Deficiency of gluconeogenic enzyme PCK1 promotes metabolic-associated fatty liver disease (MAFLD) through PI3K/AKT/PDGF axis activation in male mice

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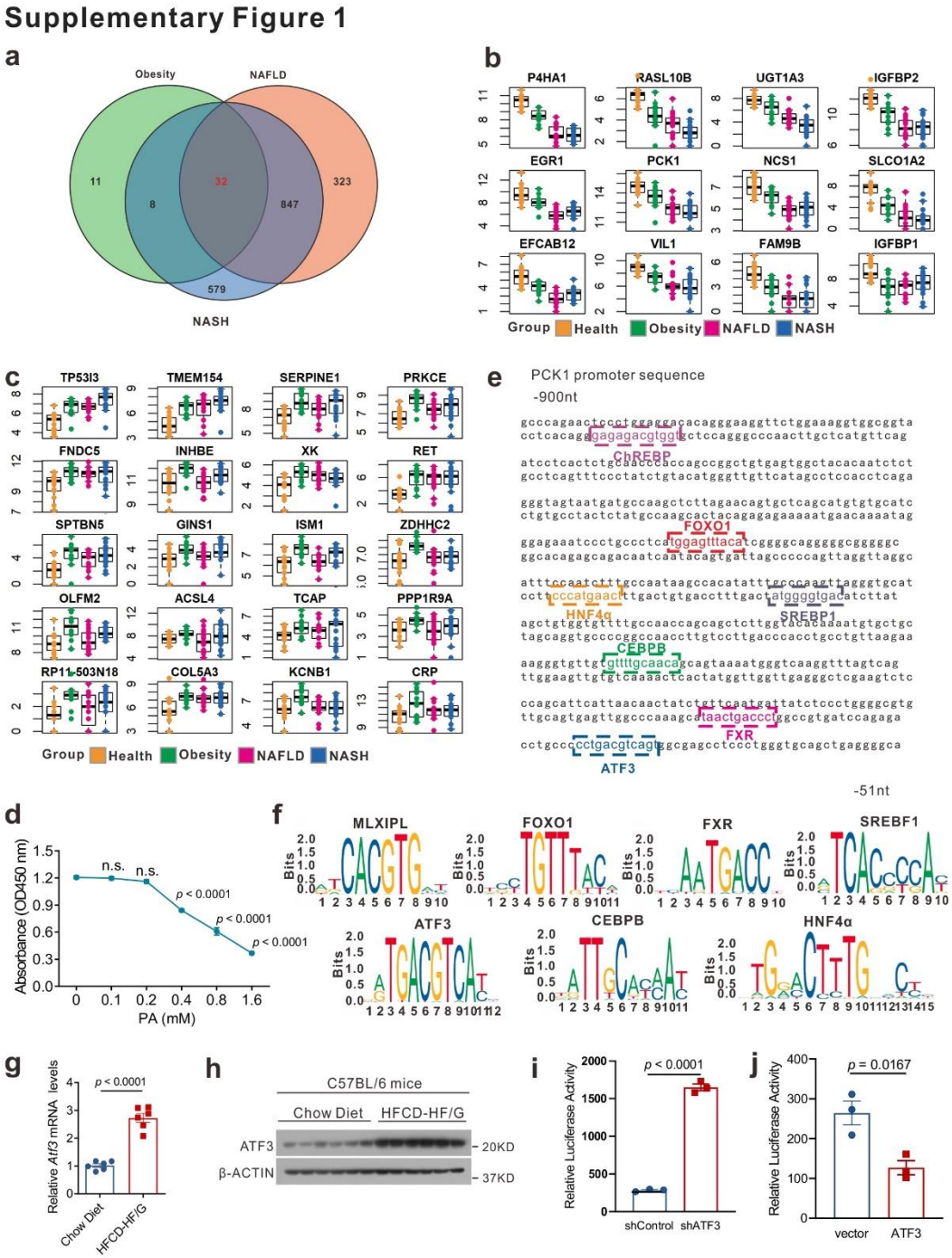
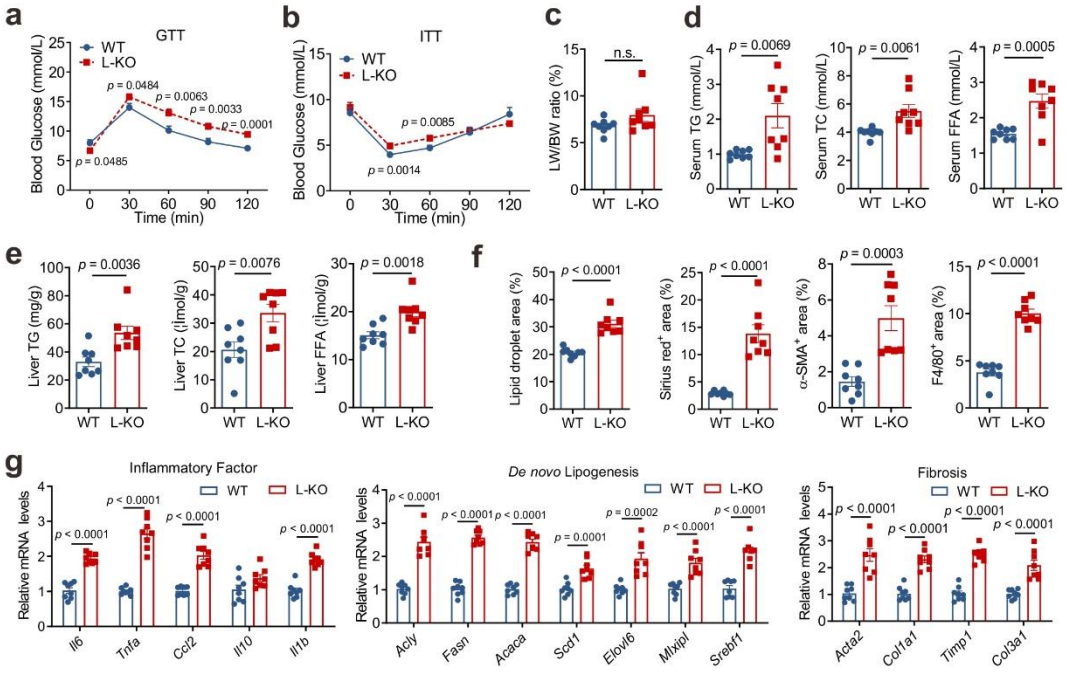
Supplementary Figures and Figure Legends



Supplementary Fig. 1 PCK1 is downregulated in NASH patients due to ATF3 upregulation in HFCD-HF/G induced mouse model. a Venn Diagram showing a total of 32 genes were significantly changed in patients with obesity,

