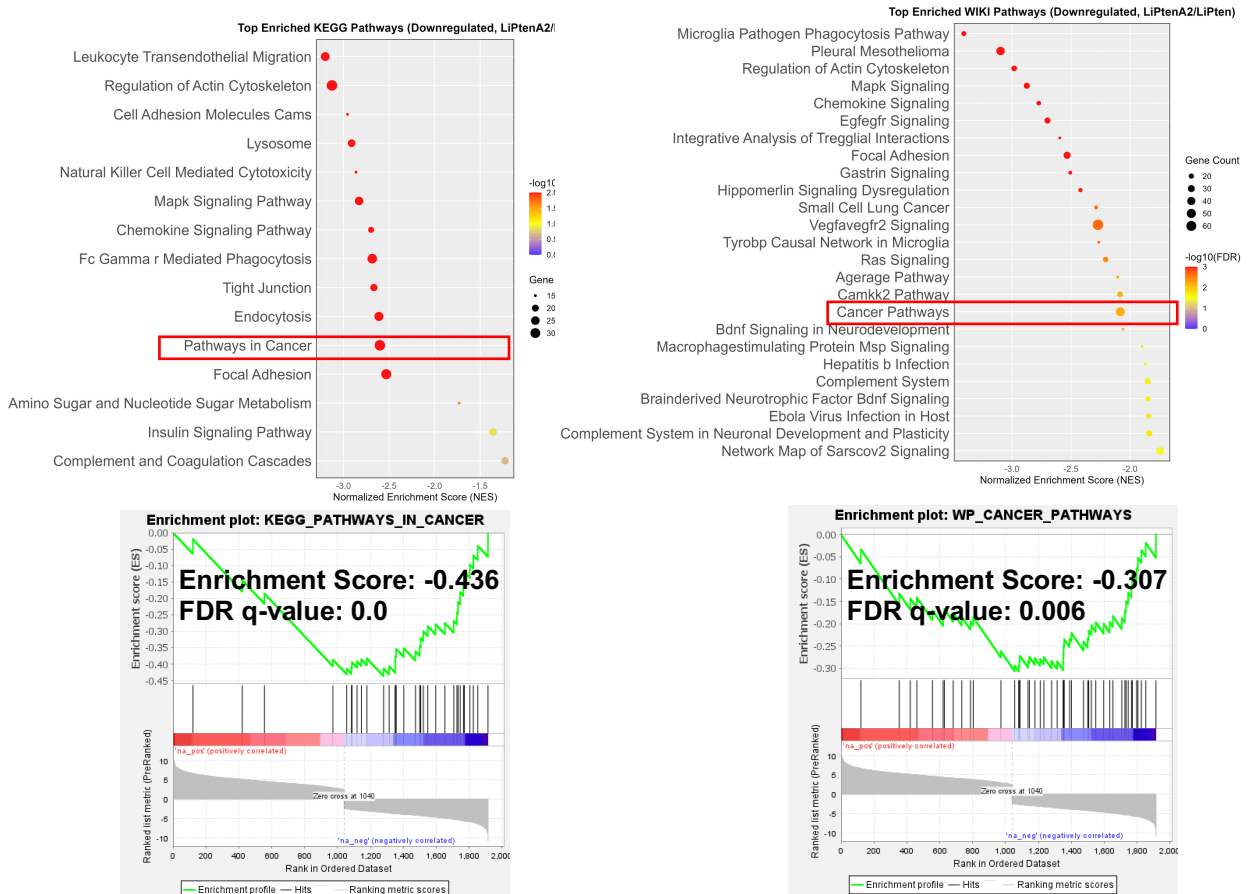


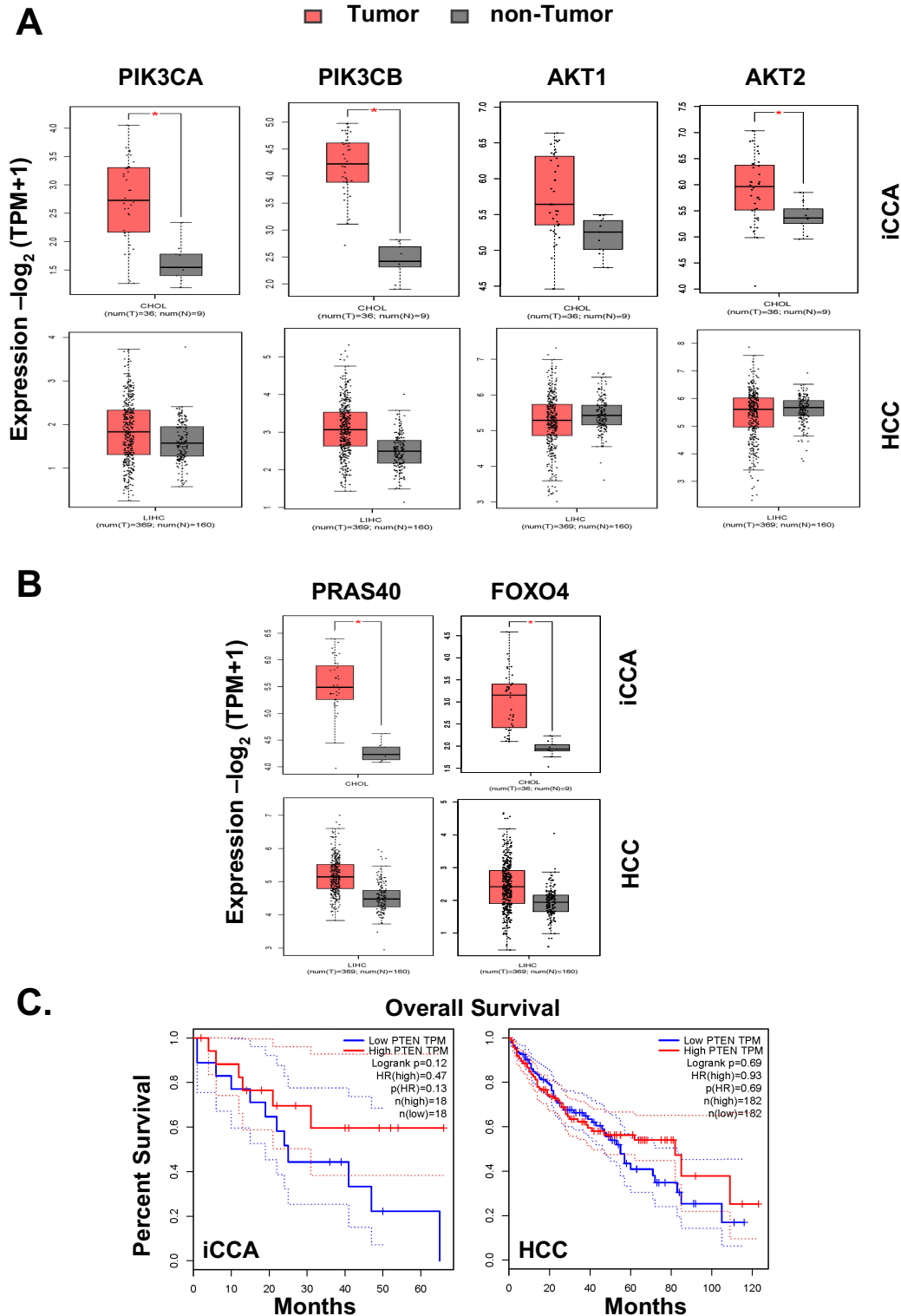
A



B

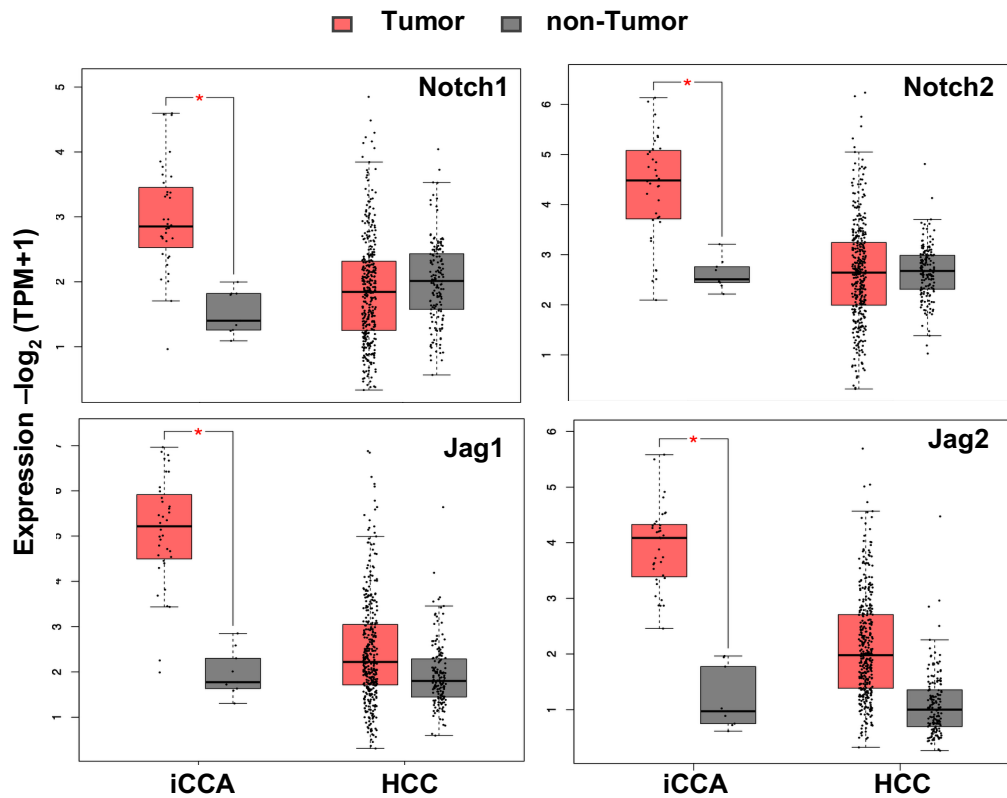


Supplemental Figure 2. Loss of AKT2 rescues oncogenesis in livers lacking PTEN. (A) PCR genotyping of tail DNA samples verified PTEN and AKT status in the respective mouse models. **(B)** Top downregulated KEGG and Wiki Pathways identified by proteomics in 12-month-old LiPtenA2 vs LiPten mice. Top, bubble plot highlighting cancer-related pathways. Bottom, enrichment plots of selected cancer pathways.



Supplemental Figure 3. PI3K/AKT signaling pathway is correlated with CCA but not HCC. Analysis of TCGA iCCA (n=36) and HCC samples (n=364) shows upregulation of PI3K and AKT isoforms (A) and AKT targets (B) mRNA expression in tumor vs. non-tumor samples in CCA but not HCC. (C) Overall survival analysis based on PTEN expression (Red, PTEN high, Blue, PTEN low) in the same iCCA and HCC samples. All analysis performed using GEPIA. *p≤0.05.

