Appendix S1

Sequences of shRNA

SHMT2: CGGAGAGTTGTGGACTTTATA

Scramble: TGTGAGGAACTTGAGATCT

Appendix S2

Composition of the perfusion buffers and brief steps isolation

Buffer 1 (Ca^{2+}-and Mg^{2+}-free HBSS): 33 mM KCl, 0.441 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 137.93 mM NaCl, 0.338 mM Na_2HPO_4, d-glucose (dextrose) (4.75 mg/ml), 26.6 μM phenol red, 0.5 mM EGTA.

Buffer 2 (Ca^{2+}- and Mg^{2+}-containing HBSS): 1.26 mM $CaCl_2$, 0.493 mM $MgCl_2$, 0.407 mM $MgSO_4$, 5.33 mM KCl, 0.441 mM KH_2PO_4, 4.17 mM $NaHCO_3$, 137.93 mM NaCl, 0.338 mM Na_2HPO_4, d-glucose (dextrose) (4.75 mg/mL), 26.6 μM phenol red, 0.72% (w/v) BSA with Collagenase (Sigma, #C5138) to a final concentration of 0.08 U/mL.

After anesthesia and disinfection, abdominal cavity was opened to expose the inferior vena cava (IVC) and a slight incision parallel to the wall of the IVC was made. Cannula filled with heparin was then inserted at the lower end of the IVC and fixed with ligature. A second ligature was placed around the IVC just below the heart to prevent the flow of buffers into whole body. Buffer 1 was perfused through the liver at a rate of 2 mL/min and a small incision in the portal vein was made to allow the free flow of perfusion buffers. Continue perfusion with buffer 1 for about 7 min with a flow rate of 7 mL/min. After changing to buffer 2 for another 8–10 min, liver was then ready to remove for cell isolation.

Supplementary Tables

Table S1. List of primary and secondary antibodies.

Antibody	Source	Category number
Akt	Cell Signaling	4691
p-Akt	Cell Signaling	9271
mTOR	Cell Signaling	2972
p-mTOR	Cell Signaling	2971
P70S6K	Cell Signaling	2708
p-P70S6K	Cell Signaling	9234
SHMT2	Proteintech Group	11099-1-AP
β-actin	GeneTex	GTX11003
HRP Goat Anti-Mouse	ABclonal	AS003
HRP Goat Anti-Rabbit	ABclonal	AS014

Supplementary Figures

Figure S1. Transfection efficiency of hepatocytes in vitro. Transfection efficiency of hepatocytes in vitro. Primary hepatocytes were transfected by Lipofectamine MessengerMAX™ according to the manufacturer's instructions, and cells transfected with GFP were used as control. A. Hepatocytes were observed under light and fluorescence microscope after transfection. B. Expression of SHMT2 in the hepatocytes analyzed by western blot. C. Quantification of the western blot analysis. (values are means ± SD, *P < 0.05)

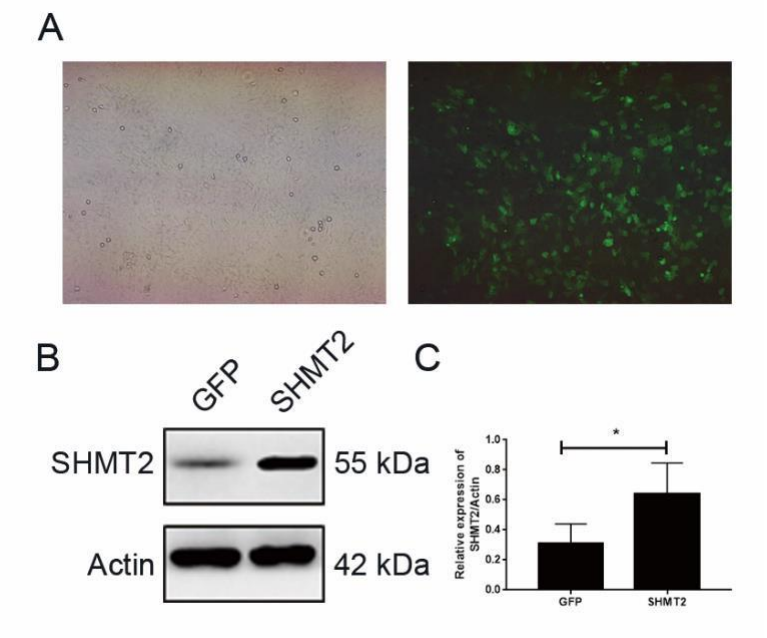

Figure S2. The retention time of metabolites. Liver tissues and cells were applied to the GC-MS analysis and retention time distribution was indicated.

