Supplementary Methods

siRNA and Plasmid Transfection

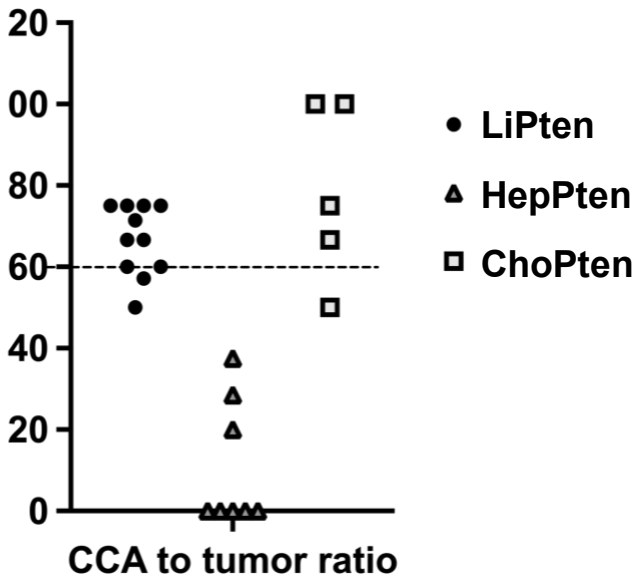
Silencer® Select siRNA targeting SOX9 (Thermo Fisher Scientific, Waltham, MA, USA, AM16708) and a non-targeting control siRNA (Thermo Fisher Scientific, AM4611) were transfected using Lipofectamine RNAiMAX (Thermo Fisher Scientific, #13778075) according to the manufacturer's instructions. shSOX9 plasmid (VectorBuilder, VB900123-7988yjz), NICD1 expression plasmid (Addgene, #20183), and corresponding empty vector controls were introduced using Lipofectamine 3000 (Thermo Fisher Scientific, #L3000008) following the manufacturer's protocol.

Antibody used for immunoblotting and immunohistochemistry.

