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Supplemental Information

Low-Dose Sorafenib Acts as a Mitochondrial Uncoupler and Ameliorates Nonalcoholic Steatohepatitis

Chongshu Jian, Jiajun Fu, Xu Cheng, Li-Jun Shen, Yan-Xiao Ji, Xiaoming Wang, Shan Pan, Han Tian, Song Tian, Rufang Liao, Kehan Song, Hai-Ping Wang, Xin Zhang, Yibin Wang, Zan Huang, Zhi-Gang She, Xiao-Jing Zhang, Lihua Zhu, and Hongliang Li

Figure S1

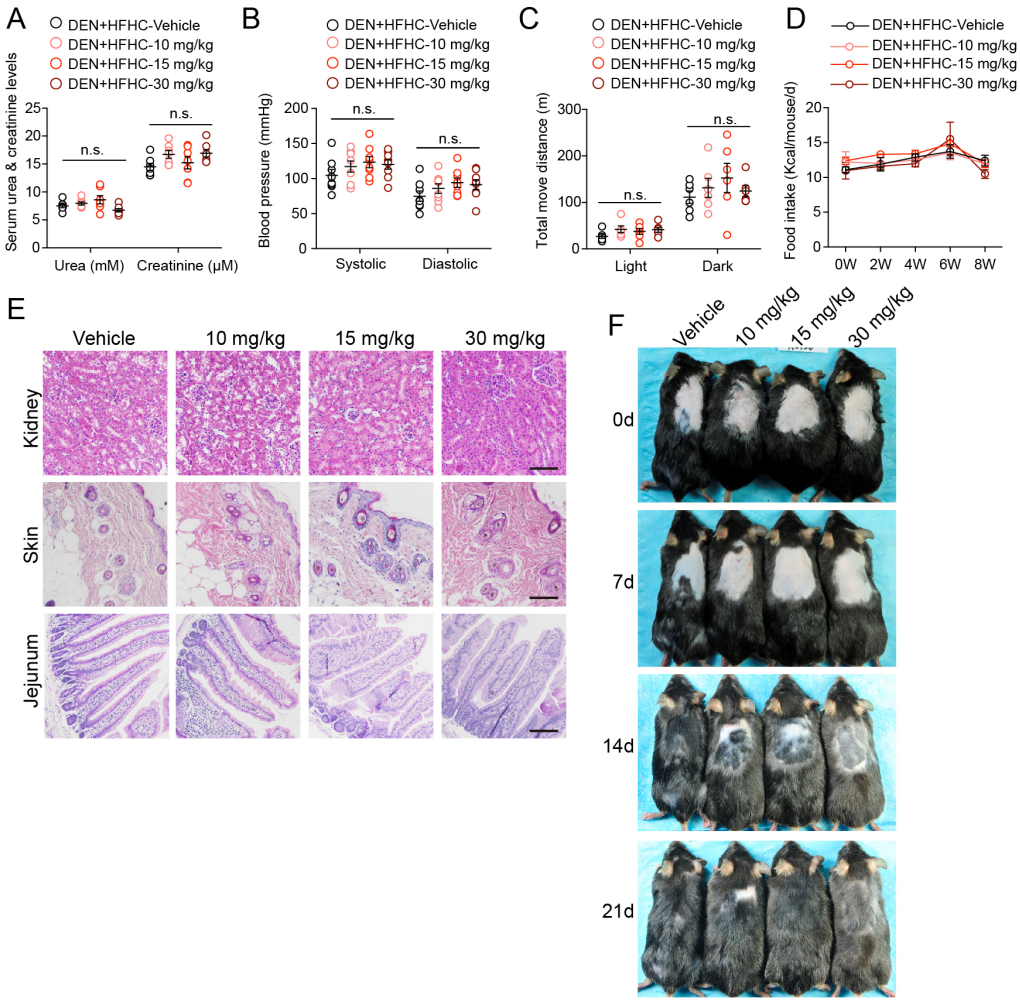

Figure S1. Effects of sorafenib on NASH-HCC mice, related to Figure 1.

(**A**) Serum urea and creatinine levels of mice in the indicated groups. *n* = 7 mice per group.

(**B**) Blood pressures of mice in each group. *n* = 8 mice per group.

(**C**) Total move distance of mice measured in metabolic cages. *n* = 6 mice per group.

(**D**) Food intake of mice in the indicated groups during the 8 weeks' treatment. *n* = 3 cages per group.

(**E**) Histology of kidney (upper), skin (middle) and jejunum (bottom) after vehicle, 10, 15 or 30 mg/kg sorafenib treatment. *n* = 6 mice per group. Scale bar, 100 μm.