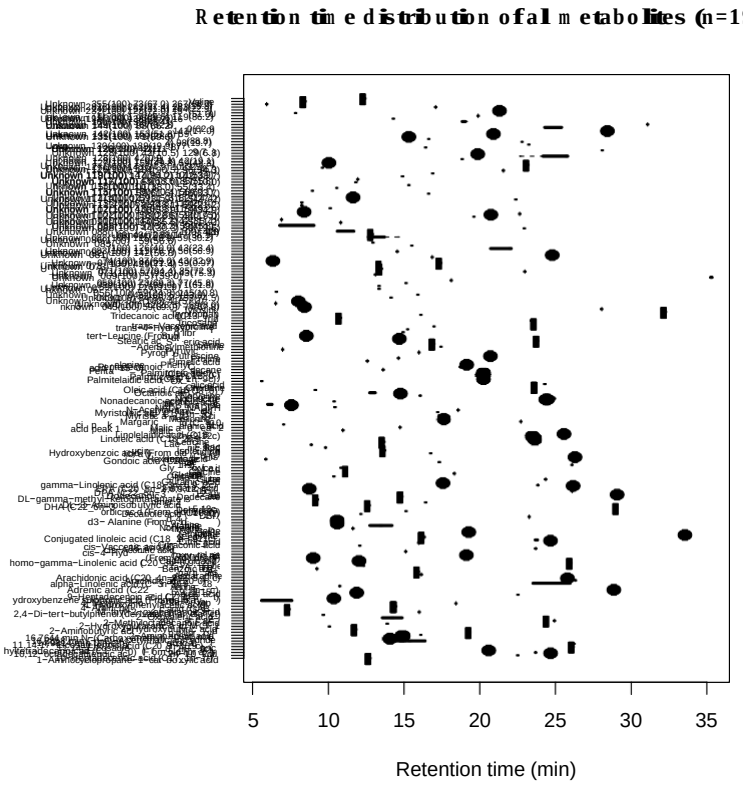

Figure S3. Transfection efficiency of AAV8 in vivo. Mice were transfected with either AAV8-scramble (as the control) or AAV8-shSHMT2 by tail vein injection two weeks before the surgeries. A. GFP in the liver tissues detected by fluorescence microscope. B. Expression of SHMT2 in the liver tissues analyzed by western blot. C. Quantification of the western blot analysis. (values are means ± SD, *P < 0.05)

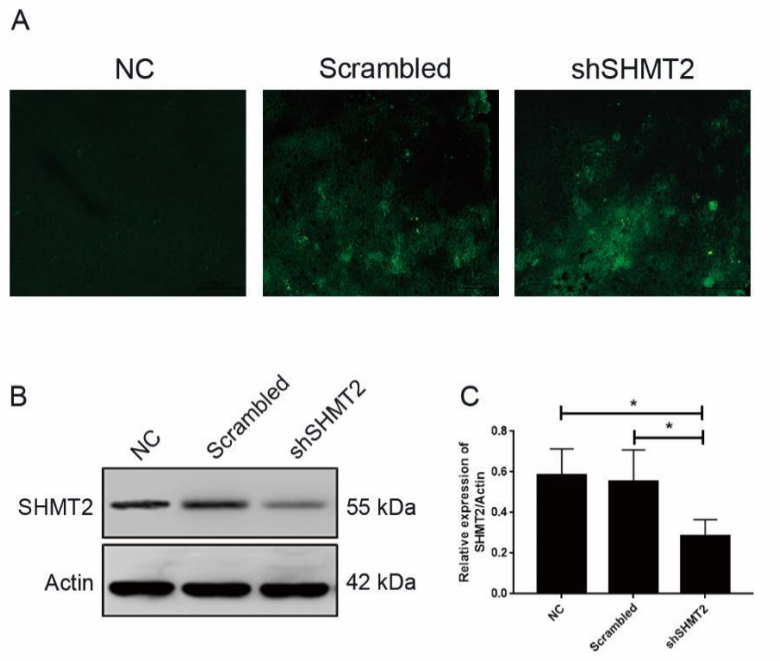

Figure S4. Representative liver samples after PH. Liver samples from NC, scramble, and shSHMT2 groups at 10 d after PH were demonstrated.