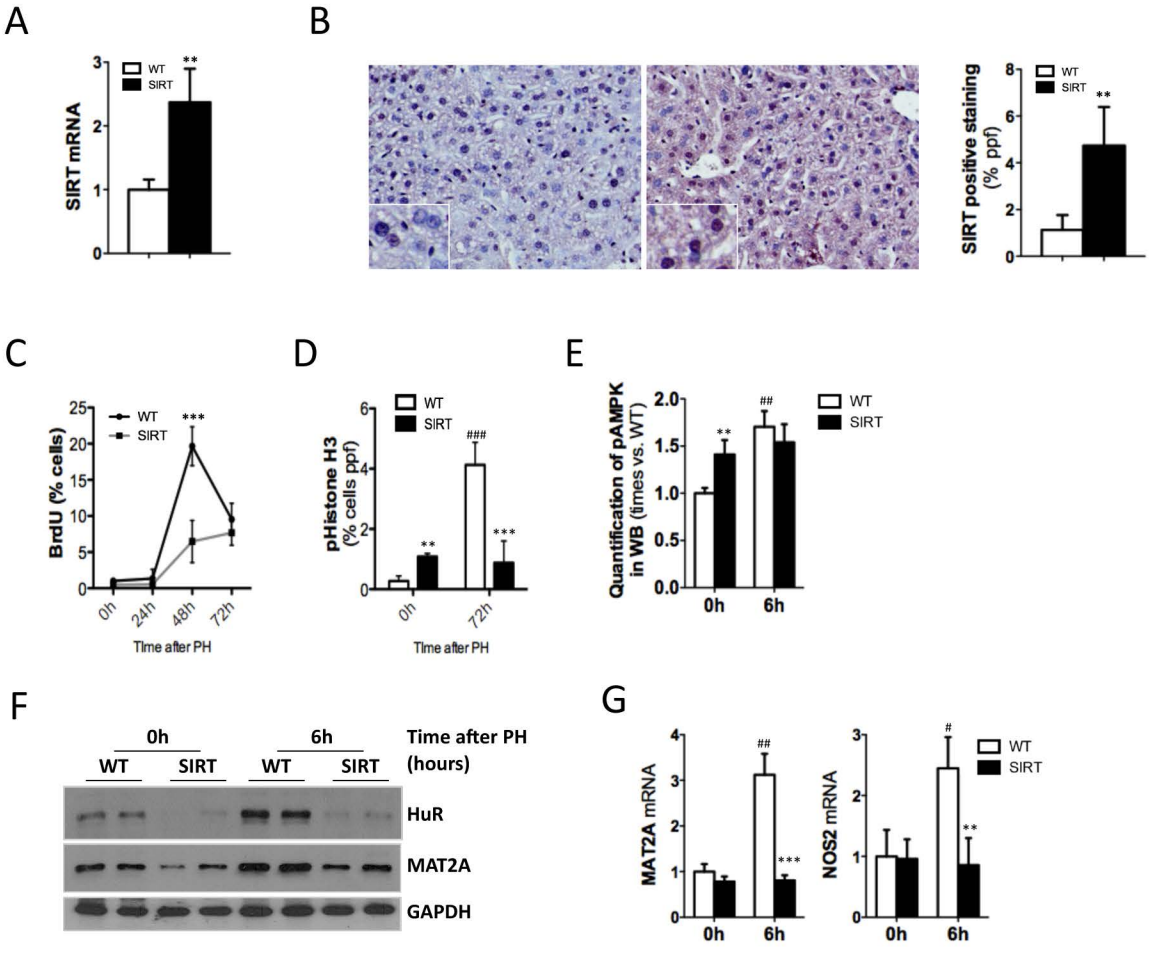
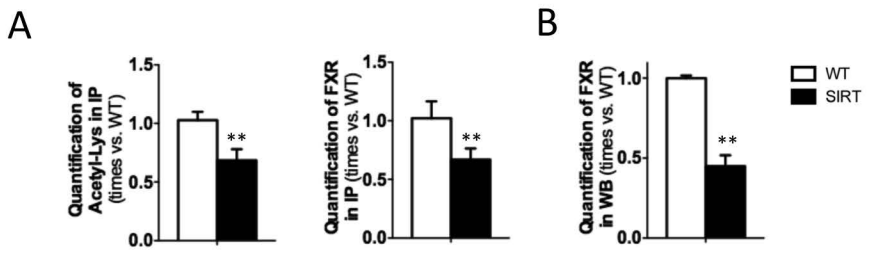


