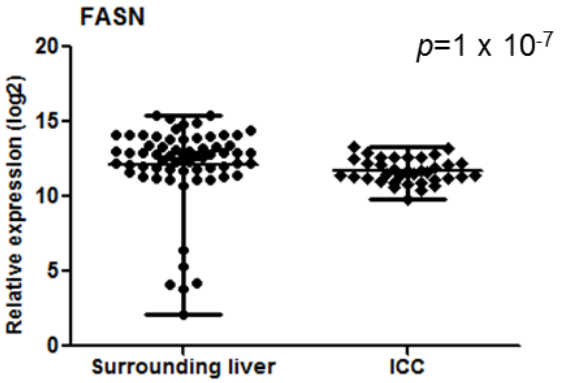
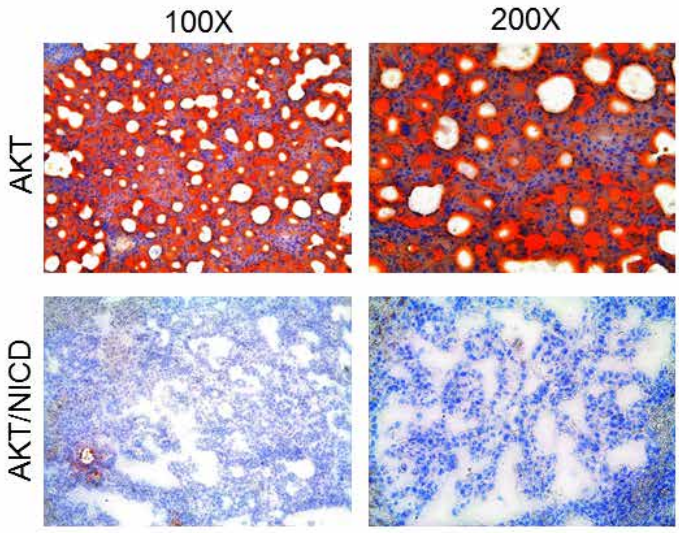
Supplementary Table 1. Clinicopathological features of intrahepatic cholangiocarcinoma (ICC) patients

Variables	
No. of patients	45
Male	35
Female	10
Etiology	
HBV	5
HCV	11
Hepatolithiasis	10
PSC	4
NA	15
Tumor differentiation	
Well	18
Moderately	15
Poorly	12

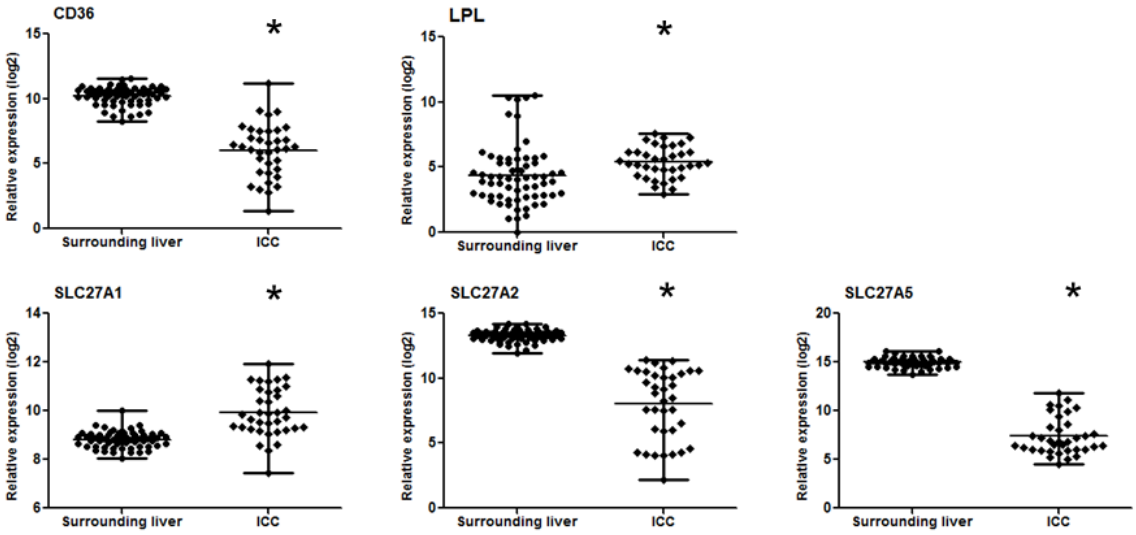
Abbreviation: NA, not available; PSC, primary sclerosing cholangitis