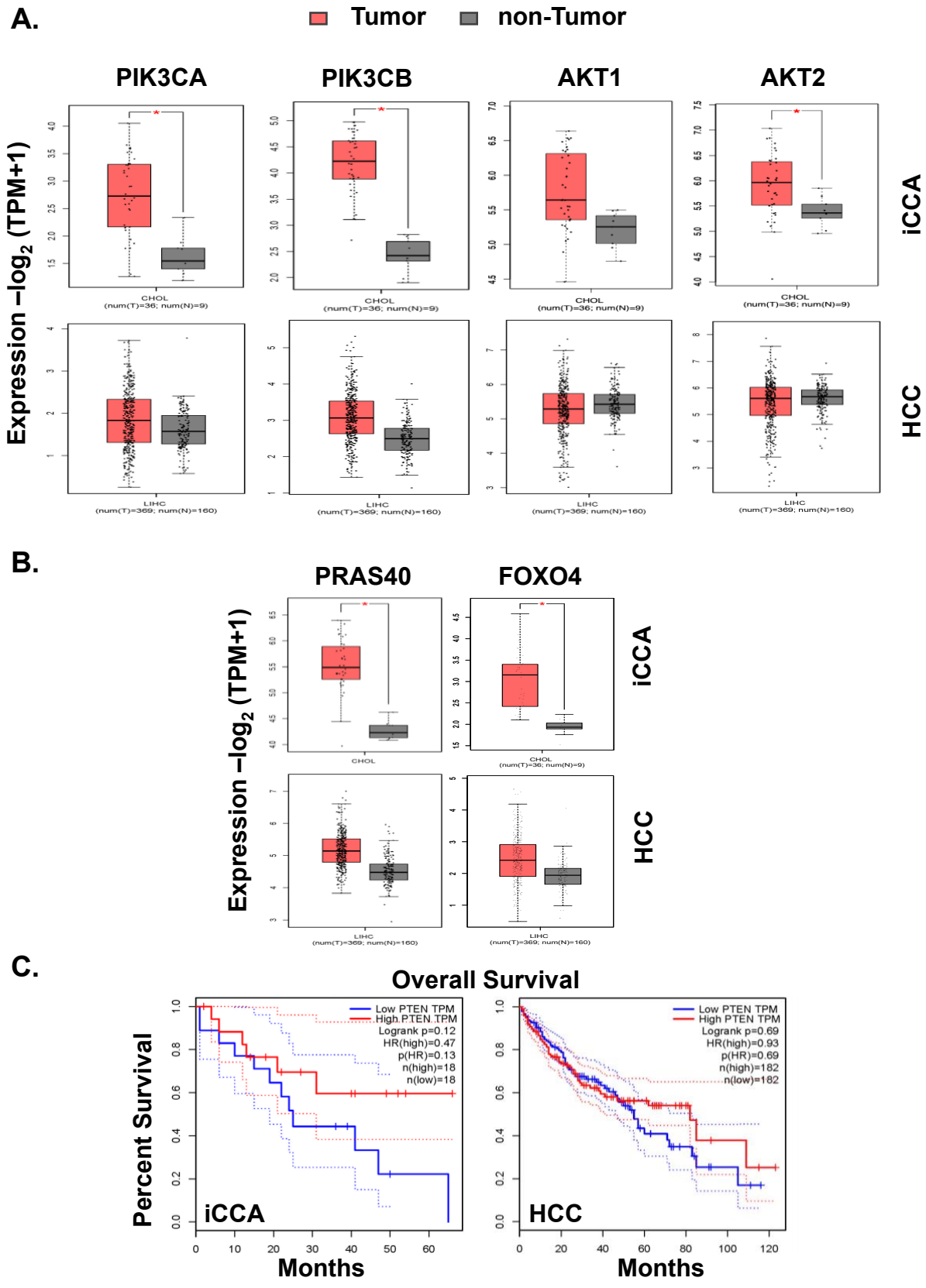
Antibody	Catalog #	Vendor	Research Resource Identifiers
activated Notch1	ab8925	Abcam	RRID: AB_306863
AKT1	2938	Cell Signaling Technology	RRID: AB_915788
AKT2	3063	Cell Signaling Technology	RRID: AB_2225186
Beta actin	A5441	Sigma	RRID: AB_476744
Beta actin	66009-1-Ig	Proteintech	RRID: AB_2687938
EPCAM	21050-1-AP	Proteintech	RRID: AB_10693684
GAPDH	G9545	Sigma	RRID: AB_796208
HepPar 1	M7158	Dako	RRID: AB_3720362
Hes1	11988	Cell Signaling Technology	RRID: AB_2728766
HNF-4-alpha	ab41898	Abcam	RRID: AB_732976
Jagged1	sc-390177	Santa Cruz Biotechnology	RRID: AB_2892141
pan-AKT1/2/3	sc-8312	Santa Cruz Biotechnology	RRID: AB_671714
Pan-CK	Z0622	Dako	RRID: AB_2650434
phospho-AKT (Ser473)	4060	Cell Signaling Technology	RRID: AB_231504
phospho-AKT (Thr308)	9275	Cell Signaling Technology	RRID: AB_329828
phospho-PRAS40 (Thr246)	2640	Cell Signaling Technology	RRID: AB_916141
Pten	9559	Cell Signaling Technology	RRID: AB_2253290
SOX9	AMAB90795	Sigma	RRID: AB_2665670
SOX9	82630	Cell Signaling Technology	RRID: AB_2665492
Vinculin	26520-1-AP	Proteintech	RRID: AB_2868558
YFP	ab6673	Abcam	RRID: AB_305643

Primers used for quantitative PCR.

Primers	**Sequence 5'-3'**
Human SOX9 F	CCAGAACAAGCCGCACGTCA
Human SOX9 R	CTGCCCCGTTCTTCACCGACT
Human EpCAM F	GCGAGTGAGAACCTACTGGA
Human EpCAM R	CGCGTTGTGATCTCCTTCTG
Human CD133 F	AAGGCATATGAATCCAAAATTGA
Human CD133 R	CCACCAGAGGCATCAGAATAA
Human GapDH F	AGGGCTGCTTTTAACTCTGGT
Human GapDH R	CCCCACTTGATTTTGGAGGGA
Mouse SOX9 F	GTGCAAGCTGGCAAAGTTGA
Mouse SOX9 R	TGCTCAGTTCACCGATGTCC
Mouse Hes1 F	ACACCGGACAAACCAAAGAC
Mouse Hes1 R	AATGCCGGGAGCTATCTTTC
Mouse Notch1 F	TGTGGCTTCCTTCTACTGCG
Mouse Notch1 R	CTTTGCCGTTGACAGGGTTG
Mouse 18S F	CGGCTACCACATCCAAGGAA
Mouse 18S R	GCTGGAATTACCGCGGCT