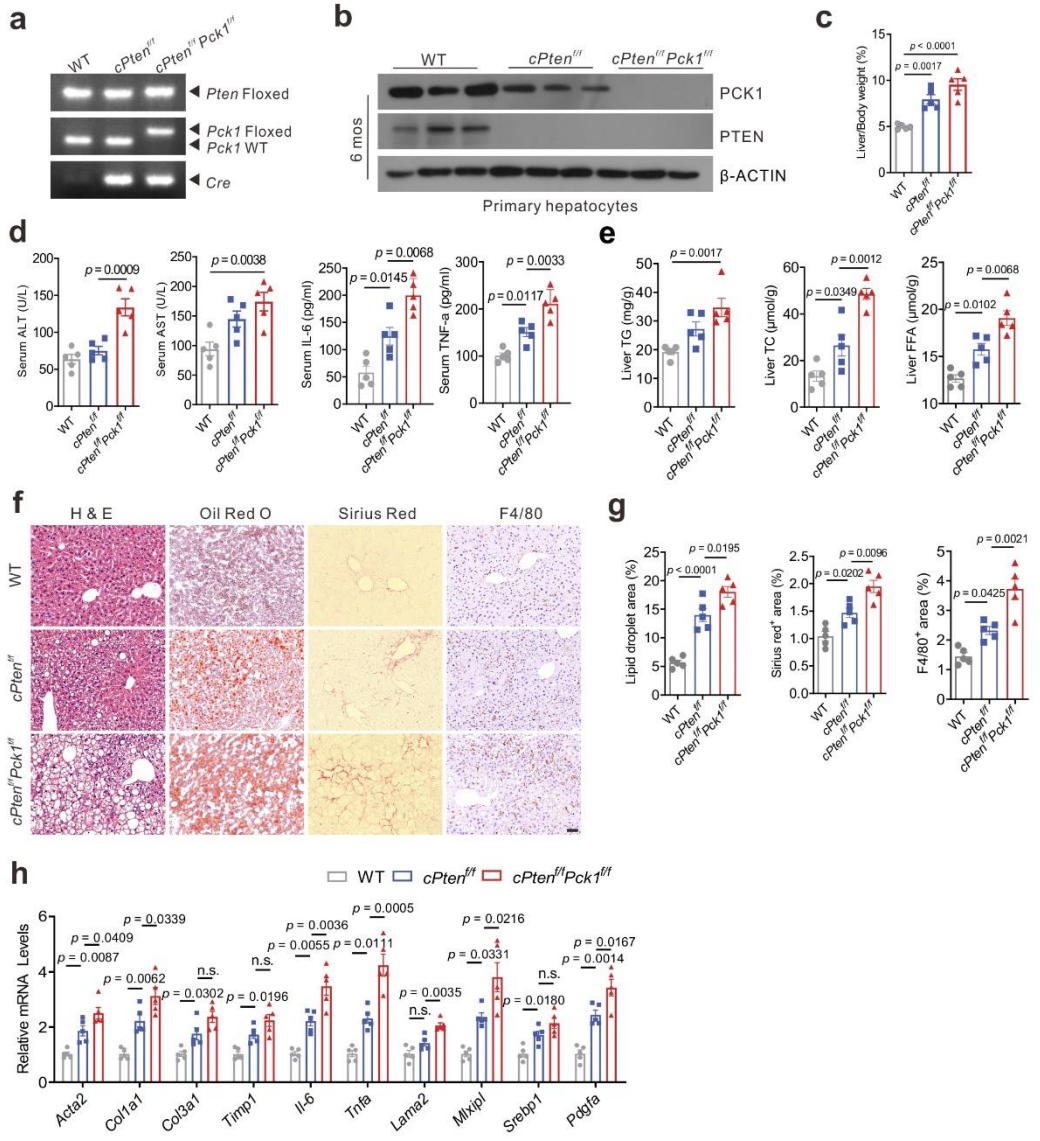
NAFLD, and NASH. **b** 12 genes were significantly downregulated in health (n = 14), obesity (n = 12), NAFLD (n = 15), and NASH (n = 16). **c** 20 genes were significantly upregulated in health (n = 14), obesity (n = 12), NAFLD (n = 15), and NASH (n = 16). The box plots show the medians (middle line) and the first and third quartiles (boxes), whereas the whiskers show 1.5× the IQR above and below the box. Unpaired, two-sided Mann–Whitney U test *P* values are depicted in the plots, and the significant *P* value cutoff was set at 0.05. **d** Cell proliferation was assessed by a CCK8 assay (n = 3). **e** Illustration of the predicted transcription factor binding sites in the 0.9 kb *PCK1* promoter region using JASPAR. **f** Potentially regulatory binding sequences of the transcription factors generated by JASPAR. **g, h** The mRNA (**g**) and protein levels (**h**) of ATF3 in mice fed with HFCD-HF/G (n = 6). **i, j** Luciferase assay of *PCK1* promoter constructs in MIHA cells knockdown (**i**) or overexpression (**j**) of ATF3 (n = 3). Results were obtained as relative luciferase activity against the activity of pGL3-Basic. Each figure represents at least three independent experiments. n was the number of biologically independent mice. Data expressed as mean ± SD; n.s., not significant. *P* values obtained via 2-tailed unpaired Student's t tests or one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.

Supplementary Figure 2



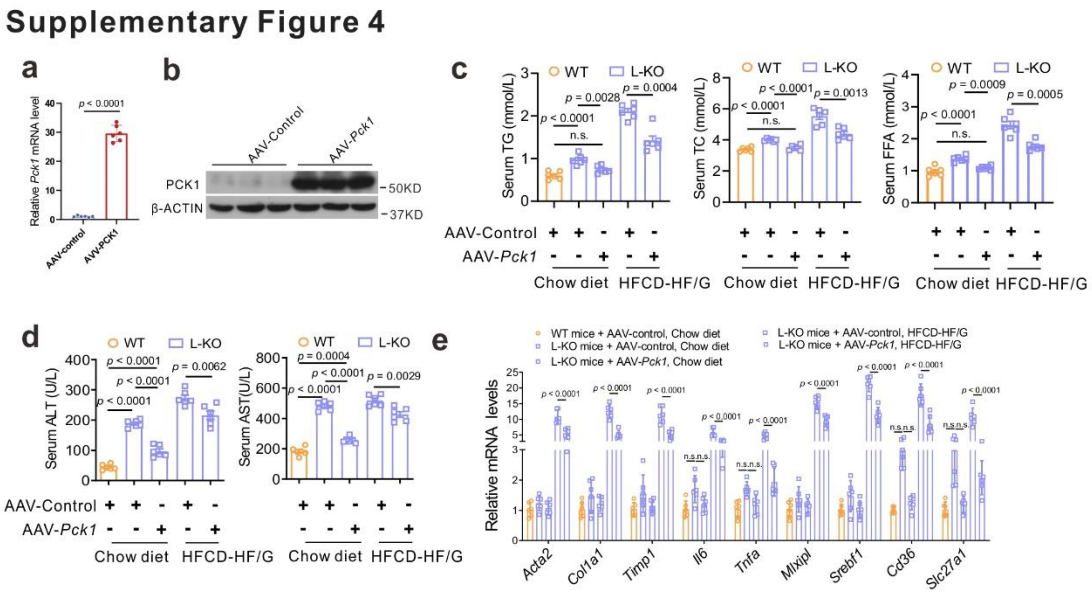
Supplementary Fig. 2 L-KO mice had abnormal lipid metabolism and severe liver injury when fed HFCD-HF/G. For **a**-**g**, WT and L-KO mice were treated with HFCD-HF/G for 24 weeks, for **a**-**b**, n=11/group; for **c**-**g**, n=8/group. **a, b** GTT (**a**) and ITT (**b**) in WT and L-KO mice after 24 weeks of HFCD-HF/G. **c** Liver weight to body weight ratio of mice from the indicated groups. **d, e** TG, TC, and FFA levels in serum (**d**) or liver tissues (**e**). **f** Quantifications of Oil red O staining, Sirius red staining, and IHC staining. **g** Genes associated with inflammatory infiltration, *de novo* lipogenesis, and fibrogenesis were measured in WT and L-KO mice. n was the number of biologically independent mice. Data expressed as mean ± SEM; n.s., not significant. *P* values obtained via 2-tailed unpaired Student's *t* tests. Source data are provided as a Source Data file.