Supplemental Figure 1



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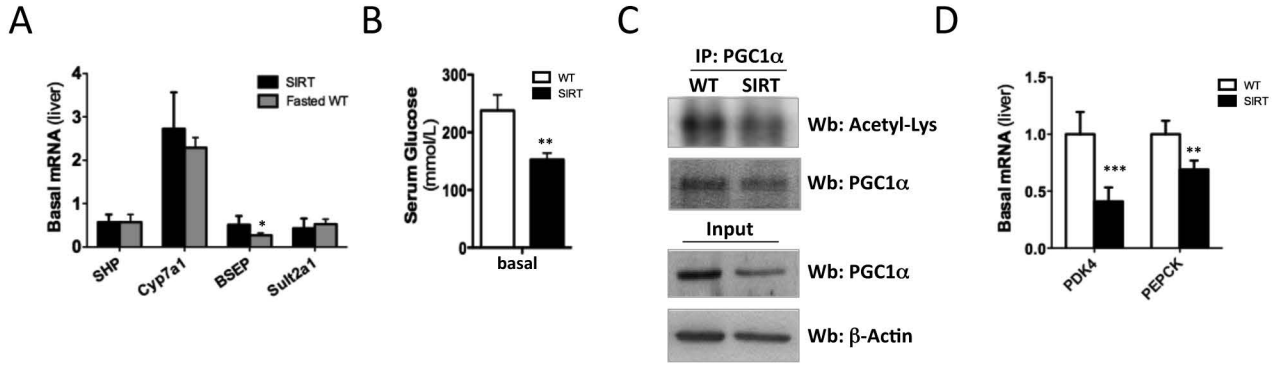
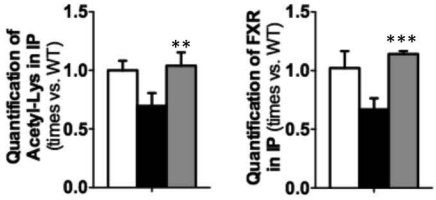


Supplemental Figure 3

A



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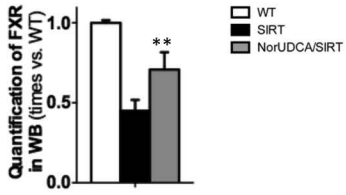

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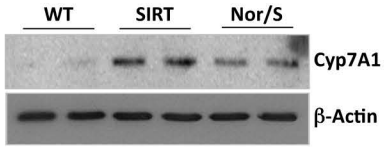
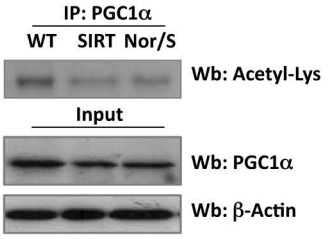
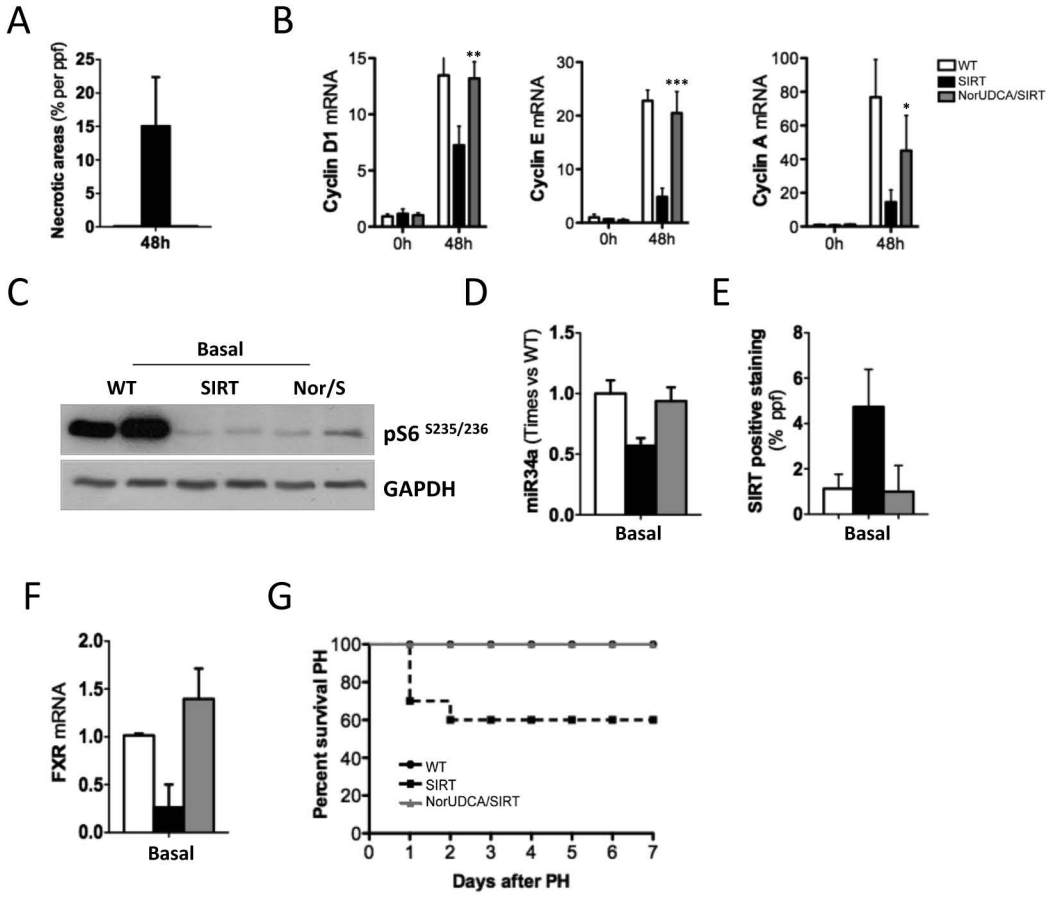


Supplemental Figure 4

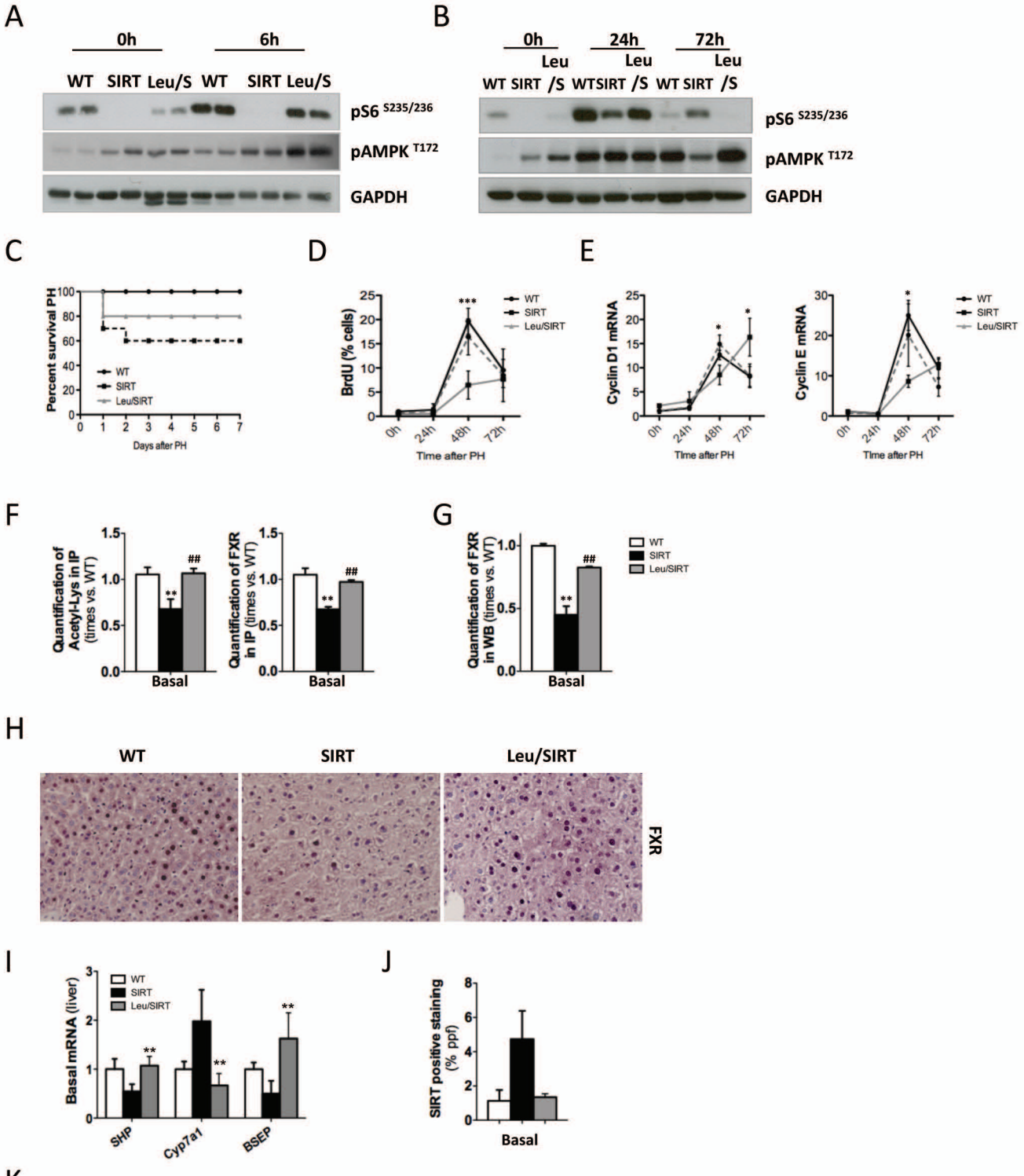
A



Supplemental Figure 5

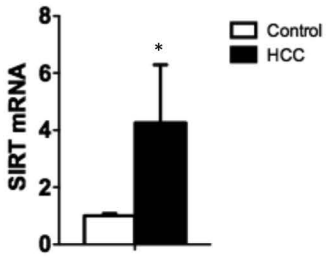


Supplemental Figure 6

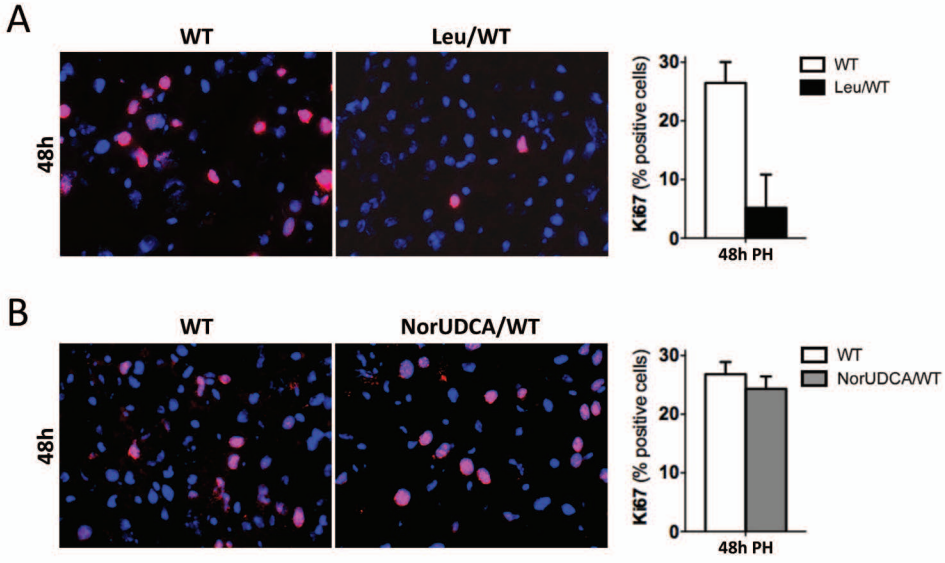


Supplemental Figure 7

A



Supplemental Figure 8



Supplemental Figure 9

BA (nmol/gr)	WT	SIRT
TSLC	0.00±0.00	0.00±0.00
TMC	103.73±21.62	85.5±10.66
TUDC	6.92±1.22	8.15±1.14
THyoC	0.00±0.00	0.00±0.00
TC	176.21±26.07	179.57±20.65
TQDC	5.94±0.89	6.03±1.00
TDC	11.18±1.90	12.68±1.18
TLC	0.20±0.01	0.24±0.04
GUDC	0.01±0.00	0.01±0.00
GC	5.52±2.25	2.21±1.62
GQDC	0.01±1.11	0.01±0.01
GDC	0.00±0.00	0.00±0.00
GLC	0.00±0.00	0.00±0.00
UDC	3.15±0.87	3.60±1.23
aMC	18.71±6.94	11.31±2.38
bMC	21.63±7.59	7.55±0.98
CA	17.08±8.17	12.92±2.37
QDC	0.20±0.03	0.94±0.59
DC	0.32±0.08	3.25±2.08
HyoDC	0.67±0.21	0.80±0.13
LC	0.04±0.02	0.57±0.30

Tauroconjugated BA
Glycoconjugate BA
Free BA

Suppl. Table 1 Bile Acid (BA) composition in livers from WT and SIRT mice at basal conditions. BA were extracted from liver samples and analyzed by high performance liquid chromatography-tandem mass spectrometry

SUPPLEMENTAL MATERIAL AND METHODS

Determination of protein expression

Protein extraction from whole liver was performed as described (1). Nuclear extracts were obtained using the ProteoExtract Subcellular Proteome Extraction kit (Calbiochem). Immunoprecipitation assays were performed in proteins extracted from livers with a buffer containing 1% NP40 using FXR and PGC1a antibodies (Santa Cruz Biotechnologies).

