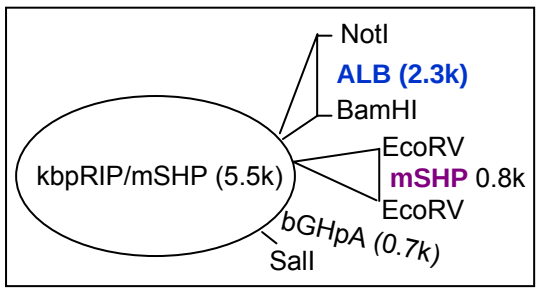
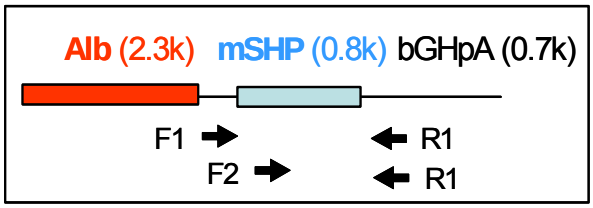
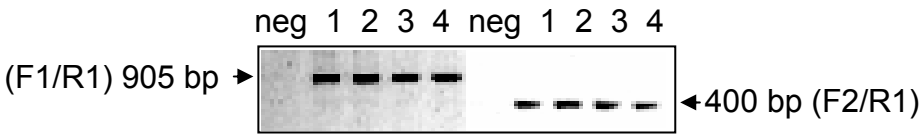
Supp. 1A



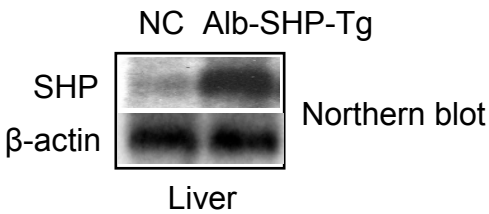
Supp. 1B



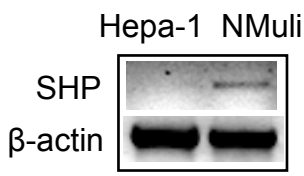
Supp. 1C



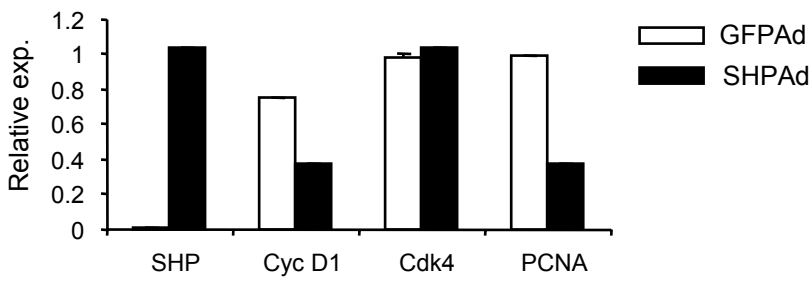
Supp. 1D



Supp. 1E

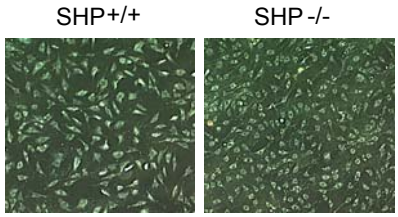


Supp. 1F

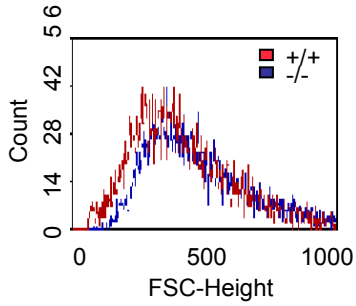


Zhang et al., Supp. Figure 1

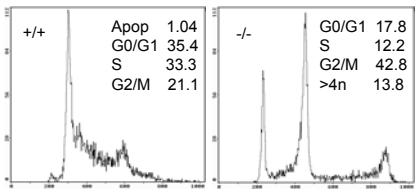
Supp. 2A



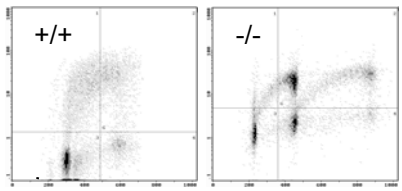
Supp. 2B



Supp. 2C



FACS



Brdu-FITC-FACS

Zhang et al., Supp. Figure 2

A Hydroxyurea (G_0/G_1 arrest)

	0h	3h	8h	12h	24h
SHP$^{+/+}$	G0/G1 84.40 S 4.91 G2/M 10.69	G0/G1 86.62 S 1.38 G2/M 12.00	G0/G1 73.56 S 22.08 G2/M 4.35	G0/G1 63.12 S 26.36 G2/M 10.53	G0/G1 33.32 S 43.64 G2/M 23.04
SHP$^{-/-}$	G0/G1 83.24 S 13.80 G2/M 2.97	G0/G1 85.65 S 11.15 G2/M 3.20	G0/G1 85.89 S 11.44 G2/M 2.67	G0/G1 43.61 S 56.39 G2/M 0.00	G0/G1 9.87 S 61.34 G2/M 28.79

B Double thymidine (G_1/S arrest)

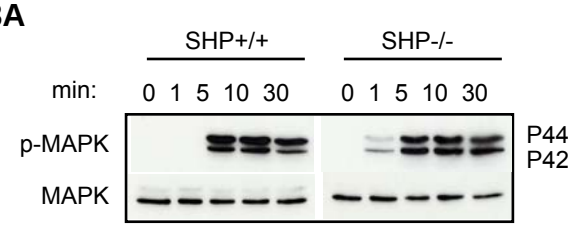
	0h	3h	8h	12h	24h
SHP$^{+/+}$	G0/G1 50.06 S 35.16 G2/M 14.78	G0/G1 72.06 S 15.17 G2/M 12.77	G0/G1 76.60 S 11.69 G2/M 11.70	G0/G1 80.24 S 6.85 G2/M 12.91	G0/G1 82.20 S 10.14 G2/M 7.66
SHP$^{-/-}$	G0/G1 20.17 S 68.50 G2/M 11.33	G0/G1 15.05 S 66.21 G2/M 18.74	G0/G1 19.47 S 53.59 G2/M 26.94	G0/G1 37.46 S 41.18 G2/M 21.36	G0/G1 43.63 S 32.76 G2/M 23.61

C Nocodazole (G_2/M arrest)

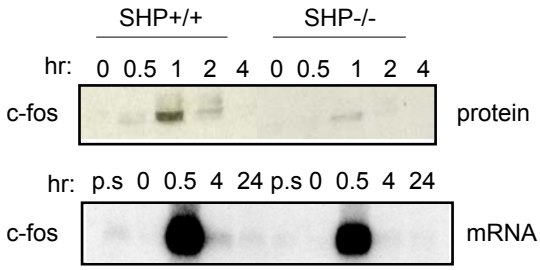
	0h	3h	8h	12h	24h
SHP$^{+/+}$	G0/G1 35.15 S 39.22 G2/M 25.63	G0/G1 32.89 S 33.17 G2/M 33.94	G0/G1 56.45 S 11.24 G2/M 32.31	G0/G1 61.53 S 22.0 G2/M 16.47	G0/G1 77.17 S 9.35 G2/M 13.47
SHP$^{-/-}$	G0/G1 25.14 S 32.06 G2/M 42.81	G0/G1 26.26 S 23.68 G2/M 50.05	G0/G1 54.96 S 11.75 G2/M 33.29	G0/G1 54.35 S 30.52 G2/M 15.13	G0/G1 80.99 S 11.59 G2/M 7.42