(**F**) Representative pictures of the dorsal surface of mice captured 0, 7, 14 and 21 days after wax depilation at the age of 20 weeks. *n* = 11 mice per group.

The data in **A**, **B** and **C** were presented as the means ± SEMs and analyzed by one-way ANOVA. n.s., no significance, $P > 0.05$.

Figure S2

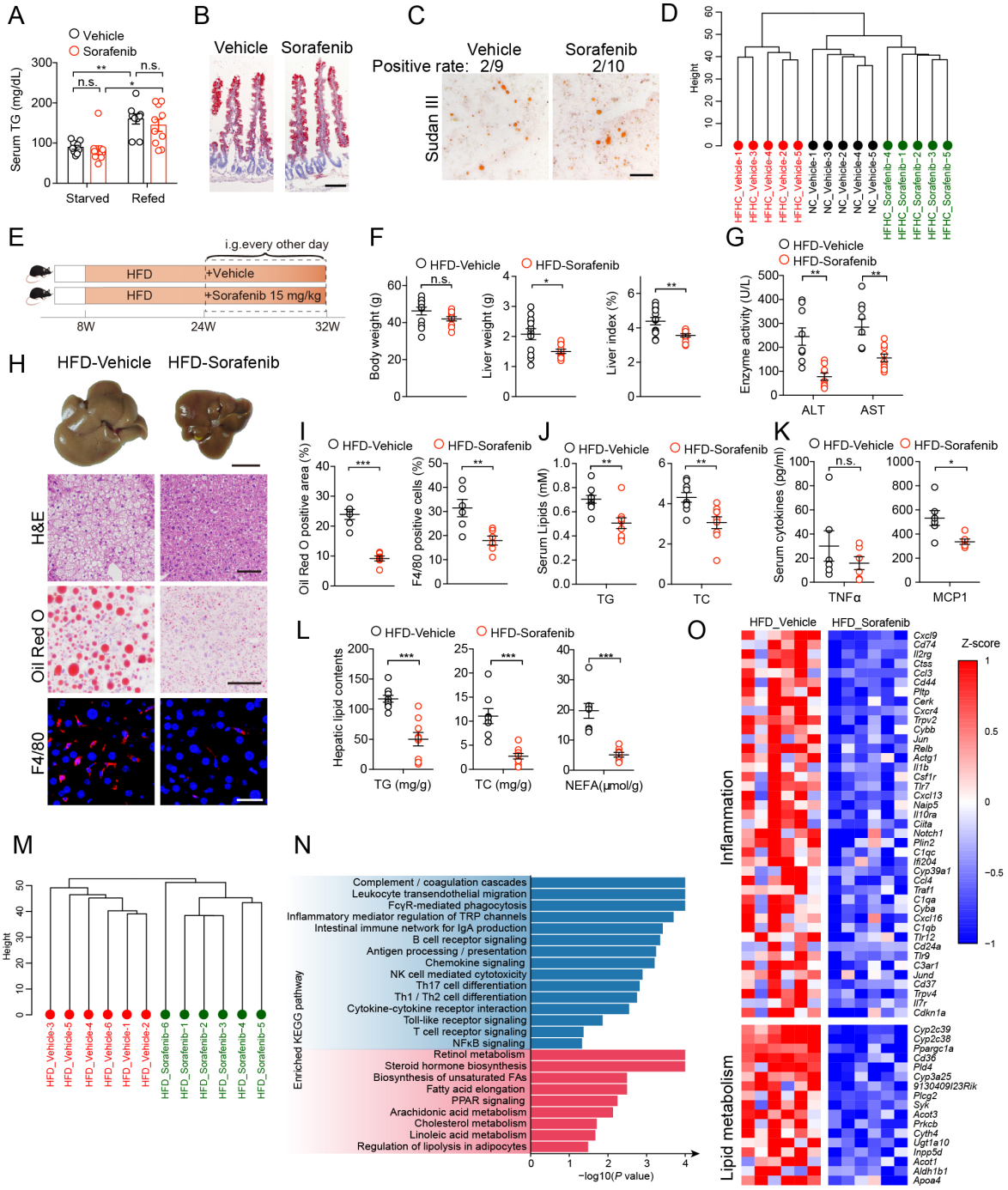

Figure S2: Effects of sorafenib on intestinal lipid absorption and HFD-induced NAFLD, related to Figure 2.

(**A**) Serum TG levels of vehicle or sorafenib treated mice before and 1 hour after HFHC diet re-feeding. *n* = 9 mice for vehicle and *n* = 10 mice for sorafenib treatment. The data were presented as the means ± SEMs and analyzed by one-way ANOVA.