Western blot analyses were performed using as described (1) using the following antibodies: pS6, p-AMPK, and Acetyl Lysine Abs from Cell signaling. pJNK1/2 was obtained from Invitrogen, GAPDH, H3K4me3 and H3K9me3 from Abcam and b-Actin from Sigma Aldrich.

Immunohistochemistry and immunocitochemistry

Parenchymal damage was assessed by H&E staining performed on paraffin-embedded liver sections. Apoptosis was evaluated by TUNEL assay performed on frozen liver sections using the in situ cell death detection kit (Roche) according to manufacturer's instructions.

To determine the mitotic status of hepatocytes IHC was performed using p Histone H3 antibody from Cell signaling.

To determine and quantify proliferation of hepatocytes mice receiver an i.p. injection of Bromodeoxyuridine (BrdU) from Sigma-Aldrich 2 hours before sacrifice. To analyze BrdU incorporation to the DNA we further performed IHC on frozen sections using a anti-BrdU Ab from Roche. Cell proliferation was quantified as % of BrdU positive cells divided by $DAPI^+$ cells.

Determination of oxidative stress

Hepatocytes isolated from WT and SIRT mice were incubated for 1 hour with with 5-(and-6)-chloromethyl-2'-7'-dichlorodihydro-fluorescein diacetate, acetyl ester (DCFH-CA) was from invitrogen.