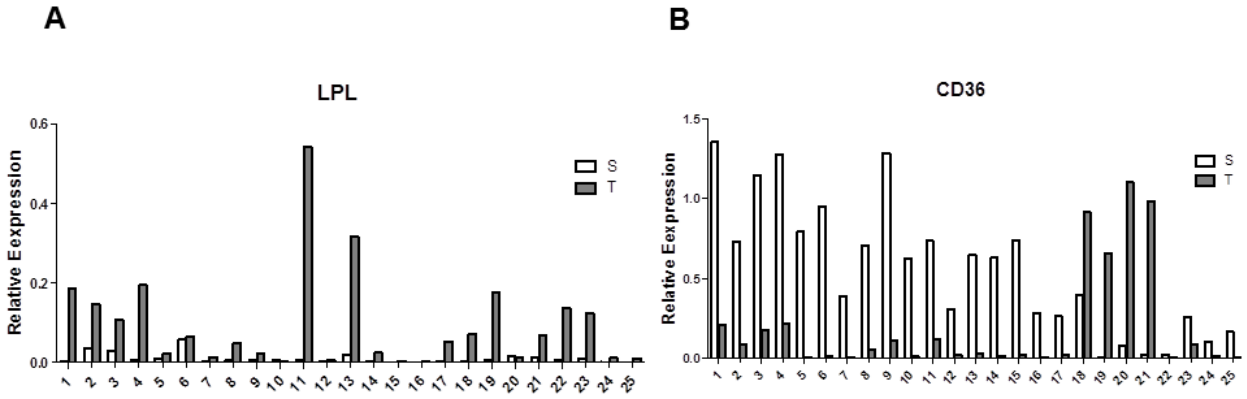
Supplementary Figure 1. Expression levels of FASN mRNA in human non-tumorous surrounding liver and ICC tissues samples. Data were retrieved from the TCGA database.



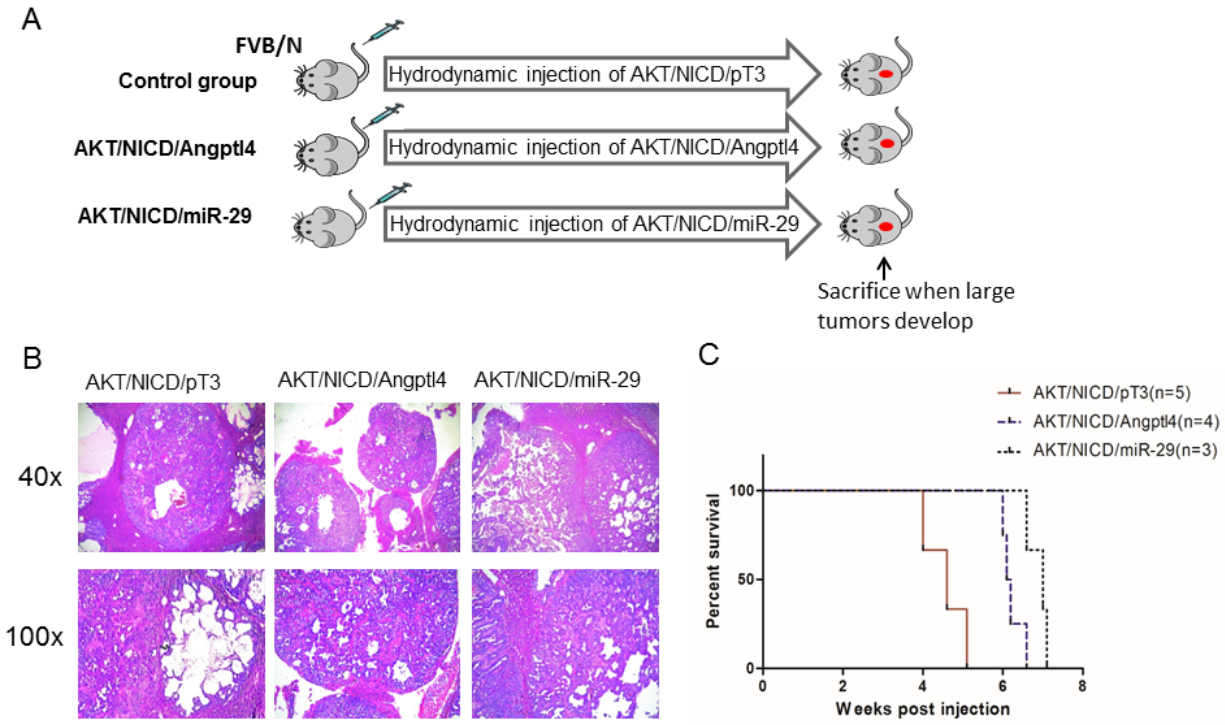
Supplementary Figure 2. Patterns of lipid storage in liver lesions from AKT and AKT/NICD mice. Oil Red O staining reveals extensive lipid accumulation in AKT injected liver tissues (upper panel), but not in AKT/NICD ICC lesions (lower panel).