(**B**) Oil Red O staining of villus of proximal jejunum from HFHC-fed mice treated with vehicle or sorafenib. *n* = 9 mice for vehicle and *n* = 10 mice for sorafenib treatment. Scale bar, 100 μm.

(**C**) Sudan III staining of fecal fat of HFHC-fed mice treated with vehicle or sorafenib. The

positive rate indicates the number of Sudan III positive mice divide by the group number. *n* = 9 mice for vehicle and *n* = 10 mice for sorafenib treatment. Scale bar, 50 μm.

(**D**) The hierarchical clustering images of sample distribution and relationship based on RNA-seq data obtained from liver tissues of mice in NC-vehicle (black), HFHC-vehicle (red) and HFHC-sorafenib (green) groups. *n* = 5 mice in each group.

(**E**) Scheme for the experimental strategy on HFD-fed mice treated with vehicle or sorafenib.

(**F**) Body weight, liver weight and liver index of HFD-fed mice treated with vehicle or sorafenib. *n* = 12 mice per group.

(**G**) Serum ALT and AST levels of HFD-fed mice treated with vehicle or sorafenib. *n* = 9 mice per group.

(**H**) Representative macroscopic and histological images of livers (scale bar, 1 cm) and liver sections from HFD-fed mice treated with vehicle or sorafenib are shown. Staining of liver sections for H&E (upper; scale bar, 100 μm), Oil Red O (middle; scale bar, 100 μm) and F4/80 (bottom; scale bar, 20 μm). *n* = 6 mice per group.

(**I**) Quantitative results for Oil Red O and F4/80 staining shown in (**H**). *n* = 6 mice per group.

(**J**) Serum concentrations of TG and TC of HFD-fed mice treated with vehicle or sorafenib. *n* = 9 mice per group.

(**K**) Serum concentrations of cytokines TNFα and MCP1 of HFD-fed mice treated with vehicle or sorafenib. *n* = 6 mice per group.

(**L**) Liver TG, TC and NEFA concentrations of HFD-fed mice treated with vehicle or sorafenib. *n* = 8 mice per group.

(**M**) Cluster images showing global sample distribution profiles and relationships based on RNA-seq data obtained from liver tissues of HFD-fed mice with vehicle (red) or sorafenib (green) treatment. *n* = 6 mice per group.

(**N**) KEGG pathway enrichment results showing the cellular pathways involved in inflammation (blue) and lipid metabolism (red) that were regulated by sorafenib on HFD-fed mice. The colors of pathways related to inflammation and lipid metabolism are in blue and red, respectively. *n* = 6 mice per group.

(**O**) Heatmap showing the genes involved in inflammation and lipid metabolism that were down-regulated by sorafenib in HFD mice. *n* = 6 mice in each group.

The data in **F**, **G**, **I**, **J**, **K** and **L** were presented as the means ± SEMs and analyzed by Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., no significance, $P > 0.05$.