Supplementary Figure 3



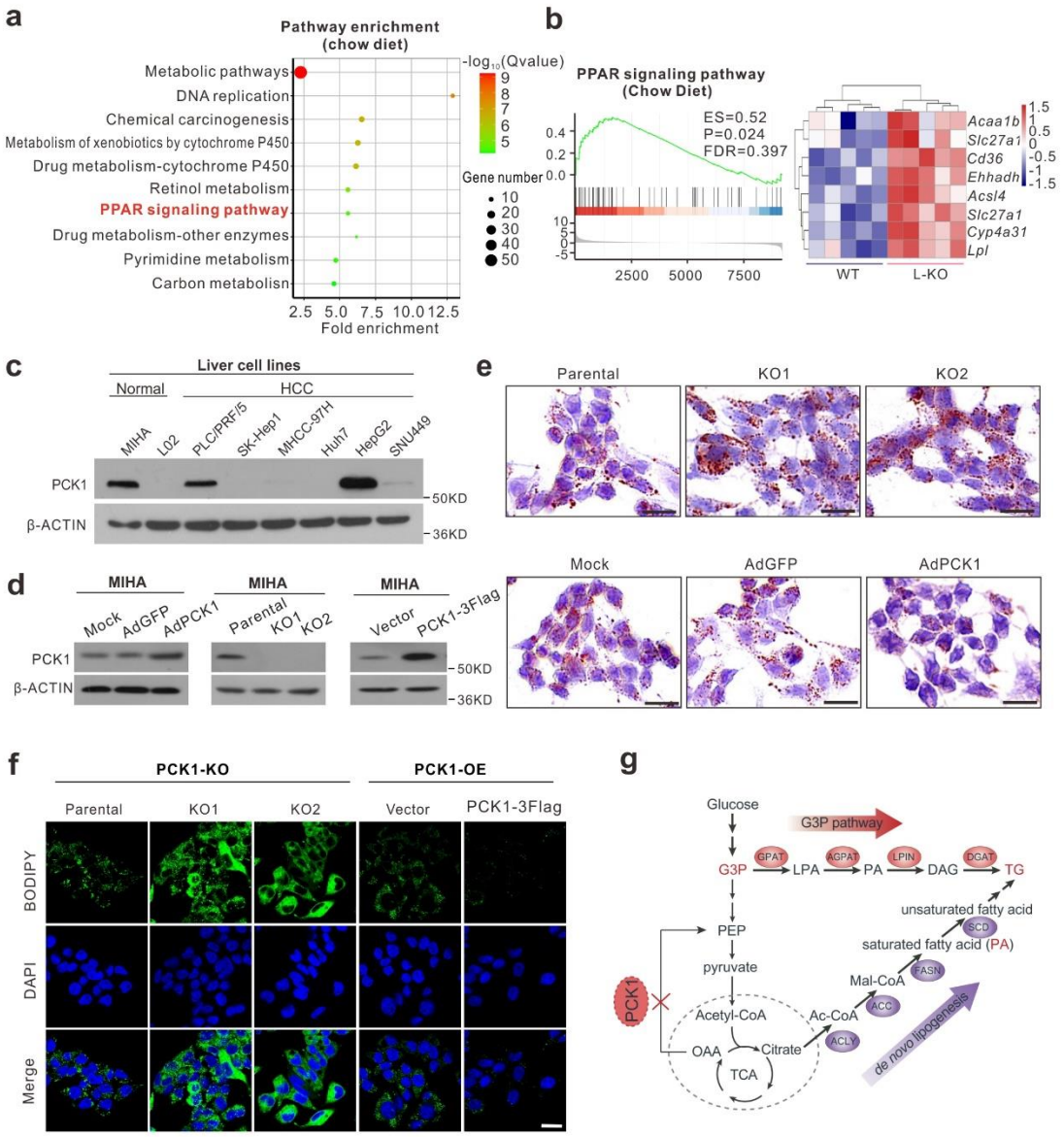
Supplementary Fig. 3 Pck1 depletion accelerates liver steatosis, inflammation and fibrosis in PTEN-null livers. a Representative PCR genotyping results from WT, *cPten*$^{f/f}$ and *cPten*$^{f/f}$*Pck1*$^{f/f}$ mice. **b** Western blot analysis of PCK1 and PTEN levels in livers from mice of the indicated genotypes, with β-actin serving as the loading control. **c** Liver weight to body weight (percentage) in three types of mice (n = 5). **d** ALT, AST, IL-6 and TNF-α levels in serum were detected using enzyme-linked immunosorbent assay

(ELISA) (n = 5). **e** Plasma levels of total triglycerides (TG), total cholesterol (TC), and free fatty acids (FFA) (n = 5). **f-g** Paraffin-embedded liver sections were stained with hematoxylin and eosin (H&E), Sirius Red, or immunostained for F4/80. Frozen sections were stained with Oil Red O. Scale bars: 50 μm. **h** Quantitative PCR analysis of liver mRNA expression (n = 5). Data are expressed as the mean ± SEM; n.s., not significant. *P* values obtained via one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.



Supplementary Fig. 4 AAV-mediated restoration of hepatic PCK1 alleviates the MAFLD phenotype in *Pck1*-null mice. For **a**-**e**, mice were administrated with AAV8-TBG-*Control* or AAV8-TBG-*Pck1* after 10 weeks of chow diet or HFCD-HF/G feeding, n=6/group. **a** qPCR analysis and western blot analysis (**b**) of exogenous *Pck1* expression levels in the livers of AAV–injected mice. *P* values obtained via 2-tailed unpaired Student's t tests. The samples were derived from the same experiment and the blots were processed in parallel. **c-d** Levels of serum TG, TC, FFA, ALT and AST concentrations were measured (n = 6). **e** qPCR analysis of genes associated with inflammatory infiltration, *de novo* lipogenesis and fibrogenesis in mice administrated with AAV8-TBG-Control or AAV8-TBG-*Pck1* (n = 6). n was the number of biologically independent mice. Data expressed as mean ± SEM; n.s., not significant. *P* values obtained via one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.

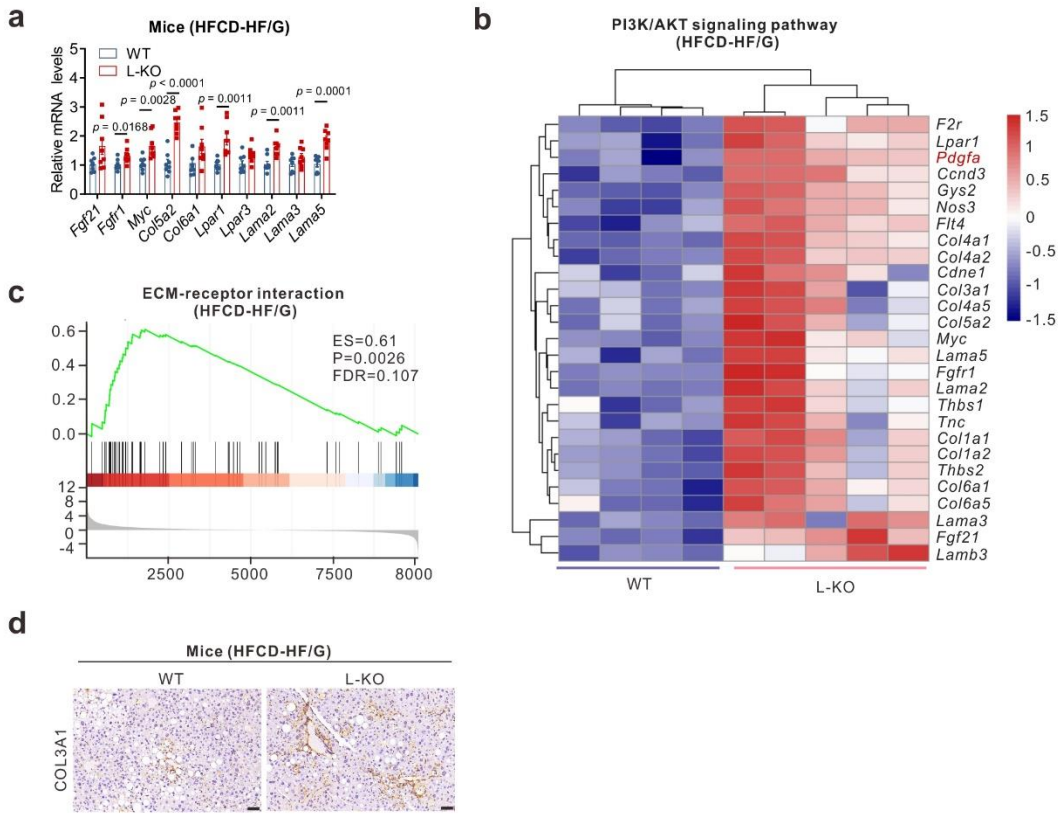
Supplementary Figure 5



Supplementary Fig. 5 Loss of PCK1 exacerbates lipid metabolism dysfunction *in vitro*. a Pathway enrichment analysis of L-KO mice fed chow diet (n=5). **b** GSEA revealed that the transcription level of PPAR signaling pathway was prominently upregulated in L-KO mice fed chow diet (n=5). **c** Expression levels of PCK1 in different cell lines were measured by western blot. The samples were derived from the same experiment and the blots were processed in parallel. **d** Protein levels of PCK1 were detected by western blot