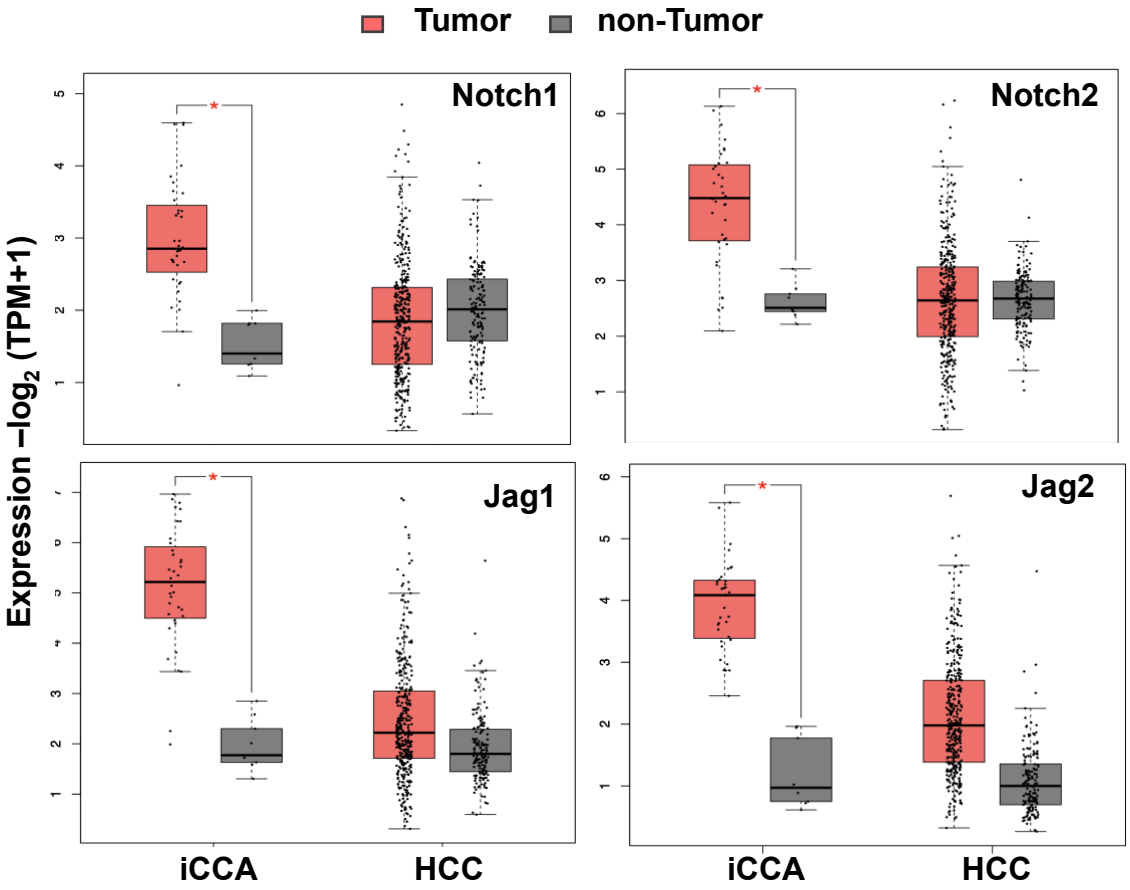
Supplemental Figure 1. Quantification of CCA development in mouse models lacking *Pten* in the liver. Percentage of the tumors that are CCA vs. total tumors developed in each mouse. Each dot is a mouse.



Supplemental Figure 2. PI3K/AKT signaling pathway is correlated with CCA but not

HCC. (A) PI3K isoforms; and **(B)** AKT isoforms and their targets mRNA expressions are elevated in tumor vs. non-tumor samples in CCA but not HCC. **(C)** Overall survival analysis of PTEN expression in iCCA (n=36) and HCC samples (n=364) available from TCGA analyzed using GEPIA. *p≤0.05.