A

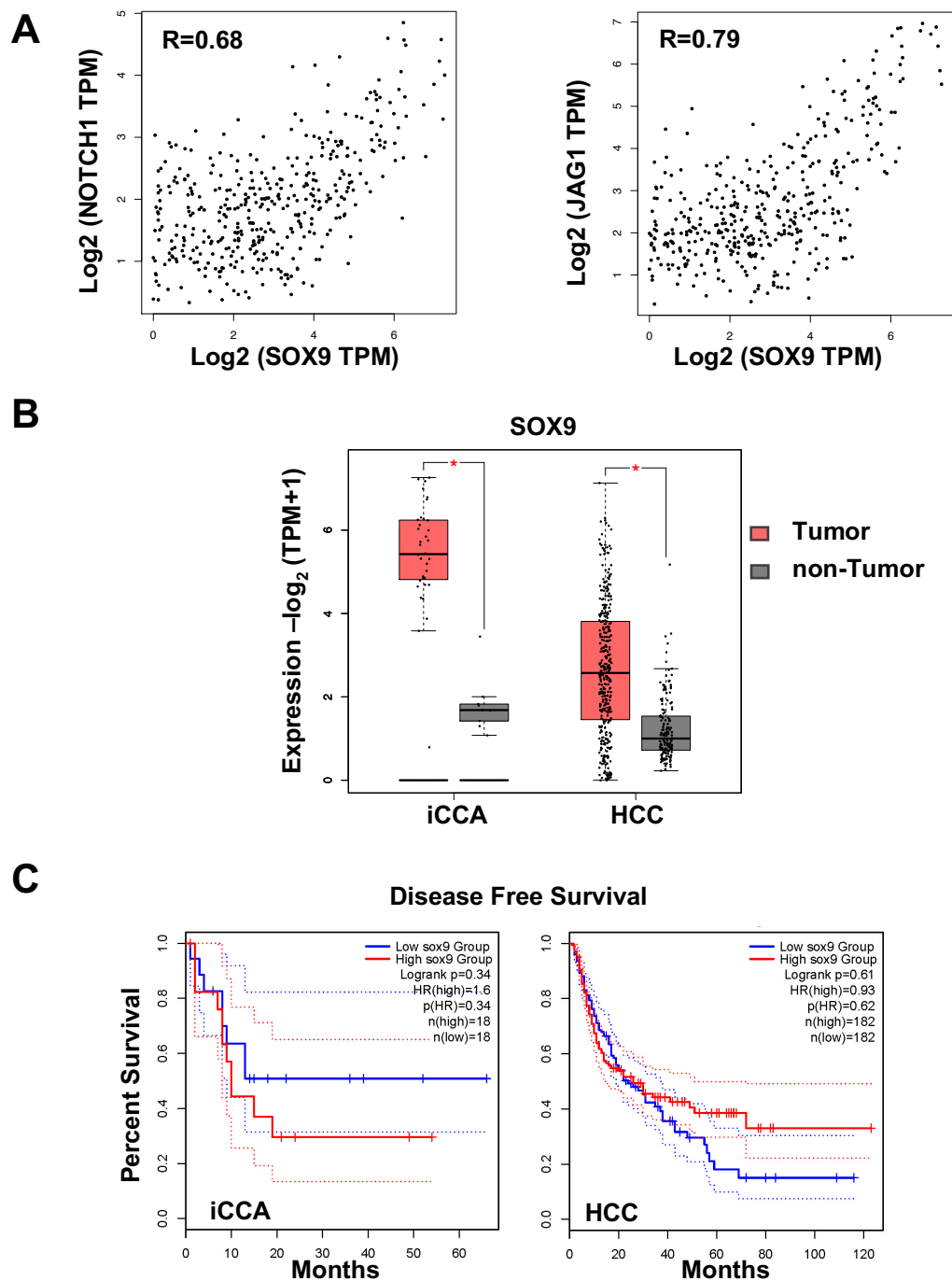


B

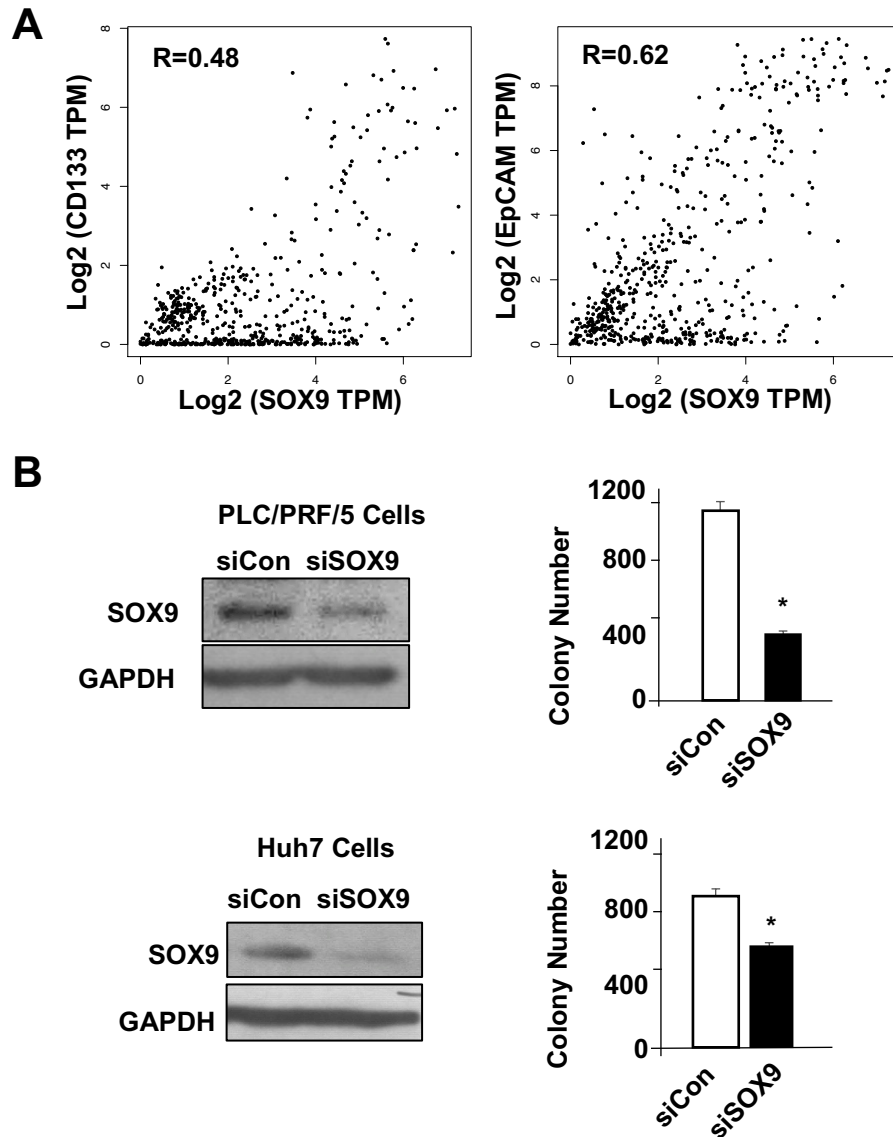
Expression Correlation (R) *				
	AKT1	AKT2	PIK3CA	PIK3CB
Notch1	0.54	0.39	0.57	0.49
Notch2	0.42	0.45	0.68	0.71
Notch3	0.35	0.33	0.55	0.48
Notch4	0.21	0.11	0.2	0.13
Jag1	0.29	0.32	0.53	0.45
Jag2	0.33	0.29	0.44	0.51
Hes1	0.35	0.2	0.42	0.41

* $P < 0.0001$. expect Notch4-AKT2 $p = 0.03$, Notch4-PIK3CA $p = 0.01$

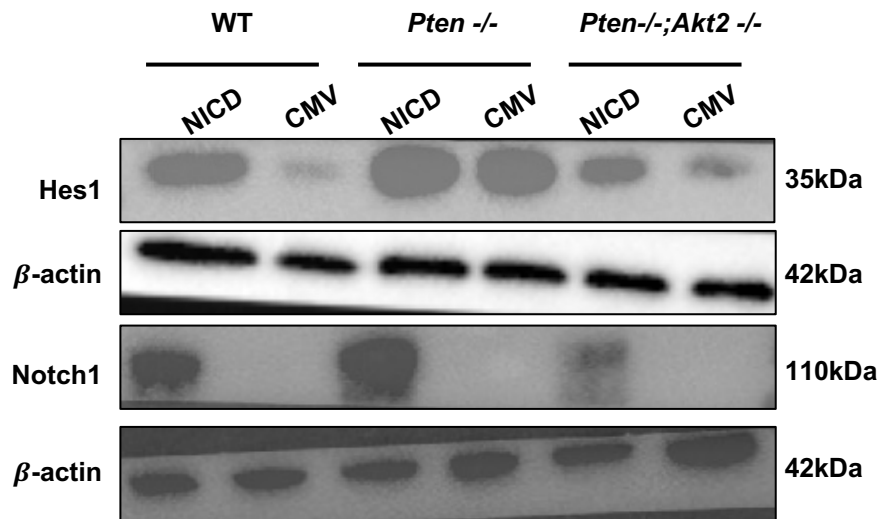
Supplemental Figure 4. Notch signaling is upregulated in CCA but not HCC and correlates well with PI3K/AKT signaling. (A) Expression of Notch1 and 2 and Notch ligand Jag1&2 mRNA in TCGA iCCA (n=36) and HCC (n=364) samples using GEPIA. **(B)** Correlation analysis of Notch signal with that of PI3K/AKT. * $P < 0.0001$ expect Notch4-AKT2 $p = 0.03$, Notch4-PIK3CA $p = 0.01$.



Supplemental Figure 5. SOX9 expression in CCA/HCC and its correlation with NOTCH signal in patient samples from TCGA. (A) Expression of NOTCH1 and JAG1 correlates well with that of SOX9 in liver cancer samples from TCGA. $p < 0.01$. **(B)** Expression of SOX9 mRNA in the same TCGA samples. **(C)** Disease-free survival of SOX9 high (red) vs. low (blue). iCCA (n=36) vs. HCC patients (n=364) using GEPIA. * $p \leq 0.05$.

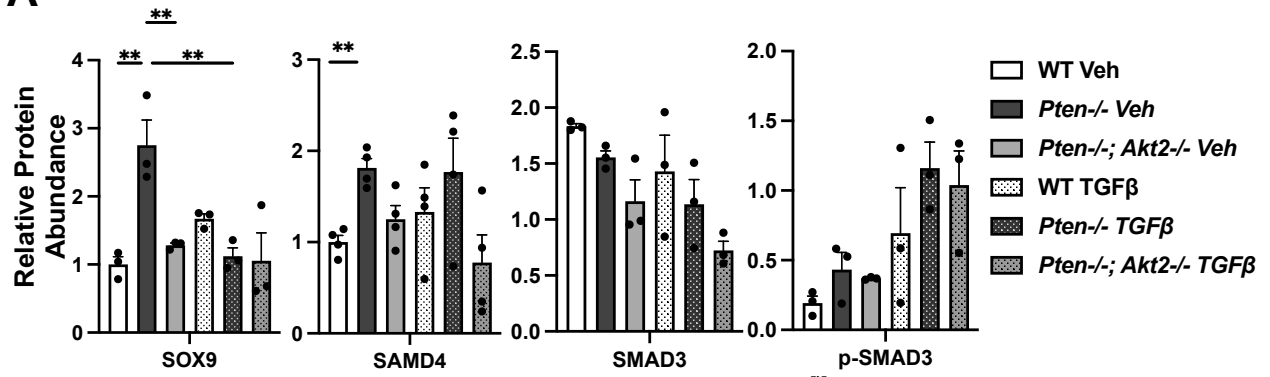


Supplemental Fig 6. Correlation of SOX9 with stem cell markers and effects of its downregulation on colony formation. (A) Expression of SOX9 correlates with that of CD133 and EpCAM in TCGA liver cancer samples. $p < 0.01$. **(B)** Colony formation in PLC/PRF/5 cells and Huh7 cells with or without siSOX9 transfection. $*p \leq 0.05$. $n = 3$.

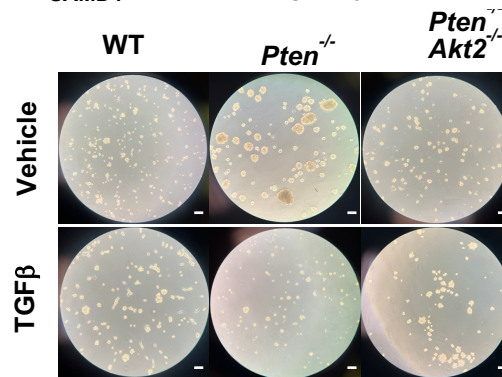


Supplemental Fig 7. NICD introduction activates Notch signaling. Immunoblotting shows NICD transfection significantly induces Notch signaling expression in WT, *Pten*^{-/-} and *Pten*^{-/-}; *Akt2*^{-/-} immortalized cells 24 hours after NICD transfection compared to CMV control. Blots are cropped for clarity.