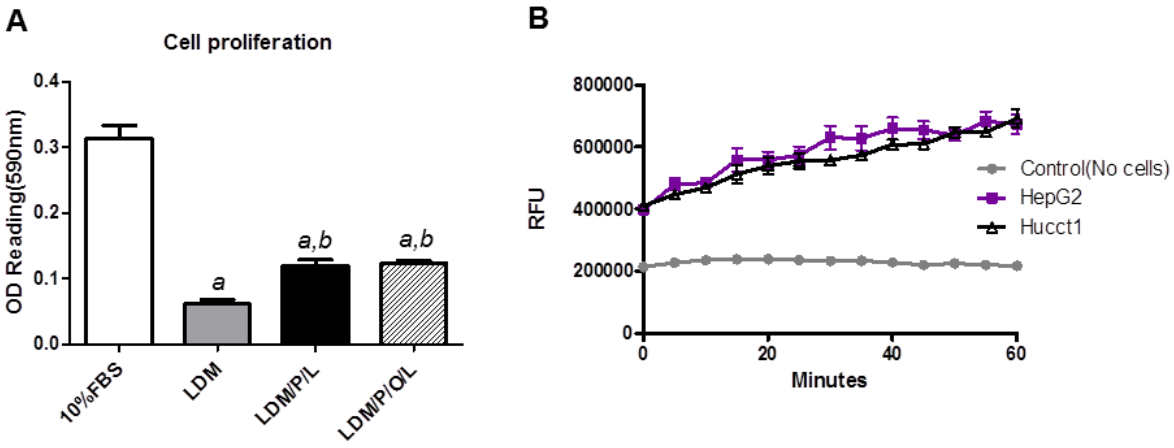
Supplementary Figure 3. Dot plot of mRNA expression of CD36, LPL, SLC27A1, SLC27A2, and SLC27A5 in human non-tumorous surrounding liver and ICC tissues samples. Data were retrieved from the TCGA database. *: $p<0.001$