Zhang et al., Supp. Table 1

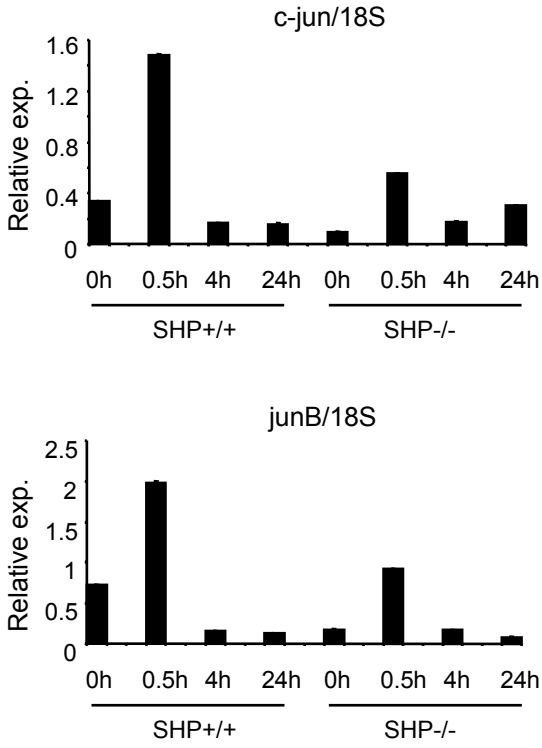
Supp. 3A



Supp. 3B

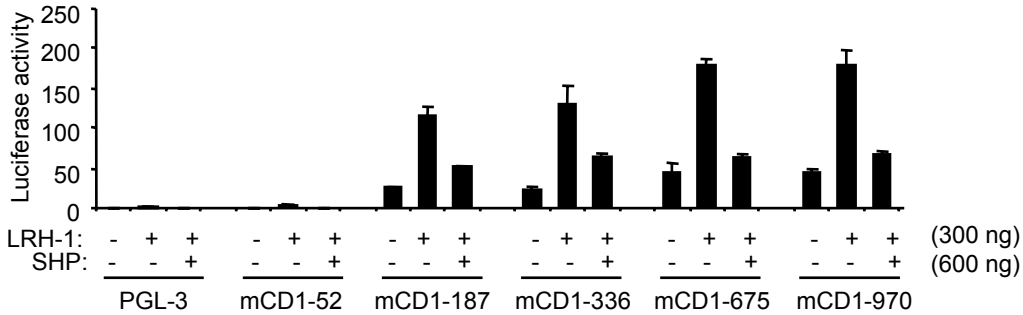


Supp. 3C



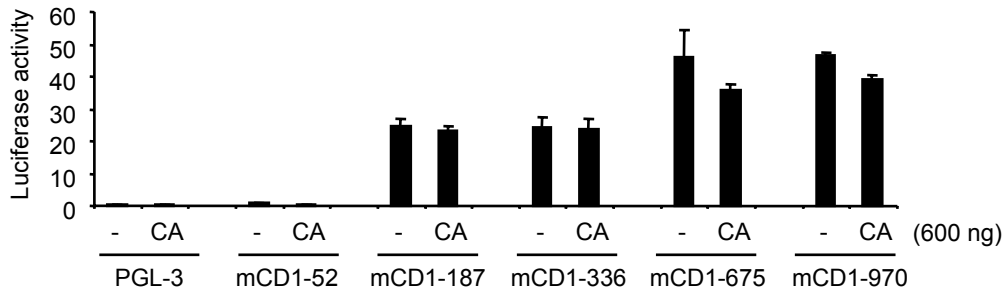
Zhang et al., Supp. Figure 3

Supp. 4A



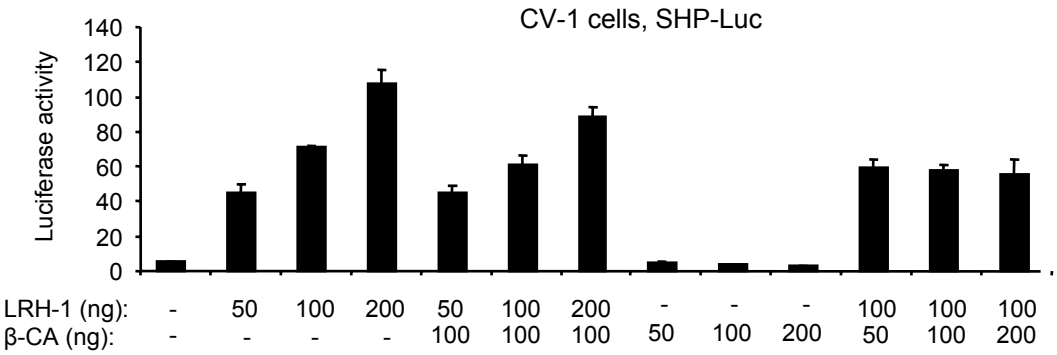
mCD1-52: contains no LRH-1 site
mCD1-187: contains one LRH-1 site
mCD1-336, 675, 970: contain two LRH-1 sites