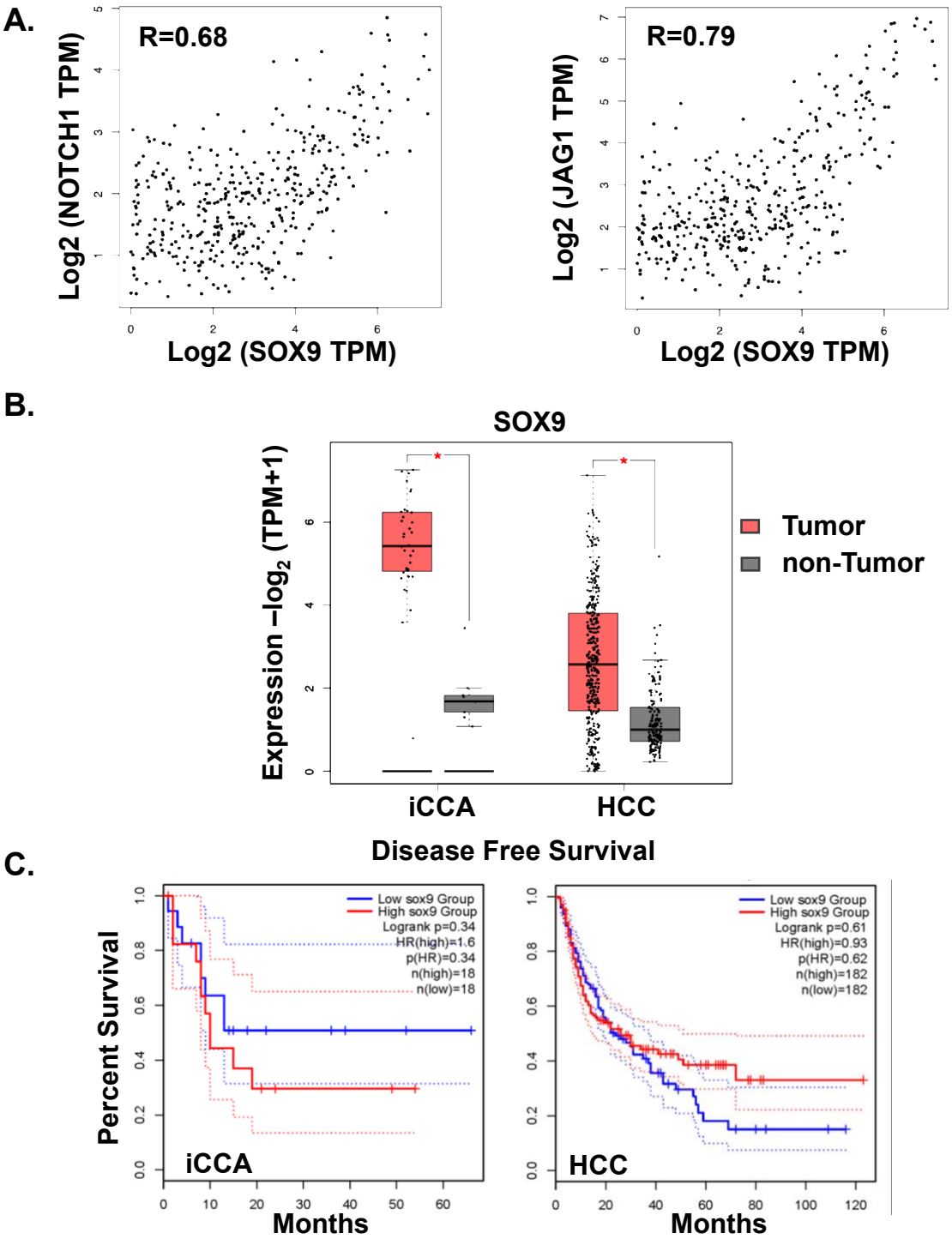
A.



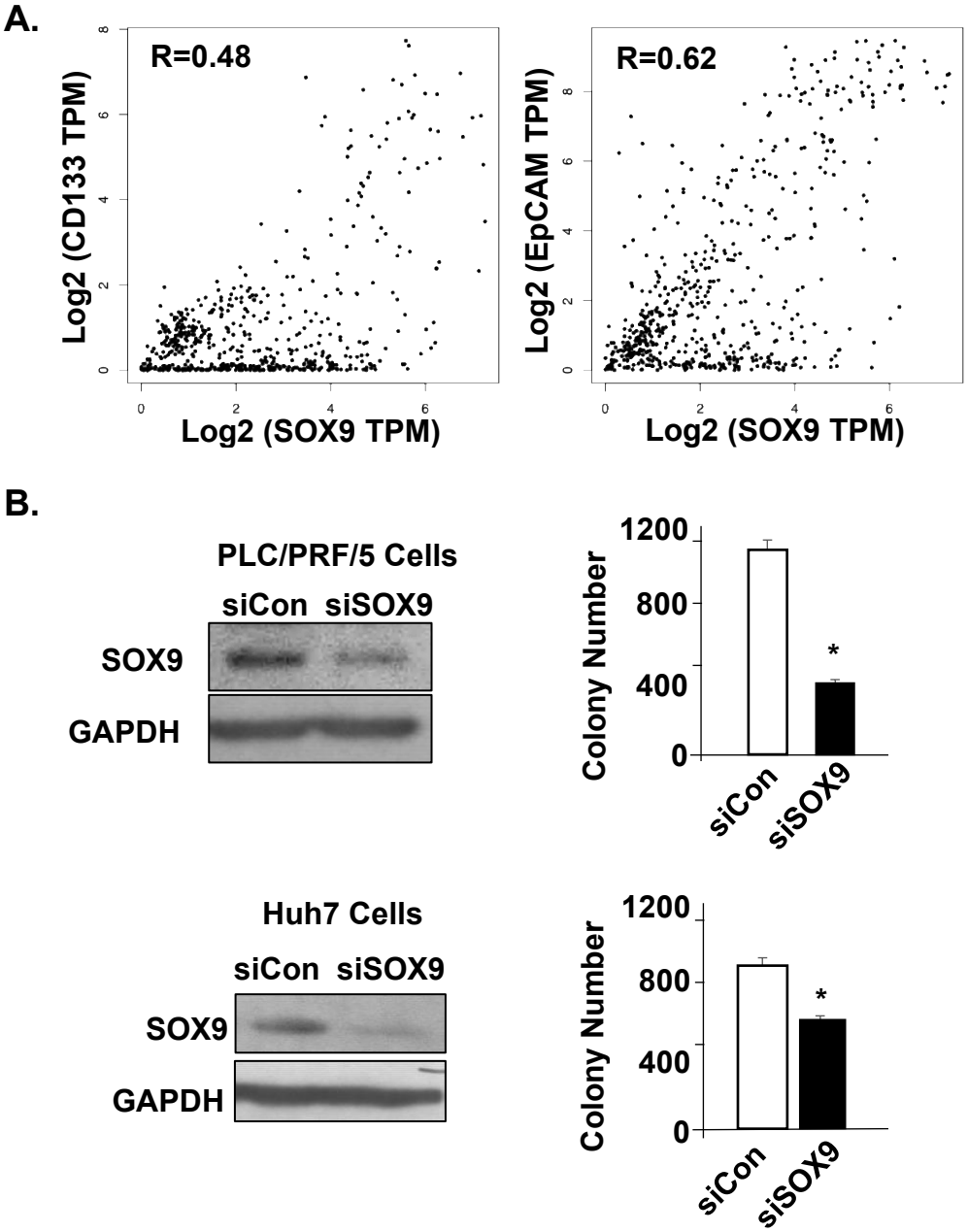
B.

Expression Correlation (R) *					
	AKT1	AKT2	AKT3	PIK3CA	PIK3CB
Notch1	0.54	0.39	0.46	0.57	0.49
Notch2	0.42	0.45	0.7	0.68	0.71
Notch3	0.35	0.33	0.51	0.55	0.48
Notch4	0.21	0.11	0.27	0.2	0.13
Jag1	0.29	0.32	0.65	0.53	0.45
Jag2	0.33	0.29	0.53	0.44	0.51
Hes1	0.35	0.2	0.38	0.42	0.41