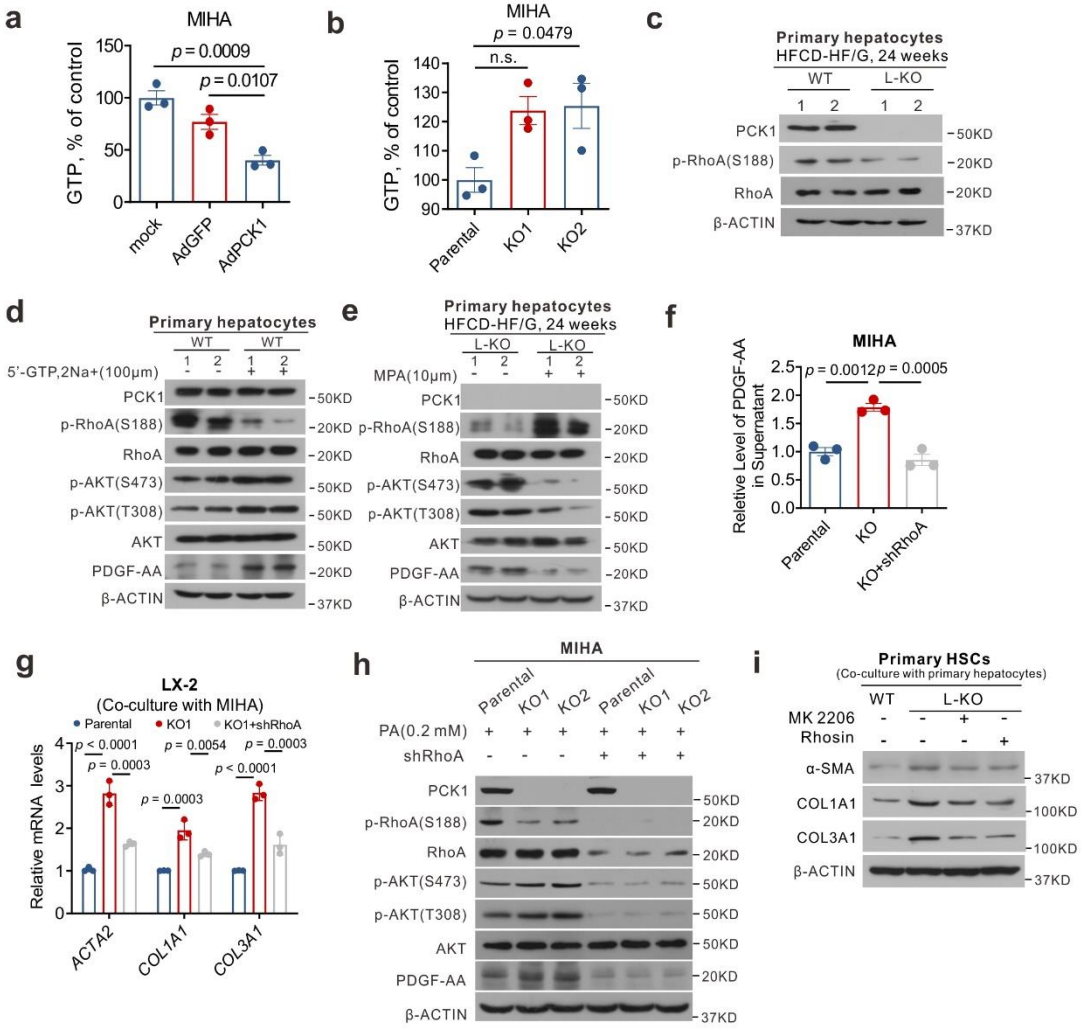
analysis in *PCK1-OE* and *PCK1-KO* MIHA cells. **e, f** Representative Oil Red O staining (**e**) and BODIPY staining (**f**) in *PCK1-OE* and *PCK1-KO* MIHA cells. Scale bars: 25 μm. The samples were derived from the same experiment. **g** Schematic presentation of the G3P pathway and DNL. G3P: glycerol-3-phosphate; PA: palmitic acid; DL: de novo lipogenesis. n was the number of biologically independent mice. Source data are provided as a Source Data file.

Supplementary Figure 6



Supplementary Fig. 6 PI3K/AKT signaling pathway and ECM-receptor interaction were activated in L-KO mice fed HFCD-HF/G. a mRNA levels of the indicated genes in the liver of WT and L-KO mice (n = 8). **b** Heatmap of expression levels of PI3K/AKT target genes in WT (n = 4) and L-KO mice (n = 5). **c** GSEA revealed the “ECM-receptor interaction” was upregulated in L-KO mice. **d** COL3A1 immunostaining in mice liver sections. Scale bars: 50 μm. n was the number of biologically independent mice. Data expressed as mean ± SEM; *P* values obtained via 2-tailed unpaired Student’s *t* tests. Source data are provided as a Source Data file.