Figure S3

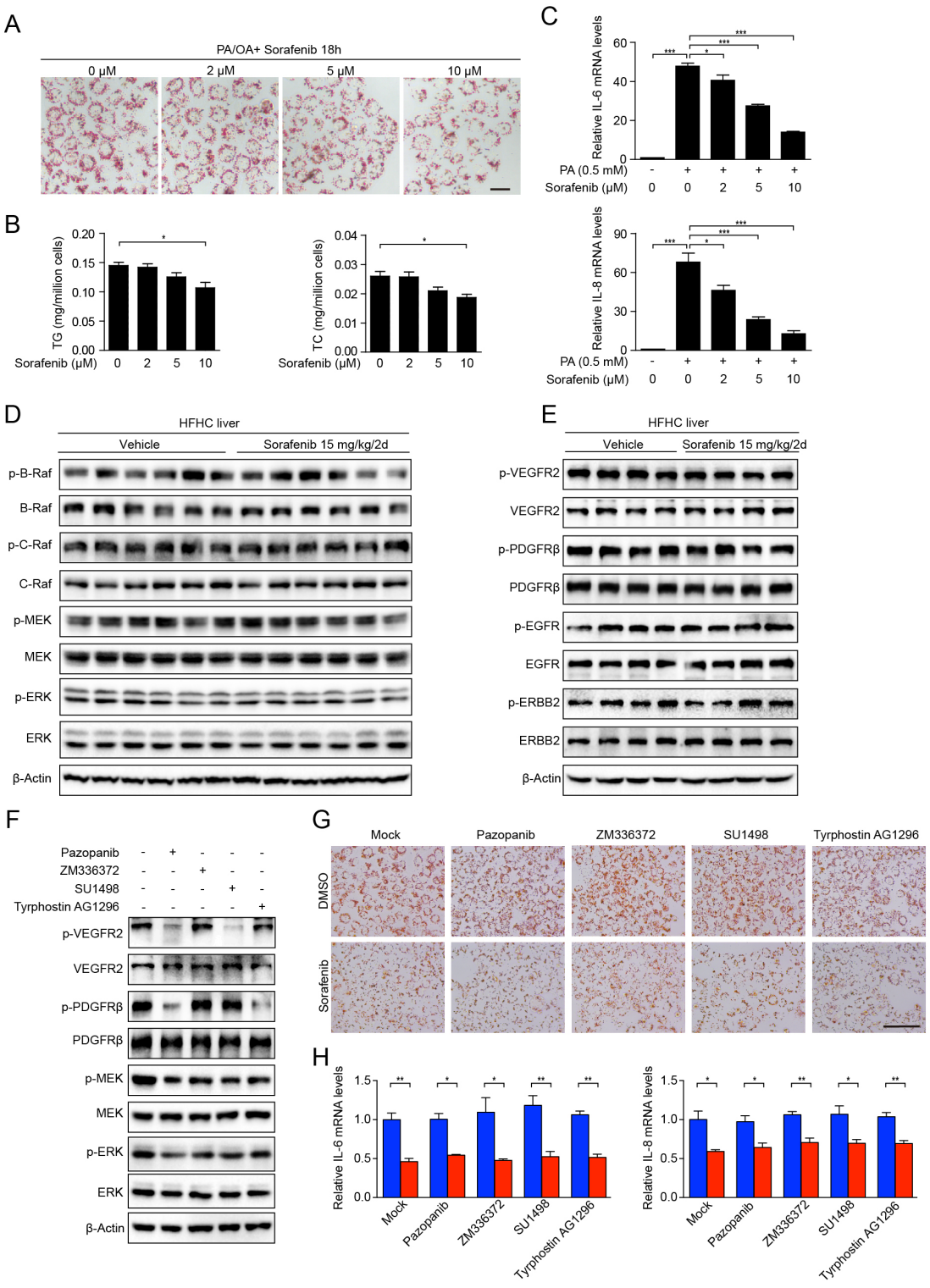

Figure S3. Sorafenib inhibits PA-induced lipid accumulation and inflammation independent of its canonical kinase targets, related to Figure 3.

(**A**, **B**) Oil Red O staining (**A**) and TG, TC contents (**B**) of the human L02 hepatocytes stimulated by PAOA together with indicated concentrations of sorafenib. *n* = 3 replicates. Scale bar, 50 μm.

(**C**) qPCR analyses of pro-inflammatory factors IL-6 and IL-8 mRNA levels in L02

hepatocytes stimulated by BSA (-) or PA (+) with indicated concentrations of sorafenib. The mRNA expression levels of target genes were normalized to that of *ACTB* (β-Actin). $n = 3$ replicates.

(**D**) Immunoblotting analyses of total and phosphorylated B-Raf, C-Raf, MEK and ERK proteins in liver tissues of HFHC-fed mice treated with vehicle or sorafenib. $n = 6$ mice per group.

(**E**) Immunoblotting analyses of total and phosphorylated VEGFR2, PDGFRβ, EGFR and ERBB2, in liver tissues of HFHC-fed mice treated with vehicle or sorafenib. $n = 4$ mice per group.

(**F**) Immunoblotting validation of the effect of tyrosine kinase inhibitors pazopanib (targeting VEGFR1-3, PDGFR, FGFR, c-KIT and c-fms), ZM336372 (RAF inhibitor), SU1498 (VEGFR inhibitor) and Tyrphostin AG1296 (PDGFR inhibitor) on VEGFR, PDGFR and RAF signaling. $n = 3$ replicates.

(**G**) Oil Red O staining of L02 hepatocytes stimulated by PAOA and treated with DMSO, pazopanib, ZM336372, SU1498, or Tyrphostin AG1296 in the absence or presence of sorafenib. Scale bar, 100 μm. $n = 3$ replicates.

(**H**) qPCR analyses of pro-inflammation factors IL-6 and IL-8 mRNA levels in L02 hepatocytes stimulated by PA and treated with DMSO, pazopanib, ZM336372, SU1498, or Tyrphostin AG1296 in the absence or presence of sorafenib. $n = 3$ replicates.