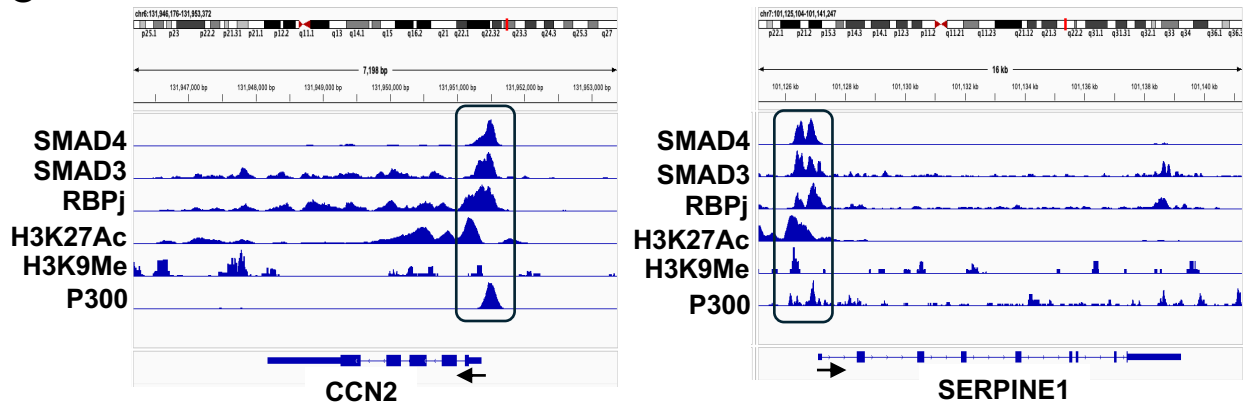
A



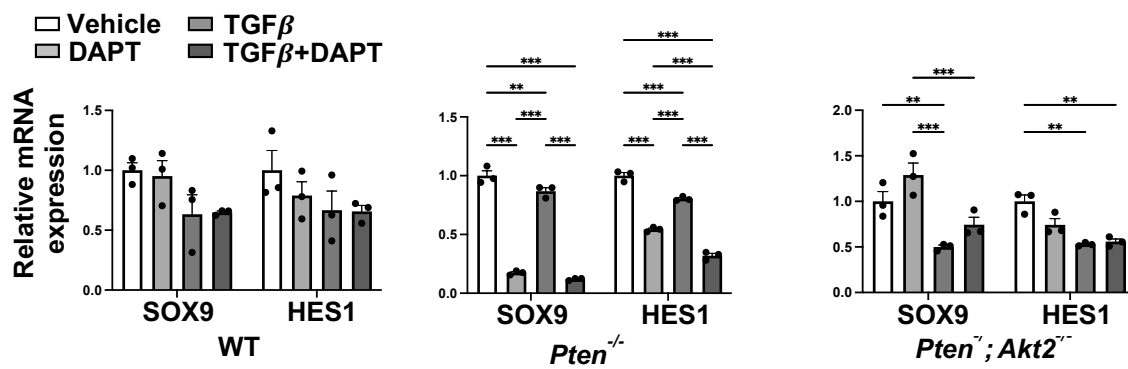
B



C



D



Supplemental Fig 8. PTEN loss induces different responses of liver tumor cells to TGF β treatment. **(A)** Quantification of relative protein abundance of immunoblotting showing expression of SOX9, SMAD4, SMAD3, and p-SMAD3 in WT, *Pten*^{-/-} and *Pten*^{-/-}; *Akt2*^{-/-} immortalized cells with or without TGF β treatment. **(B)** Representative image showing TGF β treatment inhibits sphere formation in *Pten*^{-/-} cells but not WT cells and cells lacking AKT2 (*Pten*^{-/-}; *Akt2*^{-/-}) (300,000 cells seeded per 60mm dishes). **(C)** Promoter analysis of canonical TGF β target genes. Encode ChIP-Seq data collected in HepG2 cells analyzed for promoters of CCN2 and SERPINE1, two canonical TGF β target genes, showing SMAD3 and SMAD4 co-occupy the same active regulatory regions with RBPj. These peaks are also localized at the valley of H3K27Ac peaks, marking a transcriptional activation site. **(D)** qPCR analysis of SOX9 and Hes1 mRNA expression in WT, *Pten*^{-/-} and *Pten*^{-/-}; *Akt2*^{-/-} immortalized cells treated with TGF β , DAPT or their combination under 48-hour sphere culture conditions. * p< 0.05, ** p< 0.01, *** p< 0.001.