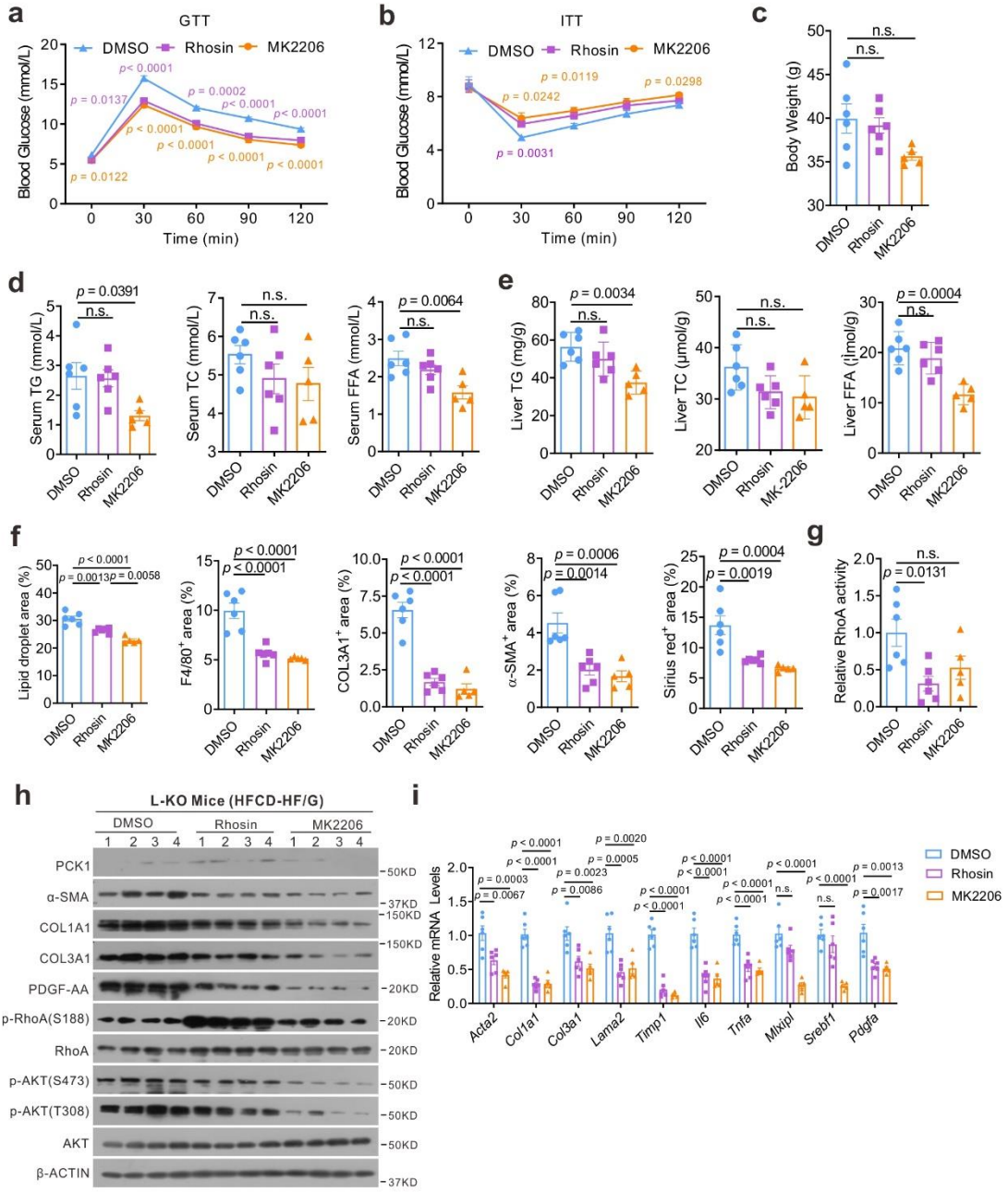
Supplementary Figure 7



Supplementary Fig. 7 PCK1 deficiency promoted the accumulation of GTP, and RhoA knockdown reversed the activation of PI3K/AKT/PDGF-AA axis. **a-b** The intracellular GTP levels in *PCK1*-OE (**a**) and *PCK1*-KO (**b**) cells treated with 0.2 mM PA were determined by HPLC. **c** Immunoblots of the indicated proteins in the primary hepatocytes isolated from WT and L-KO mice fed HFCD-HF/G. **d-e** Immunoblot analysis of AKT, p-AKT (S473 or T308), PDGF-AA, p-RhoA (S188) and RhoA in primary hepatocytes from HFCD-HF/G feeding mice. Cells were treated with 100 μM 5'-GTP, $2Na^+$ for 120 min (**d**) or 10 μM MPA for

48h (**e**). **f** Levels of PDGF-AA in the supernatant of *PCK1*-KO MIHA cells infected with either shControl or shRhoA treated with 0.2 mM PA. **g** Relative mRNA expression of *ACTA2*, *COL1A1*, and *COL3A1* in LX-2 cells co-cultured with *PCK1*-KO MIHA cells infected with either shControl or shRhoA. **h** Immunoblot analysis of indicated proteins in *PCK1*-KO MIHA cells infected with either shControl or shRhoA. **i** Isolated mouse primary hepatocytes from WT and L-KO mice fed HFCD-HF/G for 24 weeks were treated with MK2206, Rhosin or DMSO vehicle, and then co-cultured with primary hepatic stellate cell isolated from WT mice fed chow diet, and the protein levels were determined. For **a**, **b**, **f** and **g**, n = 3. Data expressed as mean ± SEM; n.s., not significant. *P* values obtained via one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.

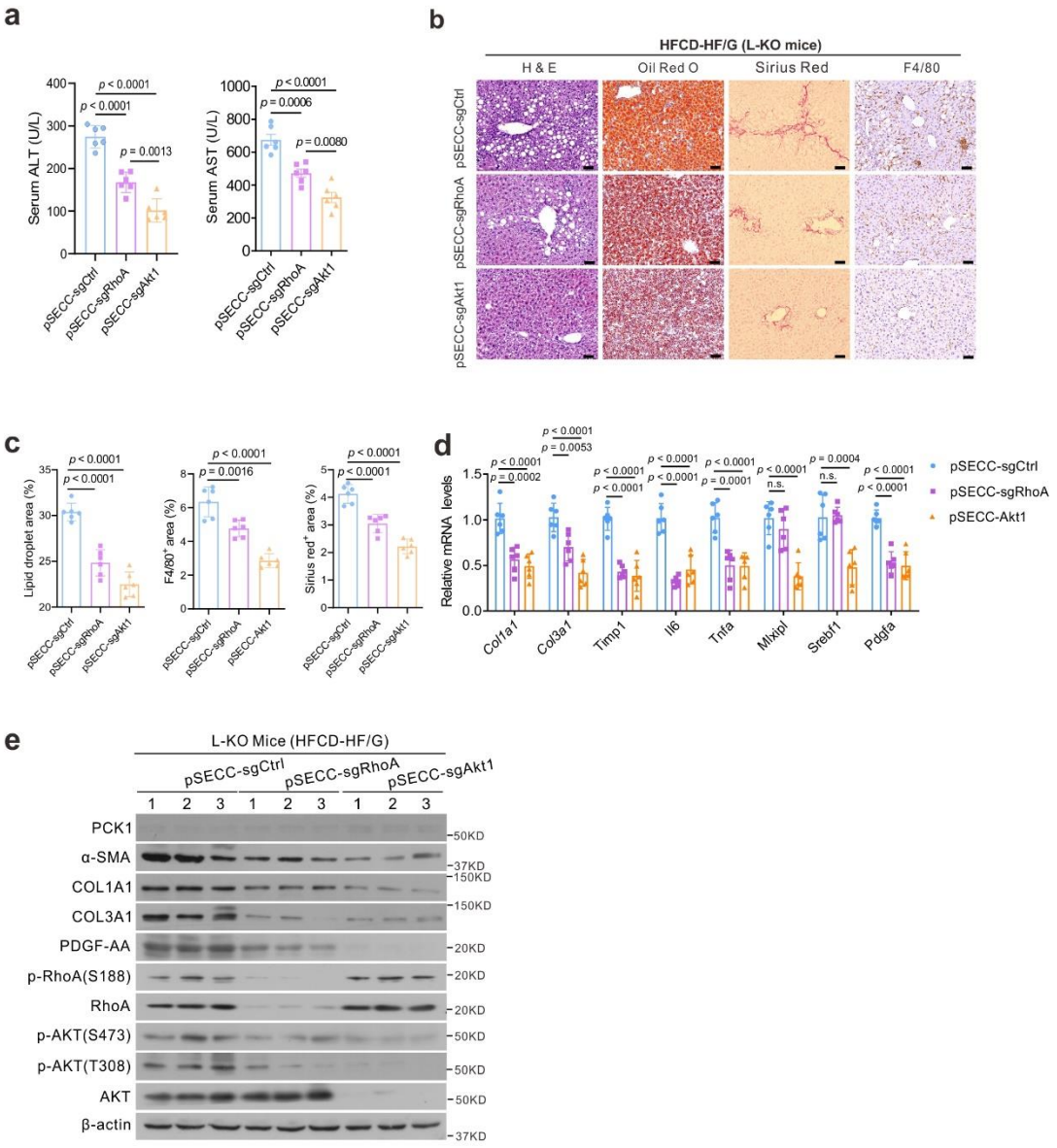
Supplementary Figure 8



Supplementary Fig. 8 RhoA/AKT inhibition partially relieves MAFLD phenotype in L-KO mice fed HFCD-HF/G. a, b Glucose levels measured during the glucose tolerance test (GTT) (**a**) and insulin tolerance test (ITT) (**b**). **c** Body weight of mice in the indicated groups. **d** Serum TG, TC, and FFA levels were measured using automated biochemical analyzer. **e** Liver TG, TC, and

FFA levels determined by colorimetry. **f** Quantification of liver sections of L-KO mice treated with DMSO, Rhosin or MK2206. **g** Reletive GTP-bound RhoA levels in mice liver tissues. **h** Expression of the indicated proteins in mice liver tissues. **i** mRNA levels of genes associated with lipid metabolism, fibrogenesis, and inflammatory infiltration. DMSO group (n = 6), Rhosin group (n = 6), MK2206 group (n = 5). n was the number of biologically independent mice. Data expressed as mean ± SEM; n.s., not statistically significant. *P* values obtained via one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.

Supplementary Figure 9



Supplementary Fig. 9 Genetic inhibition of RhoA or AKT1 protects mice from diet-induced MAFLD pathologies in L-KO mice. a Levels of AST and ALT in plasma (n = 6). **b** Representative HE staining, Oil Red O staining and immunohistochemical staining of liver tissues from L-KO mice infected with the control pSECC-sgTom lentivirus, the pSECC-sgAKT1 or the pSECC-sgRhoA lentivirus. Scale bars: 50 μm. **c** Quantification of the Oil Red O and immunohistochemical staining (n = 6). **d** Relative mRNA expression in liver

tissue of various groups was analyzed by qPCR (n = 6). **e** Western blot analysis of indicated protein expression in mice liver tissues. n was the number of biologically independent mice. Data expressed as mean ± SEM; not statistically significant. *P* values obtained via one-way ANOVA with Tukey's post hoc test. Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1. Clinical characteristics of participants with control and NASH patients.

	Control	NASH	*P* value
N (male/female)	10(5/5)	36(18/18)	NS
Age (years)	43.7 ± 4.94	44.64 ± 2.81	NS
BMI (kg/m^2)	21.34 ± 0.92	27.54 ± 0.76	<0.0001
ALT (U/L)	21.5 ± 4.54	136.2 ± 11.47	<0.0001
AST (U/L)	23.4 ± 1.56	74 ± 6.52	0.0002
γ-GT (U/L)	18.6 ± 1.69	83.17 ± 10.49	0.0025
TC (mmol/L)	2.30 ± 0.20	4.56 ± 0.20	<0.0001
TG (mmol/L)	1.09 ± 0.15	1.41 ± 0.12	NS
Glucose (mmol/L)	4.71 ± 0.17	7.79 ± 0.38	0.0001

Clinical and biochemical characteristics of NASH patients and controls. Data are expressed as mean ± SEM. Differences were analyzed by the two-tailed unpaired *t* test. BMI: body mass index; ALT: alanine transaminase; AST: aspartate transaminase; γ-GT: γ-glutamyl-transferase; TC: total cholesterol; TG: triglyceride.

Supplementary Table 2. Information on reagents.

Name	**Supplier**	**Cat no.**
MK2206	Selleckchem	S1078
Rhosin	MCE	HY-12646
Palmatic acid	Sigma Aldrich	P0500
BSA	Solarbio	A8020-100G
pAdTrack-TO4	Dr.T-C He, University of Chicago	N/A
pSEB-3Flag	Dr.T-C He, University of Chicago	N/A
AdEasy-BJ5183 E. coli	Dr.T-C He, University of Chicago	N/A
DH10B Chemically Competent E. coli	Dr.T-C He, University of Chicago	N/A
pReceiver-M02-ATF3	GeneCopoeia	EX-F0674-M02
AdGFP	Dr.T-C He, University of Chicago	N/A
AdPCK1	Lab stock[1]	N/A
pSEB-3Flag - PCK1	Lab stock[1]	N/A
LentiCRISPER-V2	Prof. Ding Xue, Tsinghua University	N/A
LentiCRISPER-*PCK1* KO1	Lab stock[1]	N/A
LentiCRISPER-*PCK1* KO2	Lab stock[1]	N/A

Supplementary Table 3. Primer sequences used in this study.

Name	Sequence	Supplier
sub-clone primer: *PCK1*-Forward	CGCGGATCCACCATGGGCCCTCCTCAGCTGCA AAACGGCC	TsingKe Biological Technology, China
sub-clone primer: *PCK1*-Reverse	CCCAAGCTTCTACATCTGGCTTATTCTTTGCTT CAAG	TsingKe Biological Technology, China
real-time PCR: *PCK1*-Forward	ATGGAGGAAGAGGGCATCCT	TsingKe Biological Technology, China
real-time PCR: *PCK1*-Reverse	ACGTACATGGTGCGACCTTT	TsingKe Biological Technology, China
real-time PCR: *β-ACTIN*-Forward	AGGCCAACCGCGAGAAGATGACC	TsingKe Biological Technology, China
real-time PCR: *β-ACTIN*-Reverse	GAAGTCCAGGGCGACGTAGCAC	TsingKe Biological Technology, China
real-time PCR: *ACTA2*-Forward	GGGGTGATGGTGGGAATG	TsingKe Biological Technology, China
real-time PCR: *ACTA2*-Reverse	GCAGGGTGGGATGCTCTT	TsingKe Biological Technology, China
real-time PCR: *COL1A1*-	GACGGCTCAGAGTCACCCA	TsingKe Biological Technology, China

Forward		
real-time PCR: *COL1A1*-Reverse	GGAGACCACGAGGACCAGA	TsingKe Biological Technology, China
real-time PCR: *COL3A1*-Forward	GCTCGGGGTAATGACGGT	TsingKe Biological Technology, China
real-time PCR: *COL3A1*-Reverse	AGGAATGCCAGCGGGAC	TsingKe Biological Technology, China
real-time PCR: *PDGFA*-Forward	TGTCAAGTGCCAGCCCTCC	TsingKe Biological Technology, China
real-time PCR: *PDGFA*-Reverse	CCGTGTCCTCTTCCCGATAAT	TsingKe Biological Technology, China
real-time PCR: *TIMP1*-Forward	GCTTCTGGCATCCTGTTGTT	TsingKe Biological Technology, China
real-time PCR: *TIMP1*-Reverse	TGGTTGACTTCTGGTGTCCC	TsingKe Biological Technology, China
real-time PCR: *SERPINH1*-Forward	GCCATGTTCTTCAAGCCACA	TsingKe Biological Technology, China
real-time PCR:	CTTTTCCTTCTCGTCGTCGTA	TsingKe Biological

SERPINH1-Reverse		Technology, China
real-time PCR: *GFAP*-Forward	GCACGCAGTATGAGGCAATG	TsingKe Biological Technology, China
real-time PCR: *GFAP*-Reverse	CCAGGTCGCAGGTCAAGGA	TsingKe Biological Technology, China
real-time PCR: *MMP2*-Forward	TTTGACGGTAAGGACGGACTC	TsingKe Biological Technology, China
real-time PCR: *MMP2*-Reverse	CCTGGAAGCGGAATGGAAAC	TsingKe Biological Technology, China
real-time PCR: *VIM*-Forward	GAGAACTTTGCCGTTGAAGC	TsingKe Biological Technology, China
real-time PCR: *VIM*-Reverse	TCCAGCAGCTTCCTGTAGGT	TsingKe Biological Technology, China
real-time PCR: *PGC1A*-Forward	GATGGCCTGTTTGATGACAG	TsingKe Biological Technology, China
real-time PCR: *PGC1A*-Reverse	TTTGGGTGGTGACACAGAAT	TsingKe Biological Technology, China
real-time PCR: *FOXO1*-Forward	TGTCAACCTATGGCAGCCAG	TsingKe Biological Technology, China
real-time PCR: *FOXO1*-Reverse	GCAGAGGCACTTGTACAGGT	TsingKe Biological Technology, China

real-time PCR: *ATF3*-Forward	TGAGTGCTTCTGCCATCGTC	TsingKe Biological Technology, China
real-time PCR: *ATF3*-Reverse	GGCTACCTCGGCTTTTGTG	TsingKe Biological Technology, China
real-time PCR: *HNF4A*-Forward	GCCTACCTCAAAGCCATCAT	TsingKe Biological Technology, China
real-time PCR: *HNF4A*-Reverse	CGGTCGTTGATGTAGTCCTC	TsingKe Biological Technology, China
real-time PCR: *CEBPB*-Forward	TCGCAGGTCAAGAGCAAGG	TsingKe Biological Technology, China
real-time PCR: *CEBPB*-Reverse	GAACAAGTTCCGCAGGGTG	TsingKe Biological Technology, China
real-time PCR: *SREBF1*-Forward	TCTGGAGGCATCGCAAGC	TsingKe Biological Technology, China
real-time PCR: *SREBF1*-Reverse	CAGCAGGTGACGGATGAGG	TsingKe Biological Technology, China
real-time PCR: *NR1H3*-Forward	GGTACAACCCTGGGAGTGAGA	TsingKe Biological Technology, China
real-time PCR: *NR1H3*-Reverse	TGGGGATGGTGGATGGAG	TsingKe Biological Technology, China

real-time PCR: *MLXIPL*-Forward	GTCGGCAATGCTGACATGA	TsingKe Biological Technology, China
real-time PCR: *MLXIPL*-Reverse	GCTGAAGAGGGAGTCAACCAC	TsingKe Biological Technology, China
real-time PCR: *NR1H4*-Forward	AACTCACCCCAGATCAACAGAC	TsingKe Biological Technology, China
real-time PCR: *NR1H4*-Reverse	GCTTCAACCGCAGACCCT	TsingKe Biological Technology, China
real-time PCR: *Il6*-Forward	GTTGTGCAATGGCAATTCTGA	TsingKe Biological Technology, China
real-time PCR: *Il6*- Reverse	AAGGACTCTGGCTTTGTCTTTCT	TsingKe Biological Technology, China
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real-time PCR: *Il10*-Forward	GGTTGCCAAGCCTTATCGG	TsingKe Biological Technology, China

real-time PCR: *Il10*-Reverse	ATTTTCACAGGGGAGAAATCG	TsingKe Biological Technology, China
real-time PCR: *Ccl2*-Forward	TGTGCTGACCCCAAGAAGG	TsingKe Biological Technology, China
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real-time PCR: *Acta2*-Forward	CCCTGAAGAGCATCCGACA	TsingKe Biological Technology, China
real-time PCR: *Acta2*- Reverse	CATCTCCAGAGTCCAGCACAA	TsingKe Biological Technology, China
real-time PCR: *Col1a1*-Forward	ACCCTGCCCGCACATG	TsingKe Biological Technology, China
real-time PCR: *Col1a1*- Reverse	CCCTCGCTTCCGTACTCG	TsingKe Biological Technology, China
real-time PCR: *Lama2*-Forward	TCCAGCCAAACCATCAGTCC	TsingKe Biological Technology, China
real-time PCR: *Lama2*- Reverse	CCACAAGAAGGTCCAATCCAAC	TsingKe Biological Technology, China
real-time PCR: *Timp1*-Forward	CCCAGAAATCAACGAGACCA	TsingKe Biological Technology, China
real-time PCR: *Timp1*-Reverse	ACGCCAGGGAACCAAGAA	TsingKe Biological Technology, China

real-time PCR: *Col3a1*-Forward	GCCCACAGCCTTCTACACCT	TsingKe Biological Technology, China
real-time PCR: *Col3a1*-Reverse	TCCCGGATAGCCACCCA	TsingKe Biological Technology, China
real-time PCR: *Acly*-Forward	CTCATTGAACCCTTCGTCCC	TsingKe Biological Technology, China
real-time PCR: *Acly* - Reverse	CCTCGGTATTCAGCTTTTCGT	TsingKe Biological Technology, China
real-time PCR: *Fasn*-Forward	CTGCCTCCGTGGACCTTATC	TsingKe Biological Technology, China
real-time PCR: *Fasn* - Reverse	GCACAGACACCTTCCCGTCA	TsingKe Biological Technology, China
real-time PCR: *Acaca*–Forward	ATTGCCTATGAACTCAACAGCG	TsingKe Biological Technology, China
real-time PCR: *Acaca* - Reverse	TGACAAGGTGGCGTGAAGG	TsingKe Biological Technology, China
real-time PCR: *Scd1*-Forward	TTCCTTATCATTGCCAACACCA	TsingKe Biological Technology, China
real-time PCR: *Scd1*- Reverse	TCGCCCCAGCAGTACCAG	TsingKe Biological Technology, China
real-time PCR: *Elovl6*-Forward	TGCGGGCTGCGGGTTT	TsingKe Biological Technology, China

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real-time PCR: *Cpt1a*-Forward	TGTCCAAGTATCTGGCAGTCG	TsingKe Biological Technology, China
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real-time PCR: *Mlxipl*-Reverse	TCAAATAAAGGTCGGATGAGGA	TsingKe Biological Technology, China
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real-time PCR: Srebf1- Reverse	TGGTAGACAACAGCCGCATC	TsingKe Biological Technology, China
real-time PCR: *Ppara*-Forward	GACATTTCCCTGTTTGTGGCT	TsingKe Biological Technology, China
real-time PCR: *Ppara*-Reverse	GCTGCGTCGGACTCGGT	TsingKe Biological Technology, China
real-time PCR: *Cd36*-Forward	ACTGTGGGCTCATTGCTGG	TsingKe Biological Technology, China
real-time PCR: *Cd36*-Reverse	TGATTTTGCTGCTGTTCTTTGC	TsingKe Biological Technology, China

real-time PCR: *Slc27a1*-Forward	GCTCCTGCGGCTTCAACA	TsingKe Biological Technology, China
real-time PCR: *Slc27a1*-Reverse	GCGCTATCGCCCTTTCG	TsingKe Biological Technology, China
real-time PCR: *Cidea*-Forward	CCTGGTTACGCTGGTGCTG	TsingKe Biological Technology, China
real-time PCR: *Cidea*-Reverse	TGGCTATTCCCGATTTCTTTG	TsingKe Biological Technology, China
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real-time PCR: *Pdgfa*-Reverse	CCTTCCTGTCTCCTCCTCCC	TsingKe Biological Technology, China
real-time PCR: *Igf1*-Forward	GGACCGAGGGGCTTTTACT	TsingKe Biological Technology, China
real-time PCR: *Igf1*-Reverse	ATAGAGCGGGCTGCTTTTG	TsingKe Biological Technology, China
real-time PCR: Vegfa-Forward	CTACTGCCGTCCGATTGAGA	TsingKe Biological Technology, China
real-time PCR: *Vegfa*-Reverse	CTGGCTTTGGTGAGGTTTGAT	TsingKe Biological Technology, China
real-time PCR: *Fgf21*-Forward	GCATACCCCATCCCTGACTC	TsingKe Biological Technology, China
real-time PCR: *Fgf21*-Reverse	GGCTGTTGGCAAAGAAACCTA	TsingKe Biological Technology, China

real-time PCR: *Fgfr1*-Forward	GGATTCTGTGGTGCCTTCTGA	TsingKe Biological Technology, China
real-time PCR: *Fgfr1*-Reverse	TTGTCTGGCCCGATCTTACTC	TsingKe Biological Technology, China
real-time PCR: *Myc*-Forward	GACTGTATGTGGAGCGGTTTCT	TsingKe Biological Technology, China
real-time PCR: *Myc*-Reverse	TCGTTGAGCGGGTAGGGA	TsingKe Biological Technology, China
real-time PCR: *Col5a2*-Forward	TGTGCGGGGCAGTGTAGG	TsingKe Biological Technology, China
real-time PCR: *Col5a2*-Reverse	TCCCAGGGTCTGTTTTGTTTG	TsingKe Biological Technology, China
real-time PCR: *Col6a1*-Forward	GGGGTCAAAGGGGCAAAG	TsingKe Biological Technology, China
real-time PCR: *Col6a1*-Reverse	GGCAATCTCAAAGTTCTGTAGGC	TsingKe Biological Technology, China
real-time PCR: *Lpar1*-Forward	TCCATACACGAATGAGCAACC	TsingKe Biological Technology, China
real-time PCR: *Lpar1*-Reverse	TGGCGAACATAGCCAAAGAT	TsingKe Biological Technology, China
real-time PCR: *Lpar3*-Forward	CTGCTCGCACTGCTCAACTC	TsingKe Biological Technology, China