The data in **B**, **C** and **H** were presented as the means ± SEMs. For statistical analysis, a one-way ANOVA was used for **B** and **C** and a Student's *t*-test was used for **H**. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Figure S4

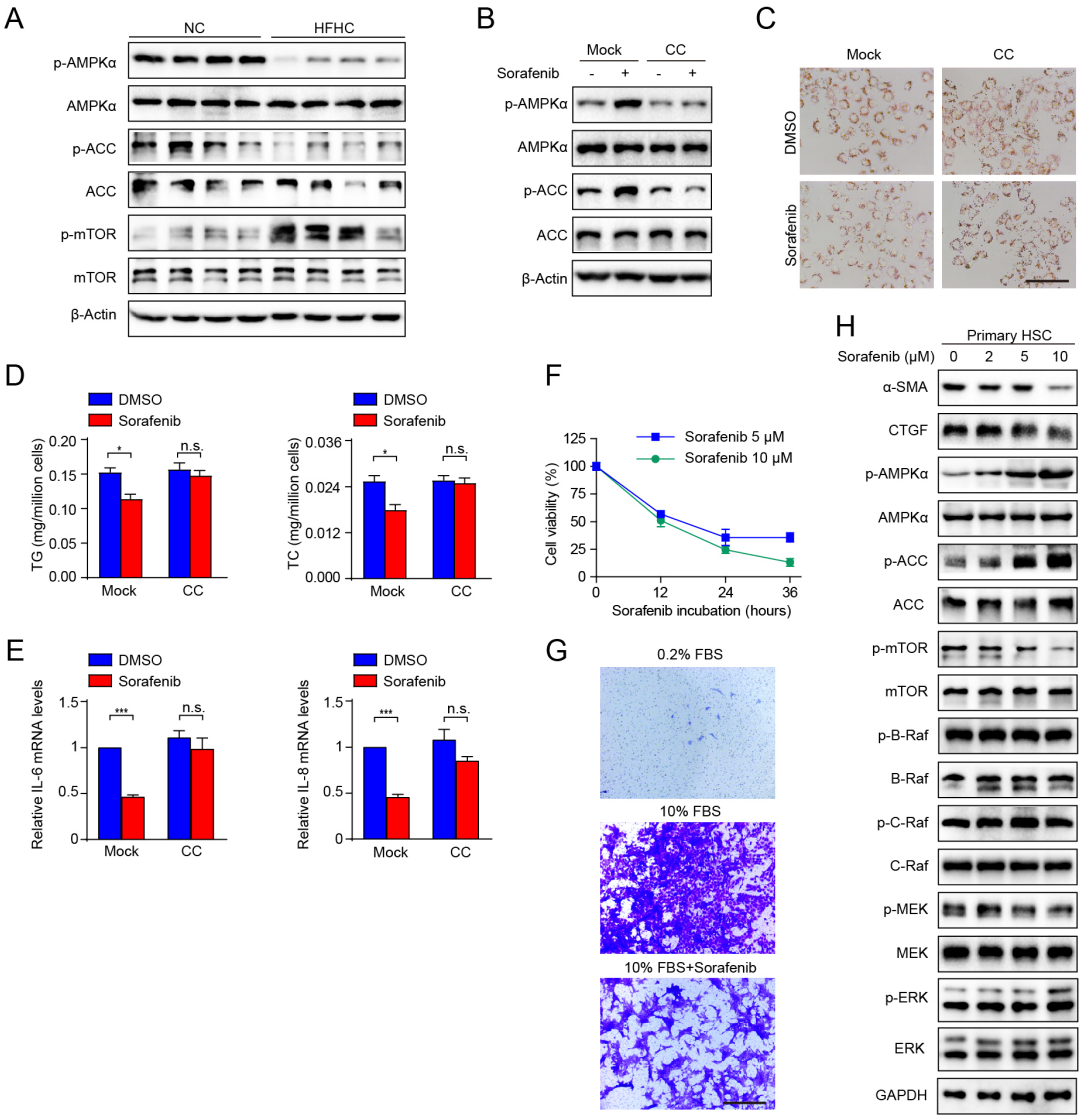

Figure S4. AMPK activation is required for the effects of sorafenib on lipid accumulation, inflammation and fibrosis *in vitro*, related to Figure 4.

(**A**) Immunoblotting analyses of total and phosphorylated AMPKα, ACC, mTOR proteins in liver tissues of mice fed on NC or HFHC diet.

(**B**) Immunoblotting analyses of total and phosphorylated AMPKα and ACC in L02 hepatocytes challenged by PA and treated with mock, Compound C (CC), sorafenib or sorafenib in combination with CC. $n = 3$ replicates.