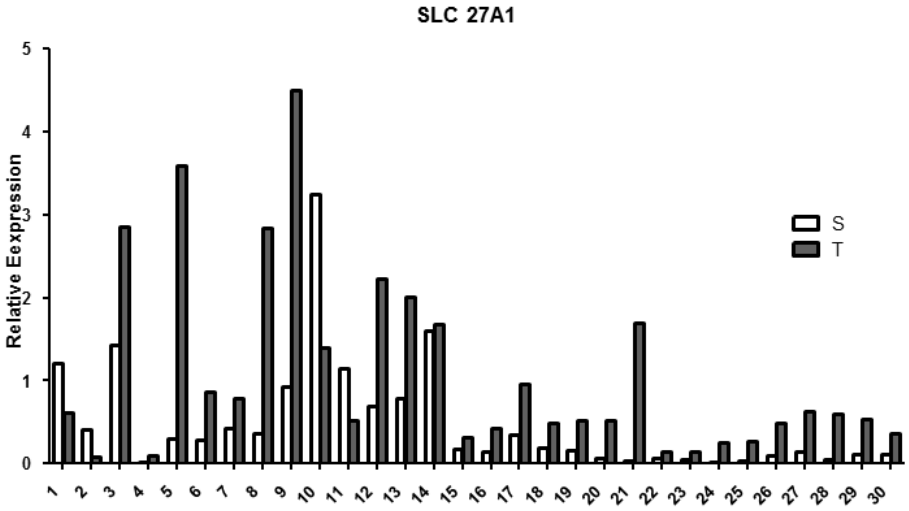
Supplementary Figure 4. Levels of LPL and CD36 mRNA in human ICC samples. Relative expression of LPL (A) and CD36 (B) in 25 paired ICC (T) and non-tumorous surrounding liver tissues (S) was analyzed by qRT real-time PCR.



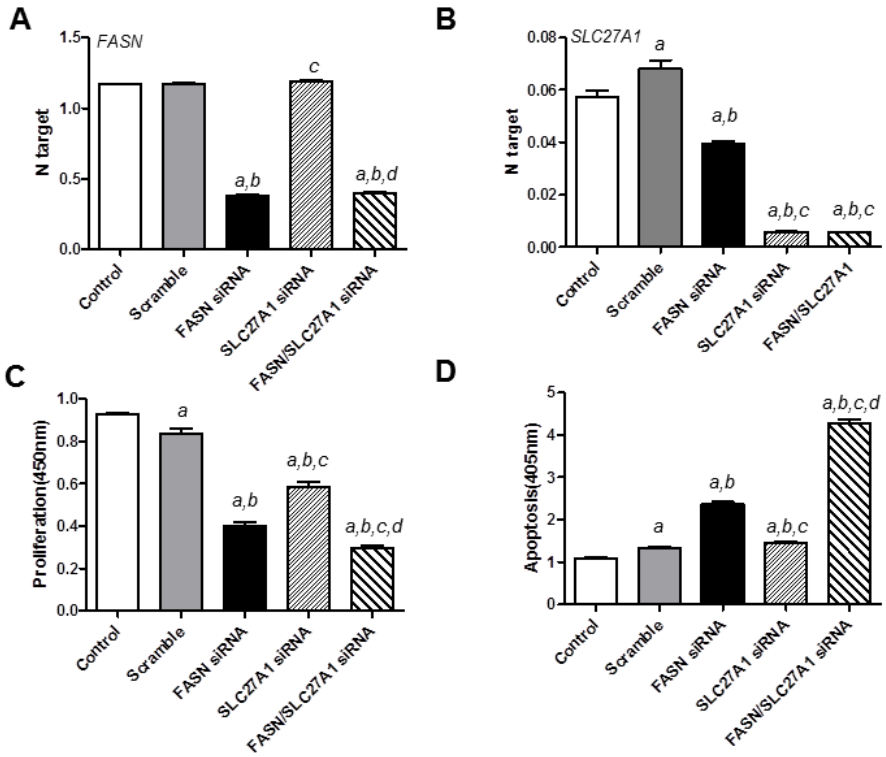
Supplementary Figure 5. Inhibition of LPL by overexpression of Angptl4 or miR-29 has limited effects on AKT/NICD driven ICC formation in mice. (A) Study design; (B) H&E images and (C) survival analysis of AKT/NICD/pT3, AKT/NICD/Angptl4, and AKT/NICD/miR-29 induced ICC in mice.