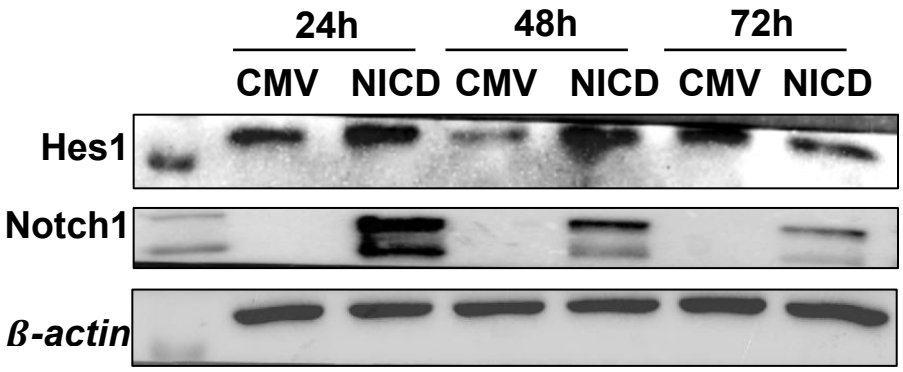
Supplemental Figure 3. Notch signaling is upregulated in CCA but not HCC and correlates well with PI3K/AKT signaling. (A) Expression of Notch1 and 2 and Notch ligand Jag1&2 mRNA in patient liver tumor and normal samples using GEPIA **(B)** Correlation analysis of Notch signal with that of PI3K/AKT. *P<0.0001 expect Notch4-akt2 p=0.03, notch4-pik3ca p=0.01.



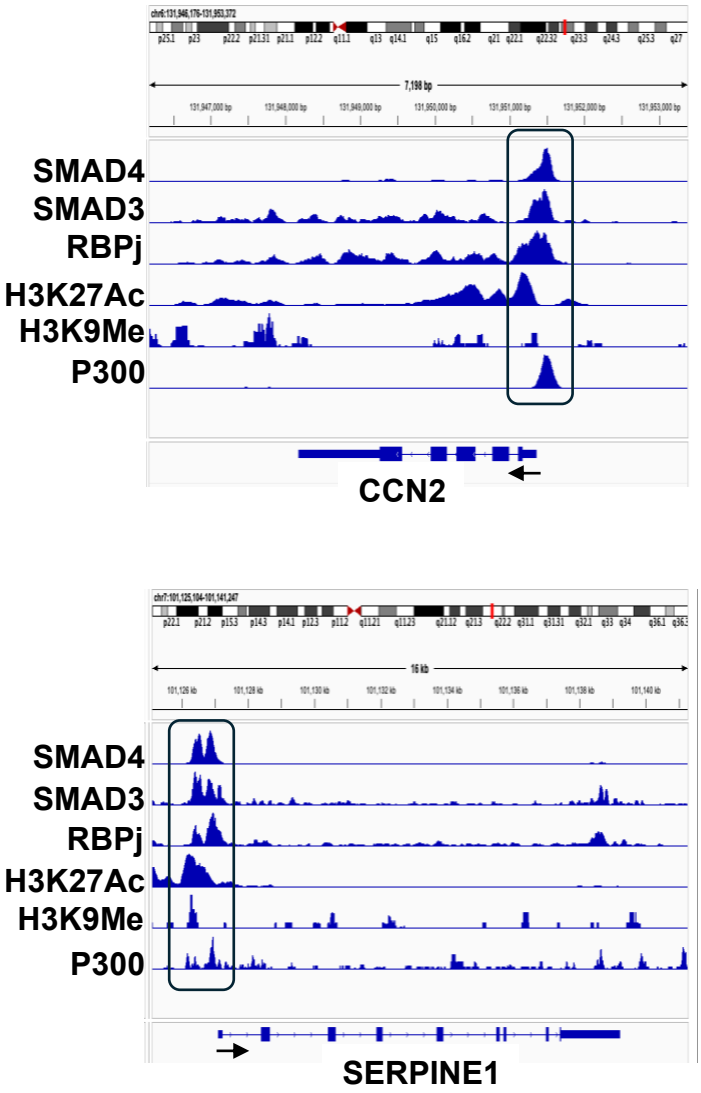
Supplement Figure 4. SOX9 expression in CCA/HCC and its correlation with NOTCH signal in patient samples from TCGA. (A) Expression of NOTCH1 and JAG1 correlates well with that of SOX9 in liver cancer. $p<0.01$. **(B)** Expression of SOX9 mRNA expression. **(C)** Disease free survival of SOX9 high (red) vs. low (blue) iCCA (n-36) vs. HCC patients (n=364) using GEPIA. *$p \leq 0.05$.



Supplemental Fig 5. 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 6. NICD introduction can activate the Notch signaling. Immounoblotting shows NICD tracfection significantly induce Notch signaling expression.



Supplemental Fig 7. Promoter analysis of **canonical TGFβ target genes.** Encode Chip-Seq data collected in HepG2 cells analyzed for promoters of CCN2 and SERPINE1, two canonical TGFβ 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.