(**C**, **D**) Oil Red O staining (**C**) and TG, TC contents (**D**) of L02 hepatocytes challenged by PAOA and treated with mock, CC, sorafenib or sorafenib in combination with CC. $n = 3$ replicates. Scale bar, 100 μm.

(**E**) qPCR analyses of IL-6 and IL-8 mRNA levels in L02 hepatocytes challenged by PA and treated with mock, CC, sorafenib or sorafenib in combination with CC. $n = 3$ replicates. The mRNA expression level of target genes was normalized to that of *ACTB* (β-Actin).

(**F**) Time-dependent effects of 5 or 10 μM sorafenib on cell viability in LX-2 cells. $n = 3$

replicates. Scale bar, 100 μm.

(**G**) Representative images showing crystal violet staining for migrated LX-2 cells challenged by FBS together with DMSO or sorafenib in the transwell assay. Scale bar, 100 μm. $n = 3$ replicates.

(**H**) Immunoblotting analyses of α-SMA, CTGF and total and phosphorylated AMPKα, ACC, mTOR, B-Raf, C-Raf, MEK and ERK proteins in rat primary hepatic stellate cells treated with indicated concentrations of sorafenib. $n = 3$ replicates.

The data in **D** and **E** were presented as the means ± SEMs, and analyzed by Student's *t*-test. *, $P < 0.05$; ***, $P < 0.001$; n.s., no significance, $P > 0.05$.

Figure S5

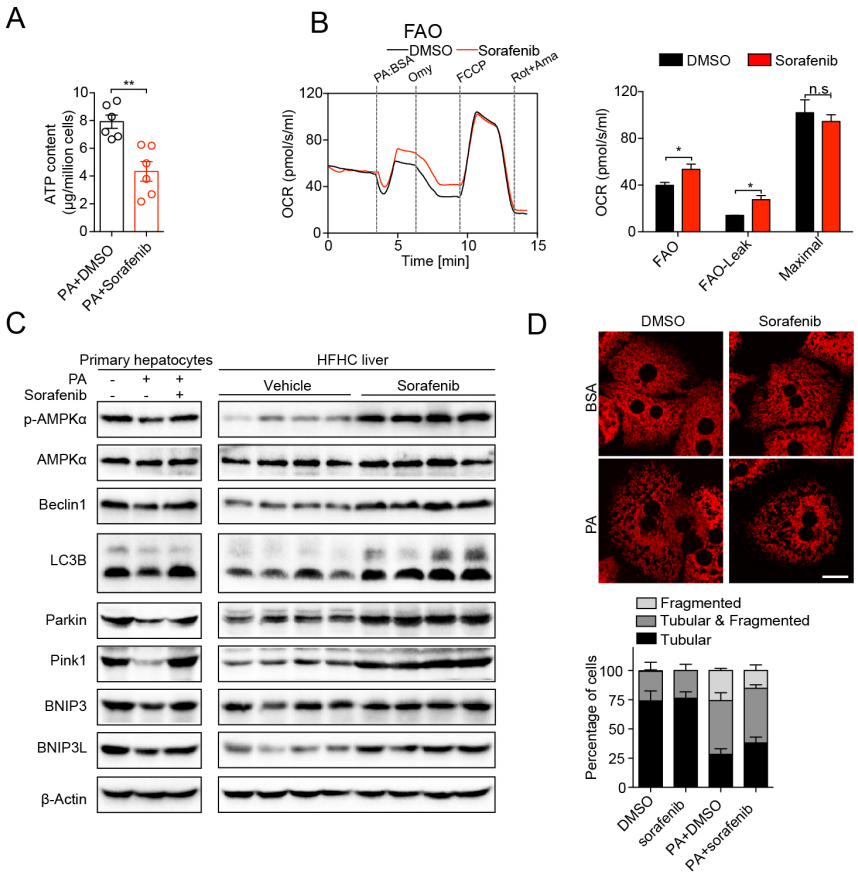

Figure S5. Effect of sorafenib on mitochondrial functions, related to Figure 6.

(**A**) ATP content in mouse primary hepatocytes stimulated by PA and treated with DMSO or sorafenib. The data were presented as the means ± SEMs and analyzed by Student's *t*-test. **, $P < 0.01$. $n = 6$ mice per group.