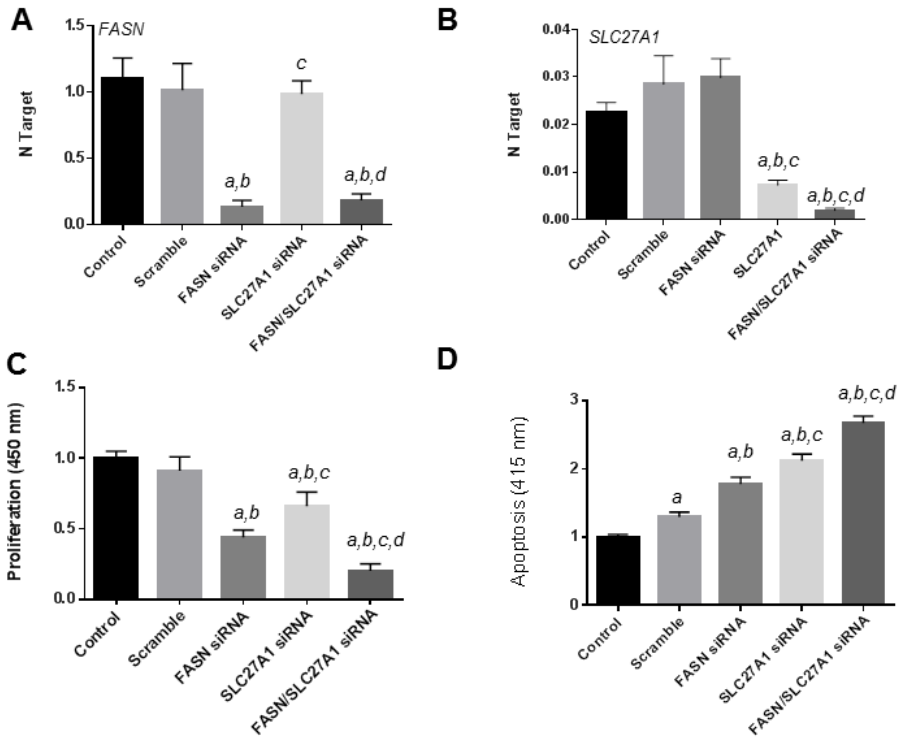
Supplementary Figure 6. (A) Requirement of exogenous fatty acids for the growth of HUCCT1 human ICC cells. Cell growth of HUCCT1 cells in the presence of 10% Fetal Bovin Serum (FBS) medium, or 10% lipoprotein depleted FBS medium (LDM). The growth inhibition effects of LDM can be partially rescued by adding exogenous fatty acids. P/L: Palmitic acid + Linoleic Acid. P/O/L: Palmitic acid + Oleic acid + Linoleic Acid. Student's T-test $p<0.05$, a) versus 10% FBS; and b) versus LDM. (B) Fatty Acid uptake assay in HUCCT1 and HepG2 cells using fluorescent fatty acid analogue.



Supplementary Figure 7. SLC27A1 expression is upregulated in human ICC samples (p<0.01). Relative expression of SLC27A1 in 30 paired ICC (T) and non-tumorous surrounding liver tissues (S) was analyzed by qRT real-time PCR.



Supplementary Figure 8. Silencing of SLC27A1 synergizes with FASN inhibition to suppress the growth of HUCCT1 human ICC cell line. (A,B) qRT-PCR analysis of FASN (A) and SLC27A1 (B) expression. (C) Cell proliferation and (D) apoptosis in HUCCT1 cells untreated (control) and transfected with scramble siRNA, FASN siRNA, SLC27A1 siRNA or FASN/SLC27A1 siRNA. Student's T-test at least $p<0.05$, a) versus control; b) versus scramble siRNA; c) versus FASN siRNA; d) versus SLC27A1 siRNA.