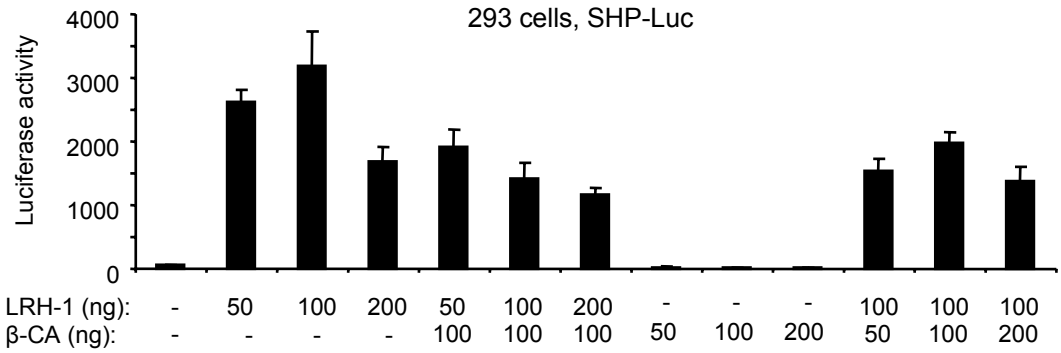
Supp. 4B

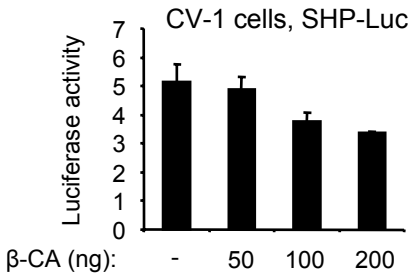


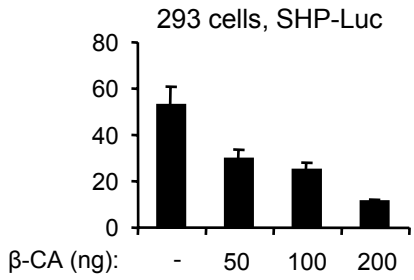
Zhang et al., Supp. Figure 4

Supp. 4C



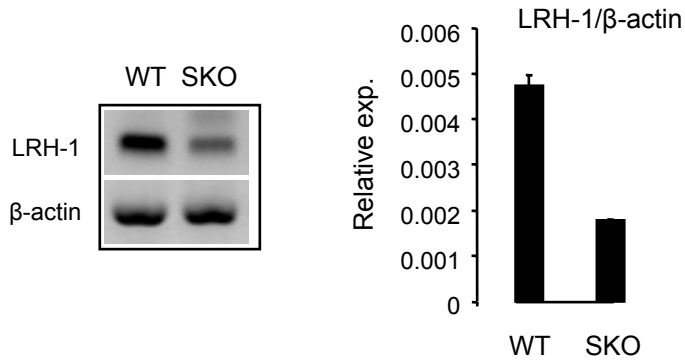






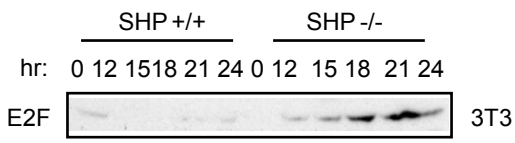
Zhang et al., Supp. Figure 4

Supp. 5A



Supp. 5B

Supp. 5C



Zhang et al., Supp. Figure 5

Supplementary Figures

Supplementary Fig. 1. Generating hepatocytes specific SHP overexpressed transgenic mice (Alb-SHP-Tg). (A) Target gene construct for overexpression of mouse SHP (mSHP) in the liver. The mouse ALB promoter was ligated into kbpa1b vector between the NotI and BamHI site to obtain kbpALB constructs. Then mSHP was ligated into the EcoRV site of the constructs. The final constructs were confirmed by enzyme digestion and sequencing. The constructs were cut by NotI/SalI and the resulting ALB-SHP fragments were gel purified and quantified for microinjection. (B) Schematic of the mouse Alb promoter-mSHP construct. The primers used to amplify the exogenous SHP transgene are indicated: F1 is located 80 bp upstream of the SHP start codon ATG, F2 is located 429 bp downstream of the SHP start codon ATG, and R1 is located 42 bp downstream of the SHP stop codon TGA. The size of the PCR product is 905 bp for F1/R1 and 400 bp for F2/R1 primers. (C) Identifying mice overexpressing mSHP in liver. Genomic DNA was extracted from the tail of each mouse and was subjected to PCR analysis. The 905 bp PCR fragment was generated by F1/R1 primers and the 400 bp by F2/R1 primers. Each number represents an individual mouse or negative (neg) control. (D) Northern blot analysis of SHP expression in livers of Alb-SHP-Tg mice. SHP is highly overexpressed (~5 fold) in livers of Alb-SHP-Tg mice as compared to non-transgene control mice (NC) (n=5, pooled RNA). (E) RT-PCR analysis of SHP mRNA in mouse hepatoma cell line Hepa-1 and normal mouse liver cell line NMuli. (F) Quantitative analysis of gene expression for Fig. 1D (normalized by β-actin). The expression of Cyc D1 was about 50% down-regulated by SHP overexpression.

Supplementary Fig. 2. (A) The *SHP*$^{-/-}$ 3T3 cells appeared smaller in size, with a spindle-like morphology, and a tendency to pile up at high cell densities. (B) Cell size analysis of *SHP*$^{+/+}$ and *SHP*$^{-/-}$ 3T3 cells by flow cytometry. Populations were gated in a

FSC-H by FL2-A dot plot and scored in a FSC-H histogram. The forward scatter, FSC-H, is indicative for the cell diameter, and a shift to the left, represents smaller cells. (C) Left: exponentially growing *SHP*$^{+/+}$ and *SHP*$^{-/-}$ fibroblasts were stained with PI and analyzed by FACS. Right: cell cycle distribution of *SHP*$^{+/+}$ and *SHP*$^{-/-}$ 3T3 cells by BrdU-FACS analysis.

Supplementary Table 1. Percentage of the cell population in G_0/G_1, S and G_2/M phase for Fig. 2A-2C. Two different cell lines for both *SHP*$^{+/+}$ and *SHP*$^{-/-}$ were examined and each line with duplicate assays. One representative result was presented.

Supplementary Fig. 3. The MEFs were examined by immunoblot analysis of cell lysates for the expression of MAPK (A) and c-fos (B, top) protein. The mRNA levels of c-fos were examined by Northern blot (B, bottom). (C) The mRNA expression of c-jun and junB was examined by real-time PCR and both genes showed marked down-regulation at early time points (0 hr and 0.5 hr).

Supplementary Fig. 4. (A) Transient transfection assay of mouse cyclin D1 promoter. LRH-1 transactivates mouse cyclin D1 (mCD1) promoter in the presence of LRH-1 binding sites. Deletion constructs of mCD1-Luc containing no LRH-1 site (mCD1-52), one LRH-1 site (mCD1-187), and two LRH-1 sites (mCD1-336, -675 and -970) were used for transient transfection. (B) Transient transfection assay of mCD1 promoter by β-catenin (CA). (C) Transient transfection assay of mouse SHP promoter cotransfected with LRH-1 and β-catenin.

Supplementary Fig. 5. (A) LRH-1 expression in wild-type and *SHP*$^{-/-}$ MEF cells by semi-quantitative PCR (left) and real-time PCR (right) analysis. LRH-1 was clearly expressed

in MEF cells, however it was down-regulated in *SHP*$^{-/-}$ cells. (B) Semi-quantitative PCR analysis of cyclin D1 expression in the *SHP*$^{-/-}$ 3T3 cells transduced with GFP or SHP adenovirus. The cells were used for foci formation assay in Fig. 5D. (C) Changes of E2F protein in immortal fibroblasts lacking *SHP*. The protein levels for E2F are induced in *SHP*$^{-/-}$ cells as compared to wild-type cells, consistent with their faster growth. Quiescence was induced by culturing cells in 0.1% serum for 72 hr (time zero), followed by stimulating cells with medium containing 10% serum. Cells were harvested after stimulation at the times indicated. Whole-cell extracts were prepared and protein levels were detected by Western blotting.