(**B**) Representative (left) and statistical (right) results of PA stimulated respiration on intact mouse primary hepatocytes treated with DMSO or sorafenib. The dotted lines indicate the time of adding PA (BSA conjugated palmitate), Omy (oligomycin), FCCP and Rot+AA (rotenone and antimycin A). FAO, fatty acid oxidation. $n = 4$ replicates. The data were presented as the means ± SEMs and analyzed by Student's *t*-test. *, $P < 0.05$; n.s., no significance, $P > 0.05$.

(**C**) Immunoblotting analyses of autophagy (Beclin1 and LC3B) and mitophagy marker proteins (Parkin, Pink1, BNIP3, BNIP3L) in mouse primary hepatocytes stimulated by PA and treated with indicated concentrations of sorafenib (left, $n = 3$ replicates) and in liver tissues of mice fed by HFHC diet and treated with vehicle or 15 mg/kg/2 days sorafenib (right, $n = 4$ mice per group).

(**D**) Mitochondrial morphological changes in primary mouse hepatocytes treated with PA or BSA in the absence or presence of sorafenib. Tubular, cells with primarily tubular mitochondria; tubular & fragmented, cells containing punctuated mitochondria with at least three clearly tubular mitochondria; fragmented, cells containing punctuated mitochondria with less than 3 tubular mitochondria. $n = 4$ replicates. Scale bar, 20 μm.

Figure S6

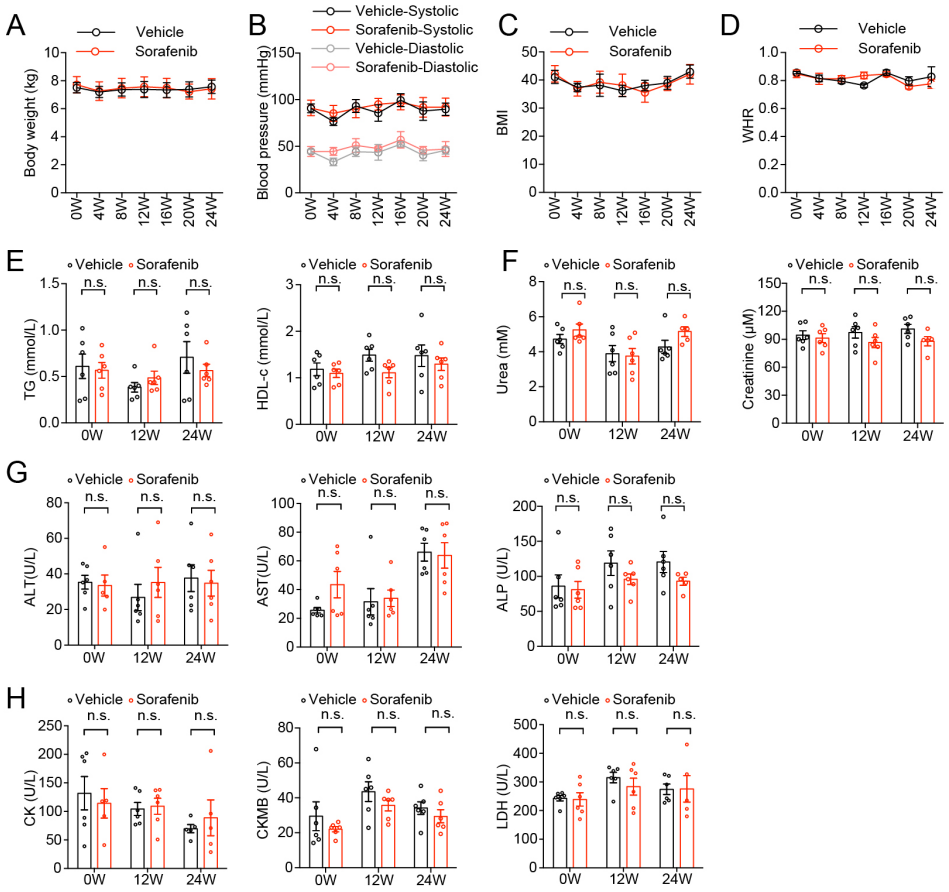

Figure S6. Efficacy of sorafenib on NASH monkeys. Related to Figure 7.

(**A**) Body weight of monkeys treated with vehicle or sorafenib. *n* = 6 monkeys per group.

(**B**) Blood pressure of monkeys treated with vehicle or sorafenib. *n* = 6 monkeys per group.

(**C**) BMI of monkeys treated with vehicle or sorafenib. *n* = 6 monkeys per group.

(**D**) Waist to hip ratio (WHR) of monkeys treated with vehicle or sorafenib. *n* = 6 monkeys per group.

(**E**) Serum concentrations of TG and HDL-c in monkeys treated with vehicle or sorafenib. *n* = 6 monkeys in each group.