Quantification of miRNA

Anti-miR-34a, scrambled RNA, and primers for q-RTPCR for measuring miR-34a were purchased from Applied Biosystems. Quantification of miRNAs was performed by qPCR according to the manufacturer‘s protocol (Applied Biosystems).

Human samples

Surgically resected specimens of 20 patients with HCC (10 hepatitis C virus and 10 alcoholic steatohepatitis (ASH)). Healthy human liver samples were used as controls for SIRT and FXR inmunostaining. The Basque Research Biobank (http://www.biobasque.org) provided the data and type of biospecimen used in this Research project.

Statistical analysis. Data are expressed as mean ± standard deviation of the mean. Statistical significance was determined by two-way analysis of variance followed by a Student's t test.

REFERENCES SUPPLEMENTAL MATERIAL

1. Beraza N, Ludde T, Assmus U, Roskams T, Vander Borght S, Trautwein C. Hepatocyte-specific IKK gamma/NEMO expression determines the degree of liver injury. Gastroenterology 2007;132:2504-2517.

SUPPLEMENTAL FIGURE LEGENDS

Suppl Figure 1 Impaired hepatocyte proliferation in SIRT mice (A) SIRT1 mRNA expression by qPCR in WT and SIRT mice at basal conditions. (B) IHC using an anti-SIRT Ab and further quantification using Frida software. (C) Quantification of BrdU positive cells in liver sections after IHC. Results presented in % or BrdU positive cells relative to DAPI stained nuclei. (D) Quantification of IHC using a phistone H3 Ab represented as % of positive cells *vs* DAPI positive nuclei per power field (ppf). (E) Quantification of western blot analysis of pAMPK expression before and 6h after PH. (F) Western blot analysis using HuR and MAT2A Ab. GAPDH was used as loading control (G) qPCR analysis of MAT2A and NOS2 at 0h and 6h after PH (Values are mean ± SD. n = 4-6 animals/time point; **P* <0.05, ***P* <0.01, ****P*<0.001 [WT *vs* SIRT], ##*P* <0.01, ###*P*<0.001 [WT *vs* WT/PH]).

Suppl Figure 2 Overexpression of SIRT1 correlates with lower acetylation and protein expression of FXR. (A) Quantification of western blot analysis after IP using a FXR Ab and an Acetyl-Lys (left panel) and quantification of western blot analysis using a FXR Ab after IP using a FXR Ab (right panel). (B) Quantification of western blot analysis using a FXR Ab. Values are mean ± SD. n = 4-6 animals/time point; ***P* <0.01 [WT *vs* SIRT]).

Suppl Figure 3 Overexpression of SIRT1 correlates with 'fasting-like' status. (A) Analysis of SHP, Cyp7a1, BSEP and Sult2a1 mRNA expression by qPCR in livers of WT animals after 8h fasting suggesting increased BA

synthesis, low transport and detoxification and an overall ‘fasting like’ status in SIRT mice. (B) Lower glucose serum levels. (C) IP using a PGC1a Ab and further western blot analysis using Acetylated Lysine Ab of liver extracts at basal conditions showing restored acetylation and protein levels of PGC1a in NorUDCA mice. Western blot analysis of nuclear extracts using PGC1a and b-Actin Abs. (D)qPCR analysis showing lower PGC1a target gene expression; PDK4 and PEPCK. (Values are mean ± SD. n = 5 animals/time point; **P <0.01 [WT *vs* SIRT]).

Suppl Figure 4 NorUDCA feeding in SIRT1 overexpressing mice restored FXR acetylation and total protein expression. (A) Quantification of western blot analysis after IP using a FXR Ab and an Acetyl-Lys (left panel) and quantification of western blot analysis using a FXR Ab after IP using a FXR Ab (right panel). (B) Quantification of western blot analysis using a FXR Ab. (C) Western blot analysis showing increased Cyp7A1 expression in SIRT mice that was lower in NorUDCA/SIRT animals. Values are mean ± SD. n = 4-6 animals/time point; **P <0.01 [SIRT *vs* NorUDCA/SIRT]).

Suppl Figure 5 NorUDCA does not restore acetylation and protein expression of PGC1a in SIRT mice (A) IP using a PGC1a Ab and further western blot analysis using Acetylated Lysine Ab of WT, SIRT and NorUDCA/SIRT diet at basal conditions. Western blot analysis of nuclear extracts using PGC1a and b-Actin Abs.

Suppl Figure 6 NorUDCA restores liver regeneration protecting the liver against necrosis and promoting Cyclins mRNA expression in SIRT mice after PH. (A) Quantification of necrotic areas in H&E stained liver sections with Frida software (Johns Hopkins University). Represented in % per power field (ppf). (B) qPCR analysis showing that Cyclin D1, Cyclin E and Cyclin A mRNA expression was restored by NorUDCA in SIRT mice after PH. (Values are mean ± SD. n = 5 animals/time point; *P <0.05, **P <0.01, ***P<0.001 [SIRT *vs* NorUDCA/SIRT]. (C) WB analysis of whole liver protein extracts using a pS6 Ab (D) Analysis of miR34a expression showing restoration of expression in NorUDCA/SIRT mice. (E) qPCR showing that FXR mRNA expression was restored by NorUDCA in SIRT mice after PH. (F) Kaplan Meier curve depicting survival of mice after PH. (Values are mean ± SD. n = 5 animals/time point; *P <0.05, **P <0.01, ***P<0.001 [SIRT *vs* NorUDCA/SIRT].

Suppl Figure 7 Leucine diet restores mTOR signaling in SIRT mice.

(A) pS6 and pAMPK levels in liver extracts analyzed by western blot analysis showing restoration of mTOR-signaling in Leu/SIRT mice at short and (B) longer timepoints after PH. (C) Kaplan Meier curve of SIRT mice fed for 2 weeks with Leu or chow diet after PH. (D) Quantification of BrdU positive cells in liver sections after IHC showing that Leucine-diet restores hepatocyte proliferation in SIRT mice. Results presented in % or BrdU positive cells (red) relative to DAPI stained nuclei (blue). (E) Analysis of mRNA expression of Cyclin D1 and Cyclin E by qPCR confirmed restoration of hepatocyte proliferation in Leu/SIRT mice after PH. (F) Quantification of western blot

analysis after IP using a FXR Ab and an Acetyl-Lys (left panel) and quantification of western blot analysis after IP using a FXR Ab and a FXR Ab (right panel). (G) Quantification of western blot analysis using a FXR Ab. (H) IHC showing restoration of FXR protein expression in Leu/SIRT mice at basal conditions (20x). (I) qPCR showing restoration of SHP, Cyp7a1 and BSEP expression in Leu/SIRT mice. (J) Quantification of SIRT IHC using using Frida software (K) TUNEL assay on isolated hepatocytes from WT, SIRT and Leu/SIRT animals cultured for 24h in serum starvation conditions (s.s.). (Values are mean ± SD. n = 4-6 animals/time point; ***P*<0.01 [WT *vs* SIRT], ##*P*<0.01 [SIRT *vs* Leu/SIRT]). *In vitro* experiments were performed 3 times in duplicate.

Suppl Figure 8 HCC samples from patients show elevated expression of SIRT. (A) qPCR analysis showing increased expression of SIRT mRNA in liver samples from HCC patients. n = 3-6 samples **P*<0.05 [Healthy control *vs* HCC]).

Suppl Figure 9 Leucine and NorUDCA have detrimental effects on healthy WT mice after PH IHC using a Ki67 Ab (left panels) and further quantification (right panels) showing (A) lower proliferation of hepatocytes 48h after PH in Leu/SIRT mice whereas (B) NorUDCA/SIRT show comparable % of proliferating hepatocytes htan WT mice 48h after PH. (Values are mean ± SD. n = 4-6 animals/time point; **P*<0.05, ***P*<0.01 [WT *vs* Leu/SIRT] and [WT *vs* NorUDCA/SIRT]).