Supplementary Figure 9. Silencing of SLC27A1 synergizes with FASN inhibition to suppress the growth of HUH28 human ICC cell line. (A,B) qRT-PCR analysis of FASN (A) and SLC27A1 (B) expression. (C) Cell proliferation and (D) apoptosis in HUCCT1 cells untreated (control) and transfected with scramble siRNA, FASN siRNA, SLC27A1 siRNA or FASN/SLC27A1 siRNA. Student's T-test at least $p<0.05$, a) versus control; b) versus scramble siRNA; c) versus FASN siRNA; d) versus SLC27A1 siRNA.

Supplemental Materials and Methods

Hydrodynamic injection and mouse monitoring

FASN$^{fl/fl}$ mice in C57BL/6 background were described previously.[28,29] *CD36*$^{-/-}$ mice in C57BL/6 background were used as previously described.[30] *AlbCre* mice in C57BL/6 background[31] were obtained from the Jackson Laboratory(Bar Harbor, ME). *FASN*$^{fl/fl}$ mice were crossed with *AlbCre* mice to eventually generate liver specific *FASN* knockout mice, *AlbCre;FASN*$^{fl/fl}$ line. All male and female mice were used in the study, and no difference was noticed when using either male or female mice in all the studies. Hydrodynamic transfection was performed as described.[32] To delete *FASN* in AKT/NICD along ICC development, two approaches were used. In the first method, 4μg AKT, 20μg NICD1, and 20μg Cre were mixed with 1.76μg pCMV-SB in 2ml of saline (experimental group). 4μg AKT, 20μg NICD1, and 20μg pT3EF1α (empty vector) were instead mixed with 1.76μg pCMV-SB in 2 ml of saline (control). Saline solution was injected into the lateral tail vein of 8 week old *FASN*$^{fl/fl}$ mice (n=5). In the second method, 4μg AKT and 20μg NICD1 plasmids were mixed with 0.96μg pCMV-SB and hydrodynamically injected into 12 week old *AlbCre;FASN*$^{fl/fl}$ (n=5) or *FASN*$^{fl/fl}$ mice (n=5). Mice were harvested 4 to 6 weeks post injection, when they become moribund. To delete *FASN* along AKT/Ras driven liver tumor development, 4μg AKT, 4μg Ras, and 20 μg Cre were mixed with 1.2μg pCMV-SB in 2 ml of saline. 4μg AKT, 4μg Ras, and 20μg pT3EF1α (empty vector) were mixed with 1.2μg pCMV-SB in 2ml of saline). Saline solution was injected into the lateral tail vein of 8 week old *FASN*$^{fl/fl}$ mice (n=5). To study whether AKT/NICD driven ICC development requires CD36, *CD36*$^{-/-}$ mice as well as *CD36*$^{+/+}$ littermates were used. 4μg AKT and 20μg NICD1 were mixed with 0.96μg pCMV-SB in 2ml of saline, and injected into 6 weeks old *CD36*$^{-/-}$ mice (n=5) as well as *CD36*$^{+/+}$ mice (n=5). To study whether miR-29 or Antgpl4 could inhibit AKT/NICD driven ICC development, FVB/N mice were used. 4μg AKT, 20μg NICD1, and 20μg Angptl4 were mixed with 1.76μg

pCMV-SB in 2ml of saline (AKT/NICD/Agnptl4 group). 4μg AKT, 20μg NICD1, and 20μg miR-29 were mixed with 1.76μg pCMV-SB in 2ml of saline (AKT/NICD/miR-29 group). 4μg AKT, 20μg NICD1, and 20μg pT3-EF1α empty vector were mixed with 1.76μg pCMV-SB in 2ml of saline (AKT/NICD/pT3 control group). The plasmid mixture was hydrodynamically injected into 6 to 8 weeks old FVB/N mice. All mice were housed, fed, and monitored in accordance with protocols approved by the Committee for Animal Research at the University of California, San Francisco.

Immunohistochemistry

Immunohistochemical staining on mouse and human liver tissue specimens was performed on 4% paraformaldehyde-fixed and paraffin-embedded sections. Deparaffinized sections were incubated in 3% H_2O_2 dissolved in 1X phosphate-buffered saline (PBS) for 30 minutes to quench the endogenous peroxidase. For antigen retrieval, slides were microwaved in 10 mmol/L citrate buffer (pH 6.0) for 12 minutes. The primary antibodies used are listed in Supplementary Table 1. The immunoreactivity was visualized with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine as the chromogen. Slides were counterstained with hematoxylin.

Protein extraction and Western blot analysis

Human and mouse liver specimens were homogenized in lysis buffer [30 mM Tris (pH 7.5), 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol, and 2 mM EDTA] containing the Complete Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Indianapolis, IN), and sonicated. Protein concentrations were determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) using bovine serum albumin as standard. Aliquots of 40 μg were denatured by boiling in Tris-Glycine SDS Sample Buffer (Invitrogen), separated by SDS-PAGE, and transferred onto nitrocellulose

membranes (Invitrogen) by electroblotting. Membranes were blocked in 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h and probed with specific antibodies (Supplementary Table 1). Each primary antibody was followed by incubation with horseradish peroxidase-secondary antibody diluted 1:5000 for 1 h and then revealed with the Super Signal West Pico (Pierce Chemical Co., New York, NY). Equal loading was assessed by reversible Ponceau Red staining (Sigma-Aldrich) and GAPDH immunoblotting.

Quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR)

Validated Gene Expression Assays for human *FASN* (ID: Hs01005622_m1), *Slc27A1* (ID: Hs01587917_m1), *CD36* (ID: Hs00169627_m1), *LPL* (Hs00173425_m1) and *β-Actin* (ID: 4333762T) were purchased from Applied Biosystems (Foster City, CA). PCR reactions were performed with 100 ng of cDNA on the whole sample collection and cell lines, using an ABI Prism 7000 Sequence Detection System and TaqMan Universal PCR Master Mix (Applied Biosystems). Cycling conditions were: 10 min of denaturation at 95°C and 40 cycles at 95°C for 15 s and at 60°C for 1 min. Quantitative values were calculated by using the PE Biosystems Analysis software and expressed as N target (NT). NT = $2^{-\Delta Ct}$, wherein ΔCt value of each sample was calculated by subtracting the average Ct value of the target gene from the average Ct value of the *RNR-18* gene

Preparation of primary mouse hepatocytes and ICC cells and FACS based fatty acid uptake assays

The primary liver cell suspension was generated as describe in our previous publication.[34] The primary liver cells were subsequently resuspended in 3 mL of a 37°C fatty acid uptake assay solution [2 μM Bodipy 3823 in 1X HBSS with 0.1% FFA-free BSA]. At prescribed time points, 500 μL of the cell suspension were transferred into 500

μL of a 4°C FFA-uptake stop solution [2 μg/mL propidium iodide (PI) in 1X HBSS]. The cells were then resuspended in a FACS buffer containing 0.5% BSA, 0.1% sodium azide in 1X PBS. Fluorescence activated cell sorting was preformed to determine the relative green fluorescent signal (bodipy tagged fatty acid analogue) in non-PI stained cells. At least 10k live cells were evaluated for green fluorescence at each time point. [35,36]

***In vitro* experiments**

The HUCCT1 and Huh28 human ICC cell lines were used for the *in vitro* study. Cells were maintained as monolayer cultures in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. For knockdown studies, cells were transfected with 50 pmol of scramble small interfering RNA (siRNA; ID # 4390846) or siRNA directed against human FASN (ID # s5032; Life Technologies, Grand Island, NY) and/or Slc27A1 (ID # L-010759-01-0005; GE Dharmacon, Lafayette, CO) according to the manufacturer's recommendations, and incubated for 48 hours. Proliferation and apoptosis were assessed using the BrdU Cell Proliferation Reagent (Cell Signaling Technology, Danvers, MA) and the Cell Death Detection Elisa Plus Kit (Roche Molecular Biochemicals, Indianapolis, IN), respectively, following the manufacturers' protocol. Experiments were repeated at least three times in triplicate.