(**F**) Kidney function of monkeys treated with vehicle or sorafenib measured by serum concentration of urea and creatinine. *n* = 6 monkeys per group.

(**G**) Liver function of monkeys treated with vehicle or sorafenib measured by enzyme activities of serum ALT, AST and ALP. *n* = 5 ~ 6 monkeys per group.

(**H**) Heart function of monkeys treated with vehicle or sorafenib measured by enzyme activities of serum CK, CKMB and LDH. *n* = 5 ~ 6 monkeys per group.

The data in **A**–**H** were presented as the means ± SEMs. For statistical analysis, a Student's *t*-test was used for **E**–**H**. n.s., no significance, $P > 0.05$.

Table S1. Dose conversions based on body surface area. Related to Figure 1 and Figure 7 and STAR Methods.

Species	K	Body weight (kg)	Body surface area (m^2)	Km (kg/m^2)	Applied dose (mg/kg)	Monkey equivalent dose (mg/kg)	Human equivalent dose	
							mg/kg	mg [b]
Mouse	9.8	0.02	0.007	2.77	10	0.62 ~ 0.80[a]	0.75	45.0[b]
					15	0.93 ~ 1.2[a]	1.12	67.5[b]
					30	1.86 ~ 2.4[a]	2.25	134.99
Monkey	11.8	8	0.472	17	1	——	0.94 ~ 1.2[a]	56.4 ~ 72[a,b]
Human	10.6	60	1.623	37	——	——	13.3	800[b]

a, oral bioavailibity of sorafenib is 38%-49%.

b, dose for a 60-kg person.

Table S2. Organ indexes of sorafenib treated NASH-HCC mice. Related to Figure 1.

	Vehicle(n = 12)	10 mg/kg (n = 12)	15 mg/kg (n = 11)	30 mg/kg (n = 12)
Body weight (g)	36.42 ± 1.43	36.00 ± 0.68	32.86 ± 1.39	32.7 ± 0.81
Liver index (%)	6.76 ± 0.28	5.62 ± 0.31*	5.59 ± 0.25*	5.70 ± 0.28
White adipose tissue index (%)	4.91 ± 0.25	4.43 ± 0.34	3.67 ± 0.45	4.15 ± 0.33
Heart index (%)	0.44 ± 0.02	0.41 ± 0.02	0.49 ± 0.02	0.42 ± 0.02
Kideny index (%)	1.12 ± 0.04	1.08 ± 0.04	1.23 ± 0.05	1.18 ± 0.03
Lung index (%)	0.57 ± 0.06	0.51 ± 0.04	0.65 ± 0.07	0.53 ± 0.03
Spleen index (%)	0.36 ± 0.02	0.36 ± 0.03	0.38 ± 0.02	0.31 ± 0.02
Pancreas index (%)	0.60 ± 0.03	0.59 ± 0.03	0.70 ± 0.04	0.58 ± 0.04

Organ index= organ weight / body weight × 100%

The data were presented as the means ± SEMs and analyzed by One-way ANOVA. *, $P < 0.05$ *v.s.* Vehicle group.

Table S3. Oligonucleotide sequences for the generation of gene knockout L02 cell lines. Related to STAR Methods.

Gene	Target sequence	Genotyping primers (5'-3')
BRAF	sg-1: CCCCACCAAATTTGTCCAAT	F: GCAAAGCTAATTCTCTCTTCCCA
	sg-2: GAGGCCCTATTGGACAAATT	R: GGAACACTGGCAGTTACTGTG
CRAF	sg-1: CAGCGCCGGGCATCAGATGA	F: CTTAATGTGCTCCACAGGCAGA
	sg-2: GCTTGGAAGACGATCAGCAA	R: GATCCTTAATGTGCTCCACAGG
PRKAA1	sg-1: TACTCAATCGACAGAAGATT	F: CAGCCTAGGAAGAAACTTTTC
	sg-2: GTTGGCAAACATGAATTGAC	R: ACTCCTGACCTCAAGTGATCT
PRKAA2	sg-1: CATACCGAAATCGGCTATCT	F: CTTAATGTGCTCCACAGGCAGA
	sg-2:AATGCCAAGATAGCCGATTT	R: CTTTGCTGATGAATGCAGGAGGT

Table S4. Primers used for qPCR. Related to STAR Methods.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
IL-6	GAGTAGTGAGGAACAAGCCAGA	AAGCTGCGCAGAATGAGATGA
IL-8	CTAGGACAAGAGCCAGGAAGAA	GGGGTGGAAAGGTTTGGAGTA
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT