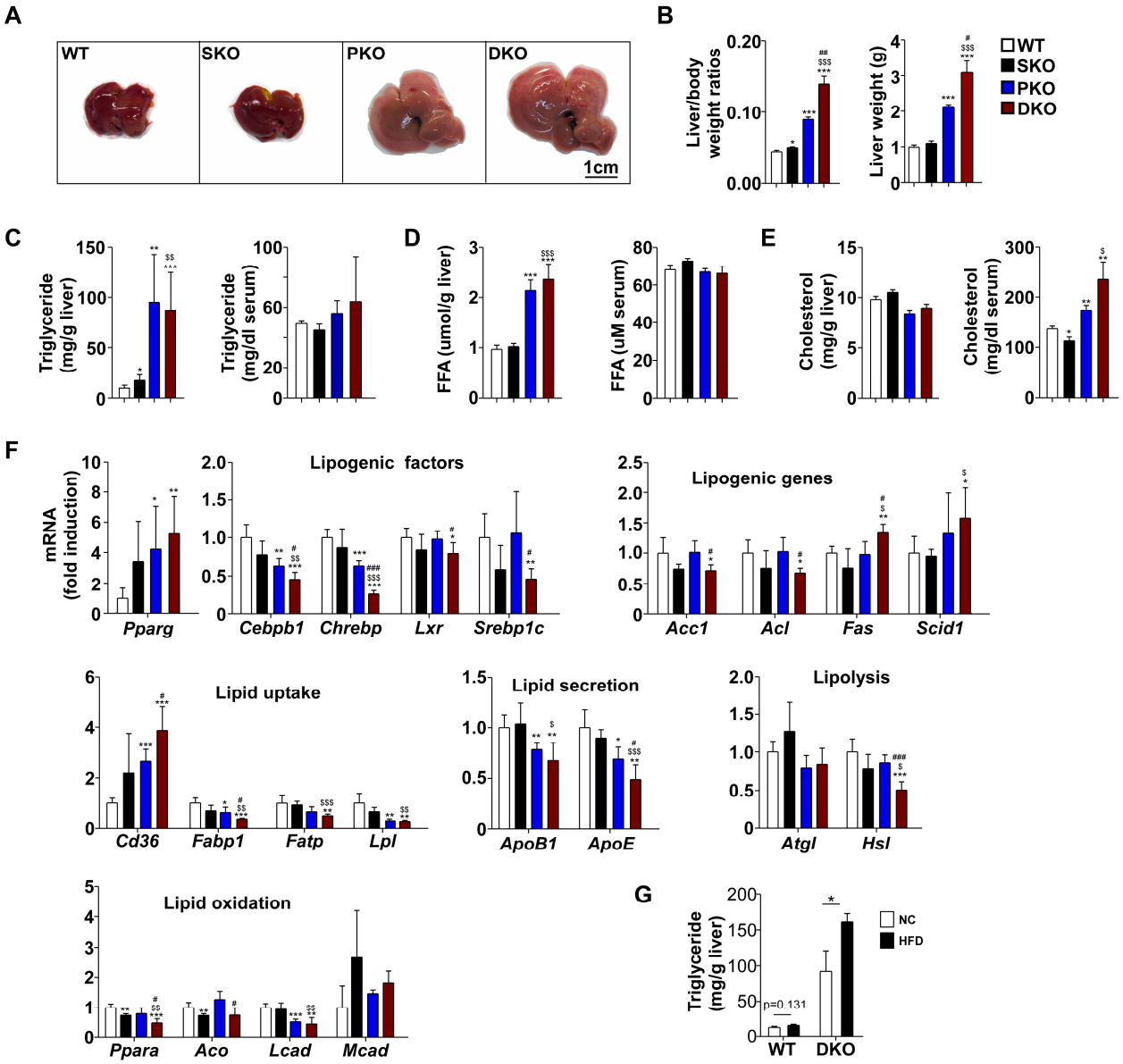


Figure S1. *DKO* mice develop hepatic steatosis, related to Figure 2. (A) Macroscopic view of representative livers. **(B)** Liver/body weight ratios and liver weight were compared (n=6). **(C, D, E)** Hepatic and serum triglycerides, free fatty acids and cholesterol levels were determined and compared among 2-month-old mice (n=6). **(F)** mRNA levels of key genes in lipid metabolism was determined using qPCR and compared among 1-month-old mice (n=5). **(G)** 1-month-old WT and DKO mice were fed high fat diet for 1 month. Hepatic triglycerides were measured and compared among normal chow (NC) and high fat diet (HFD) fed *WT* and *DKO* mice (n=5~6). Data are shown as means ± S.D. **(A-F)** *, **, or *** indicates *SKO*, *PKO* or *DKO* vs. *WT*. $, $$, or $$$ indicates *DKO* vs. *SKO*. # or ### indicates *DKO* vs. *PKO*. *, $ or # indicates p<0.05. ** or $$ indicates p<0.01. ***, $$$ or ### indicates p<0.001. **(G)** * indicates p<0.05.

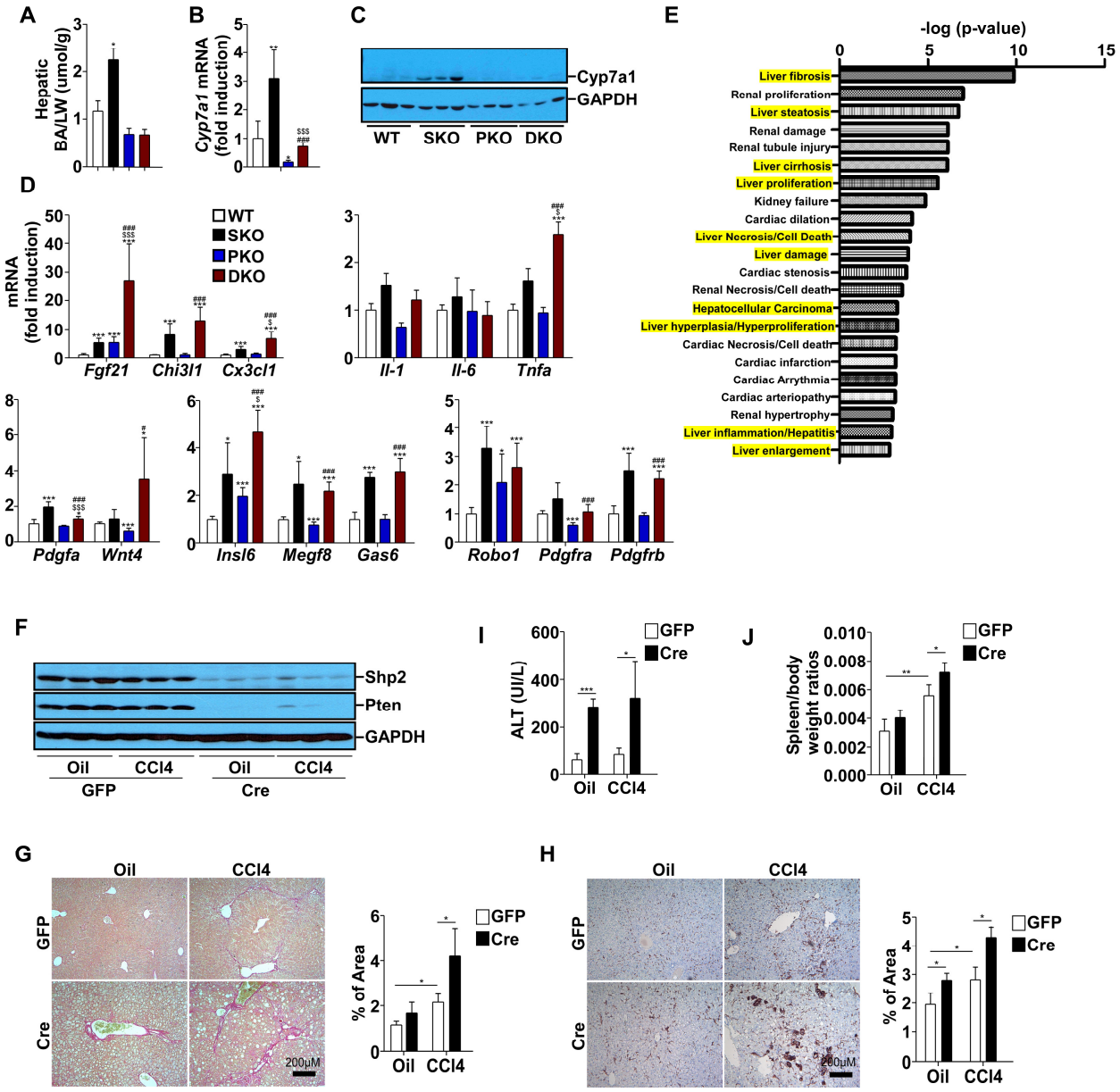


Figure S2. Dual deletion of *Shp2* and *Pten* in hepatocytes leads to early-onset NASH, related to Figure 3. (A) Hepatic bile acid levels were determined and compared (n=6). **(B)** mRNA level of *Cyp7a1* was determined by qPCR and compared (n=5). **(C)** Cyp7a1 was determined by immunoblot in liver lysates**. (D)** mRNA levels of secretory factors and related receptors were determined by qPCR and compared (n=5). **(E)** Analysis of toxicogenomics changes in *DKO* livers. Bar chart was generated using IPA-Tox® with microarray data (*DKO* vs. *WT*). Toxicology changes related to liver were highlighted. All samples used in this figure were obtained from 2-month-old mice. **(A, B, D)** Data are shown as means ± S.D. *, **, or *** indicates *SKO*, *PKO* or *DKO* vs. *WT*. $ or $$$ indicates *DKO* vs. *SKO*. ### indicates *DKO* vs. *PKO*. * or $ indicates $p<0.05$. ** indicates $p<0.01$. ***, $$$ or ### indicates $p<0.001$. **(F-J)** 2~3-month-old *WT* (*Pten*$^{fl/fl}$:*Shp2*$^{fl/fl}$:*Alb-Cre*$^{-}$) were infected with AAV-GFP or AAV-Cre. One week after infection, mice were I.P. with olive oil or CCL4 twice a week for 4 weeks. **(F)** Expression of *Shp2* and *Pten* was examined by immunoblot analysis of liver lysates. **(G, H)** Right, Picro-Sirius Red staining **(G)** and F4/80 **(H)** immunostaining of liver sections. Left, quantification of percentage of positive Sirius Red and F4/80 staining area (n=3~4). **(I)** Serum ALT was measured and compared (n=3~ 4). **(J)** Spleen/body weight ratios were compared (n=3~ 4). Data are presented as Mean±SD. (*$p<0.05$, ** $p<0.01$,*** $p<0.001$)

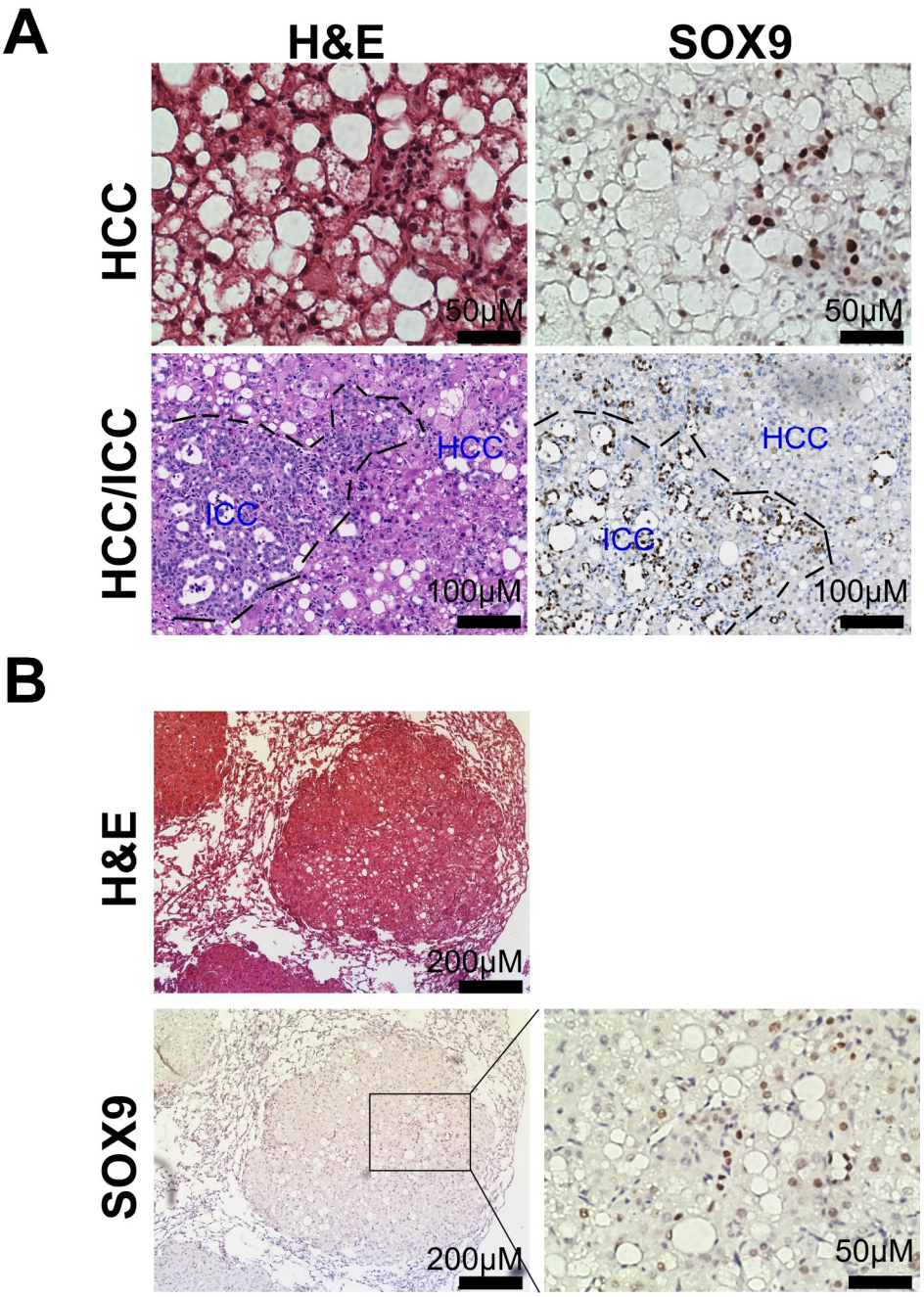


Figure S3. Microscopic view of representative tumors in *MUP-uPA* mice, related to Figure 4. Data are from *MUP-uPA* mice 5 months after transplantation with *DKO* hepatocytes. **(A)** Representative H&E and SOX9 staining on sections of tumors. **(B)** H&E and SOX9 immunostaining on lung sections.

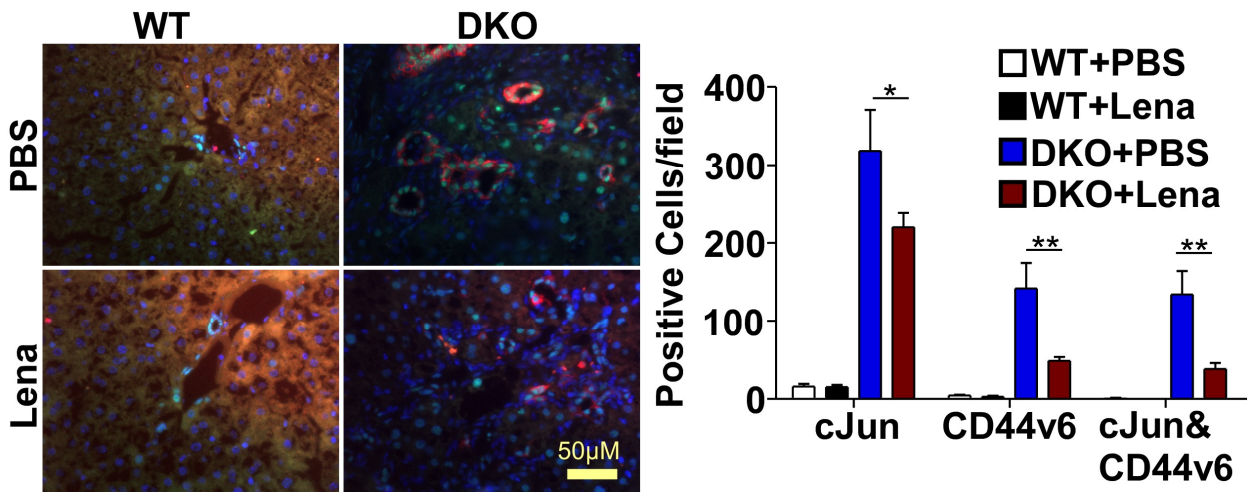


Figure S4. Lenalidomide treatment reduces liver TIC population in *DKO* mice, related to Figure 5. Left, representative co-immunostaining of CD44v6/cJun on liver sections. Right, quantification of cJun, CD44v6 and cJun/CD44v6 positive cells (n=3). Lena: lenalidomide. Data are shown as means ± S.D.. * indicates $p<0.05$. ** indicates $p<0.01$.

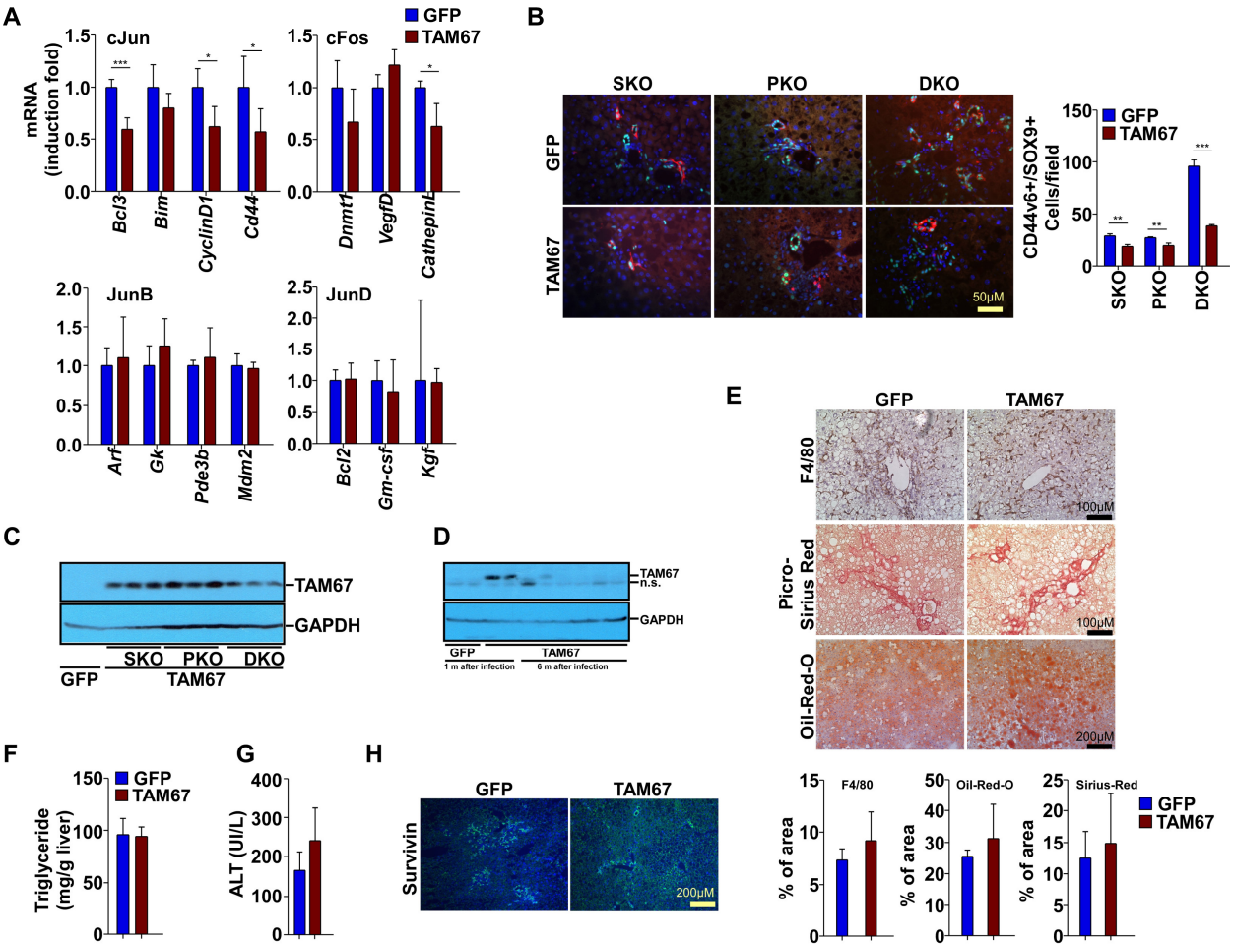
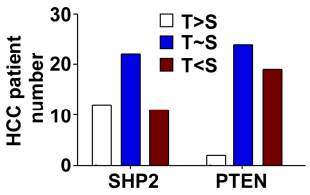


Figure S5. TAM67 overexpression does not suppress NASH in *DKO* mice, related to Figure 6. (A) mRNA levels of cJun, cFos, JunB and JunD downstream targets were determined by qPCR and compared between mice infected with AAV-GFP or AAV-TAM67 (n = 4). **(B)** Left, representative co-immunostaining of CD44v6/SOX9 on liver sections from *SKO*, *PKO* and *DKO* mice infected with AAV-GFP or AAV-TAM67. Right, quantification of CD44v6/SOX9 positive cells (n=4). **(C, D)** TAM67 expression was determined by immunoblot analysis of liver lysates. **(E)** Top, immunostaining for F4/80, Picro-Sirius Red and Oil-Red-O staining were performed on liver sections from mice infected with AAV-GFP or AAV-TAM67. Bottom, quantification of percentage of positive F4/80, Sirius Red, and Oil-Red-O staining area (n=5). **(F, G)** Liver triglyceride and serum ALT levels were determined and compared between mice infected with AAV-GFP or AAV-TAM67 (n=6). **(H)** Immunostaining for Survivin was performed on liver sections from mice infected with AAV-GFP or AAV-TAM67. Data are shown as means ± S.D.. * indicates $p<0.05$. ** indicates $p<0.01$. *** indicates $p<0.001$.

A



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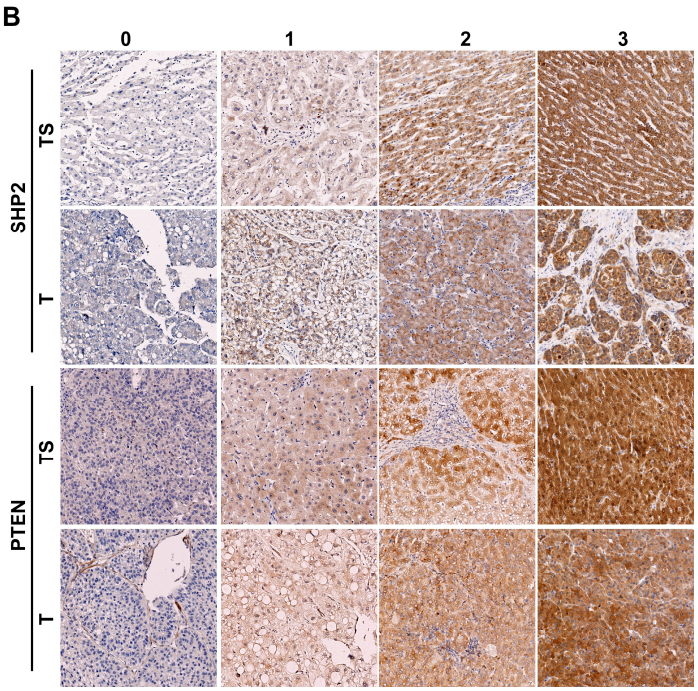

C

Patient Number(tumor tissue)		PTEN staining score		
		[0,1]	(1,2]	(2,3]
SHP2 staining score	[0,1]	173	45	0
	(1,2]	56	44	7
	(2,3]	5	4	1

Patient Number(tumor-surrounding tissue)		PTEN staining score		
		[0,1]	(1,2]	(2,3]
SHP2 staining score	[0,1]	133	55	17
	(1,2]	69	42	9
	(2,3]	4	4	2

Figure S6. Analysis of SHP2 and PTEN expression in HCC patients, related to Figure 7. (A) Expression of SHP2 and PTEN was analyzed by immunostaining and compared in 45 pairs of human HCC and tumor-surrounding tissuearray samples. Liver cancer tissue array (LV1504) was purchased from US Biomax (T: tumor; S: tumor-surrounding tissue). **(B)** Tissue microarray (TMA) of paired human HCC tumor and tumor-surrounding tissue were stained for SHP2 or PTEN and scanned with Hamamatsu Slide Scanner (Microscopy Core, UCSD) (20X objective was used). Representative TMA images are shown here. Score: 0: negative staining; 1: weak staining; 2: moderate staining; 3: strong staining. **(C)** 3x3 matrix representation of PTEN and SHP2 status in tumor and tumor surrounding tissues.

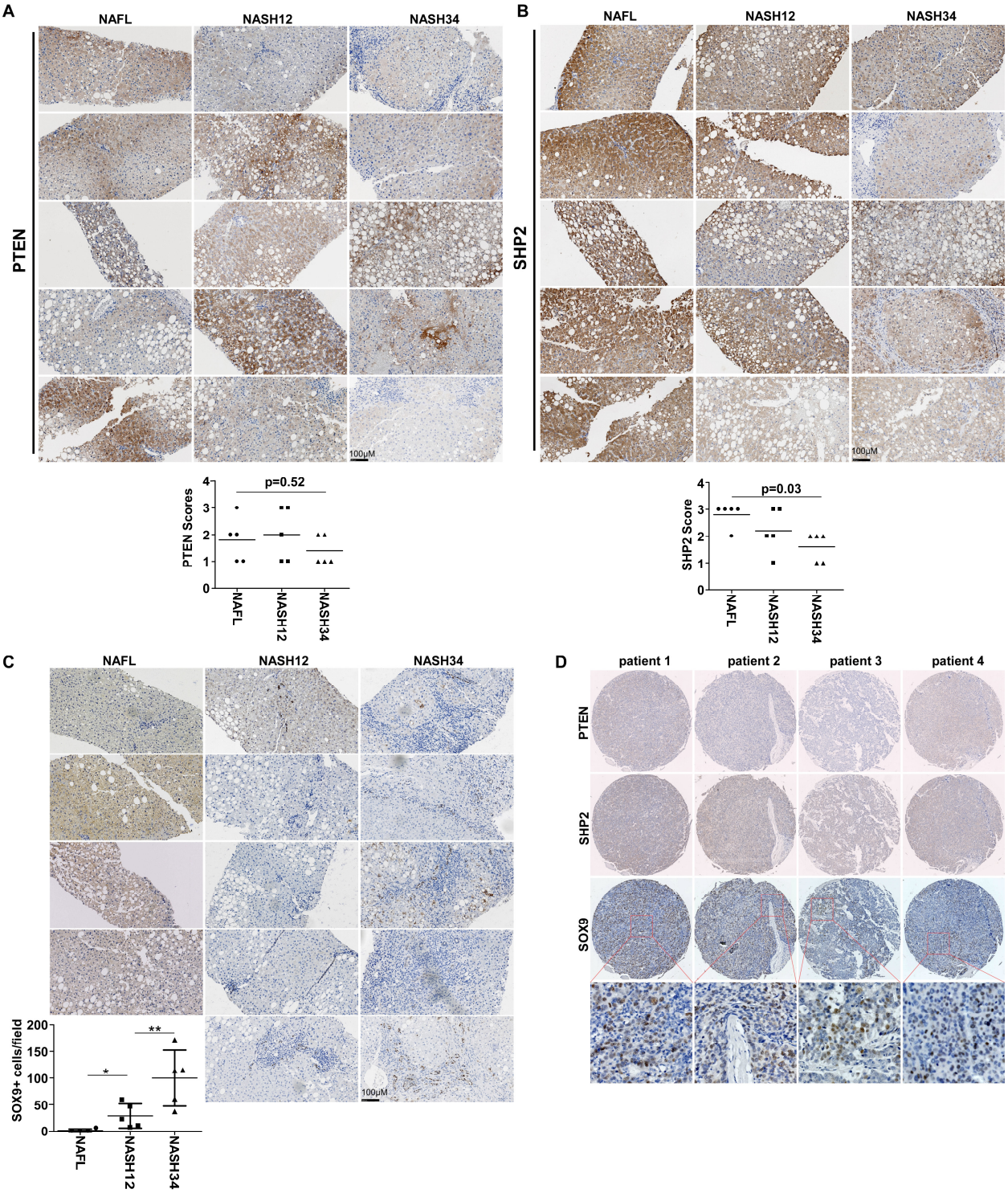

Figure S7. Analysis of SHP2, PTEN and SOX9 expression in NASH or HCC patients, correlated to Figure 7. **(A-B)** Expression of PTEN **(A)** and SHP2 **(B)** was analyzed by immunostaining and compared in NAFL, NASH12 (less severe NASH patients) and NASH34 (more severe NASH patients) human patients (n=5). Representative images are shown here. Scores from 0 to 3 were given to each sample with 0: negative staining; 1: weak staining; 2: moderate staining; 3: strong staining. Data are shown as means ± S.D.. p-value was calculated using one-way ANOVA. **(C)**Expression of SOX9 was analyzed by immunostaining and compared in NAFL, NASH12 and

NASH34 (NAFL: simple steatosis; NASH12 with stage 1-2 fibrosis; and NASH34: NASH with stage 3-4 fibrosis) human patients (n=4~5). Data are shown as means ± S.D.. *, $p<0.05$. **, $p<0.01$. **(D)** Expression of SHP2, PTEN and SOX9 was analyzed by immunostaining and compared in 350 pairs of human HCC samples.

See also Table S6.

Table S1, related to Figure 1,Tumor incidences in mice of various genotypes

Genotype	Age (mo) of	NO. of mice	Tumor
WT	1-18	60	Not observed
SKO	1-11	37	Not observed
	12	6	HCA (2)
	18	7	HCA (6)
PKO	1-3	13	Not observed
	4-6	15	Not observed
	7	12	Bile ductal hyperplasia (6);HCA (3); ICC (1)
	9	7	HCA (4);ICC (4)
	12	11	HCA (11);ICC (11)
	13-16	7	HCA (7); ICC (7); HCC (3);HCC/ICC (2)
DKO	1-2	11	Not observed
	4-5	9	HCA (6); HCC (2)
	7	10	HCA (10); ICC (2); HCC (6);HCC/ICC (2)
	12	11	HCA (11);ICC (9); HCC (9);HCC/ICC (9);lung metastasis (3)

Table S2 , related to Figure 5,Oncogene with altered expression

ILMN_Gene	Fold change	P-value
cJun	**2.55**	**0.005**
Kras	1.33	0.009
Nras	1.58	0.009
Lmo2	3.83	0.018
Tpr	1.74	0.021
Bcl2	1.32	0.042
Pparg	1.16	0.043
Mdm2	1.06	0.047
Ccnd1	2.29	0.047

Table S3 , related to Figure 7, HCC patients information

Characteristics	Parameters	Number		P-Value	Statistical Method
		$SHP2^{Low}$	$SHP2^{High}$		
SHP2 staining score	Mean (SD)	0.65 (0.42)	1.73 (0.40)	1.94E-72	Two-tailed Student's t test
Overall Survival Time (Months)	Mean (SD)	34.3 (14.4)	38.2 (12.8)	0.0126	
Disease-Free Survival Time (Months)	Mean (SD)	29.3 (19.8)	34.2 (19.6)	0.0301	
Sex distribution	Male	193	104	0.5995	χ2 tests
	Female	37	16		
Age (years)	Range	10--78	16--79	0.4949	
	Median	53	53		
	Mean (SD)	52.28 (11.29)	53.17 (12.00)		
HBV	N/A	6	3	0.9996	
	HBV (-)	33	18		
	HBV (+)	191	99		
Cirrhosis	Cirrhosis (-)	24	16	0.5274	
	Cirrhosis (+)	206	104		
TNM stage	T1	151	91	0.1463	
	T2	73	27		
	T3	6	2		
Differentiation stage	N/A	1	0	0.3876	
	Grade1	3	3		
	Grade2	162	92		
	Grade3	63	24		
	Grade4	1	1		
Tumor Number	N/A	4	1	0.9864	
	Single	201	105		
	Multiple	25	14		
Tumor Size (cm)	Mean (SD)	5.6 (3.7)	4.6 (3.2)	0.0072	Two-tailed Student's t test
AFP (ng/ml)	Mean (SD)	6773 (16409)	2672 (10421)	0.0133	
ALT(UI/L)	Mean (SD)	65.6 (110.7)	45.9 (38.4)	0.06	

Table S4 , related to Figure 7, HCC patients information					
Characteristics	**Parameters**	**Number**		**P-Value**	**Statistical Method**
		$PTEN^{Low}$	**$PTEN^{High}$**		
PTEN staining score	Mean (SD)	0.16 (0.24)	1.34 (0.50)	3.52E-93	Two-tailed Student's t test
Overall Survival Time (Months)	Mean (SD)	33.6 (14.5)	40.2 (11.3)	6.79E-05	
Disease-Free Survival Time (Months)	Mean (SD)	28.8 (17.9)	36.3 (23.6)	0.0013	
Sex distribution	Male	193	91	0.1061	χ2 tests
	Female	41	10		
Age (years)	Range	13--79	16--79	0.3112	
	Median	53	52		
	Mean (SD)	53.1 (11.1)	51.7 (11.6)		
HBV	N/A	8	1	1	
	HBV (-)	35	15		
	HBV (+)	191	85		
Cirrhosis	Cirrhosis (-)	26	14	0.5869	
	Cirrhosis (+)	208	87		
TNM stage	T1	157	75	0.345	
	T2	71	25		
	T3	6	1		
Differentiation stage	N/A	1	0	0.0251	
	Grade1	1	5		
	Grade2	170	74		
	Grade3	60	22		
	Grade4	2	0		
Tumor Number	N/A	3	2	0.7973	
	Single	207	87		
	Multiple	24	12		
Tumor Size (cm)	Mean (SD)	5.4 (3.7)	5.0 (3.3)	0.407	Two-tailed Student's t test
AFP (ng/ml)	Mean (SD)	6020 (15584)	2957 (10817)	0.0733	
ALT (UI/L)	Mean (SD)	63.5 (104.6)	49.1 (66.2)	0.2	

Table S5, related to Figure 7, HCC patients information

Table S6 , related to Figure S7, NAFL and NASH patients information

Characteristics	Parameters	Number			P-Value	Statistical Method
		NAFL	NASH12	NASH34		
Sex distribution	Male	3	2	0	0.1225	χ2 tests
	Female	2	3	5		
Age (years)	Range	33--65	35--66	53--65	0.3462	One-way ANOVA
	Median	47	61	60		
	Mean (SD)	48.4 (12.5)	52 (15.2)	59.4 (4.4)		
BMI	Mean (SD)	30.8 (4.1)	31.6 (4.0)	29.5 (5.8)	0.785	
ALT (UI/L)	Mean (SD)	41.8 (19.5)	39.4 (22.1)	45 (21.45)	0.9155	
Serum glucose (mg/dL)	Mean (SD)	94.8 (13.8)	100.2 (24.4)	152 (77.9)	0.1551	
Serum insulin (mIU/L)	Mean (SD)	17.4 (6.3)	23.6 (6.5)	27.2 (15.3)	0.3447	
Serum triglycerides (mg/dL)	Mean (SD)	183.6 (74.6)	149.4 (55.8)	142.5 (91.2)	0.6665	

Table S7, List of primers and antibodies