real-time PCR: *Lpar3*-Reverse	CTGGCTGCCCGTCTCG	TsingKe Biological Technology, China
real-time PCR: *Gpat3*-Forward	CTTCCAGACAGCAGCCTCAA	TsingKe Biological Technology, China
real-time PCR: *Gpat3*-Reverse	TCCCCATCAATCCACCGT	TsingKe Biological Technology, China
real-time PCR: *Gpat4*-Forward	CAAGCCCTACACCAACGGAA	TsingKe Biological Technology, China
real-time PCR: *Gpat4*-Reverse	TGGCAGGAGGAAGCAATACC	TsingKe Biological Technology, China
real-time PCR: *Agpat2*-Forward	CTGCTGTTGCTGCTTGTGC	TsingKe Biological Technology, China
real-time PCR: *Agpat2*-Reverse	CCTCCAGTTTCTTCTGTCCG	TsingKe Biological Technology, China
real-time PCR: *Agpat3*-Forward	GCTTGCCTACCTGAAGACCC	TsingKe Biological Technology, China
real-time PCR: *Agpat3*-Reverse	CCAAACCGCTCGCACATC	TsingKe Biological Technology, China
real-time PCR: *Lpin1*-Forward	TGCTCATCCACCAGAGTAAGG	TsingKe Biological Technology, China
real-time PCR: *Lpin1*-Reverse	TCCGTGAGGTCGTCCAGAT	TsingKe Biological Technology, China

real-time PCR: *Lpin2*-Forward	CCCCTCCTGGGATTCTGTC	TsingKe Biological Technology, China
real-time PCR: *Lpin2*-Reverse	TGAAAAGGCGAGCACTGGTA	TsingKe Biological Technology, China
real-time PCR: *Lpin3*-Forward	GGATGACCCAAACCTCGTG	TsingKe Biological Technology, China
real-time PCR: *Lpin3*-Reverse	TTGCGGCTTTCTCCCTCT	TsingKe Biological Technology, China
real-time PCR: *Dgat1*-Forward	AAGACGGGCGGACCAGC	TsingKe Biological Technology, China
real-time PCR: *Dgat1*-Reverse	CACCAGGATGCCATACTTGATA	TsingKe Biological Technology, China
real-time PCR: *Dgat2*-Forward	CTGCGGGGTGAGCGTC	TsingKe Biological Technology, China
real-time PCR: *Dgat2*-Reverse	ACCTTTCTTGGGCGTGTTC	TsingKe Biological Technology, China
real-time PCR: *Mmp1*-Forward	GGCTGAAAGTGACTGGGAAAC	TsingKe Biological Technology, China
real-time PCR: *Mmp1*-Reverse	TGGCAAATCTGGCGTGTAA	TsingKe Biological Technology, China
real-time PCR: *Mmp9*-Forward	GCCCTGAACCTGAGCCA	TsingKe Biological Technology, China

real-time PCR: *Mmp9*-Reverse	ACTTCCCATCCTTGAACAAATAC	TsingKe Biological Technology, China
real-time PCR: *Lama2*-Forward	TCCAGCCAAACCATCAGTCC	TsingKe Biological Technology, China
real-time PCR: *Lama2*-Reverse	CCACAAGAAGGTCCAATCCAAC	TsingKe Biological Technology, China
real-time PCR: *Lama3*-Forward	CCGCTCGGGCTCCTATT	TsingKe Biological Technology, China
real-time PCR: *Lama3*-Reverse	ACATGCTGCTTGCACTGACA	TsingKe Biological Technology, China
real-time PCR: *Lama5*-Forward	GGAATATGTCGGTGAGGATTCA	TsingKe Biological Technology, China
real-time PCR: *Lama5*-Reverse	TCCAGGTAGAAGATGGCTAGATG	TsingKe Biological Technology, China
real-time PCR: *β-Actin*-Forward	CGTTCAATACCCCAGCCATG	TsingKe Biological Technology, China
real-time PCR: *β-Actin*-Reverse	GACCCCGTCACCAGAGTCC	TsingKe Biological Technology, China
ChIP qPCR: *PCK1*-Forward 1	CCCAAAGCATAACTGACCCTG	TsingKe Biological Technology, China
ChIP qPCR: *PCK1*-Reverse 1	TTAAATACTGTGGAAAAGAATAGCC	TsingKe Biological Technology, China

ChIP qPCR: *PCK1*-Forward 2	TGGTTGAGGGCTCGAAGTC	TsingKe Biological Technology, China
ChIP qPCR: *PCK1*-Reverse 2	ACGGCCAGGGTCAGTTATG	TsingKe Biological Technology, China
ChIP qPCR: *PCK1*-Forward 3	CCCAGCATTCATTAACAACTATCT	TsingKe Biological Technology, China
ChIP qPCR: *PCK1*-Reverse 3	TGCTTGGTGGCAGAACCTC	TsingKe Biological Technology, China
sgRNA: *PCK1*-Forward	CACCGGCTGAAGAAGTATGACAAC	TsingKe Biological Technology, China
sgRNA: *PCK1*-Reverse	AAACGTTGTCATACTTCTTCAGCC	TsingKe Biological Technology, China
TA cloning: *PCK1*-Forward	AACCTGTGGATCTCCCTTC	TsingKe Biological Technology, China
TA cloning: *PCK1*-Reverse	CAAATCAATGTTCCGCTCA	TsingKe Biological Technology, China
shRNA: *ATF3* -Forward 1	TGCAAAGTGCCGAAACAAGATTCAAGAGATCTT GTTTCGGCACTTTGCTTTTTTC	TsingKe Biological Technology, China
shRNA: *ATF3* -Reverse 1	TCGAGAAAAAAGCAAAGTGCCGAAACAAGATC TCTTGAATCTTGTTTCGGCACTTTGCA	TsingKe Biological Technology, China

shRNA: *ATF3* -Forward 2	TGAGAAACCTCTTTATCCAATTCAAGAGATTGG ATAAAGAGGTTTCTCTTTTTTC	TsingKe Biological Technology, China
shRNA: *ATF3* -Reverse 2	TCGAGAAAAAAGAGAAACCTCTTTATCCAATCT CTTGAATTGGATAAAGAGGTTTCTCA	TsingKe Biological Technology, China
shRNA: *ATF3* -Forward 3	TGGACTCCAGAAGATGAGAGTTCAAGAGACTC TCATCTTCTGGAGTCCTTTTTTC	TsingKe Biological Technology, China
shRNA: *ATF3* -Reverse 3	TCGAGAAAAAAGGACTCCAGAAGATGAGAGTC TCTTGAACTCTCATCTTCTGGAGTCCA	TsingKe Biological Technology, China
Genotyping of *Pck1*-Forward	TCTGTCAGTTCAATACCAATCT	TsingKe Biological Technology, China
Genotyping of *Pck1*-Reverse	AATGTTCTCTGCAAGTCCTGGTG	TsingKe Biological Technology, China
Genotyping of *Pten*-Forward	ATCCCCACCAATGAACAAAC	TsingKe Biological Technology, China
Genotyping of *Pten*-Reverse	CTCCTCTACTCCATTCTTCCC	TsingKe Biological Technology, China
Genotyping of *Cre*-Forward	CACCCTGTTACGTATAGCCG	TsingKe Biological Technology, China

Genotyping of *Cre*-Reverse	GAGTCATCCTTAGCGCCGTA	TsingKe Biological Technology, China

Supplementary Table 4. The antibody information.

Name	Supplier	Cat no.	Clone no.	Dilution
PCK1	Bioworld Technology, USA	BS6870	Polyclonal	1:2000
PPARα	Proteintech, USA	15540-1-AP	Polyclonal	1:1000
CD36	Abcam, USA	Ab133625	EPR6573	1:1000
FATP1	Affinity Biosciences, USA	DF7716	Polyclonal	1:1000
α-SMA	Cell Signaling Technology, USA	19245T	D4K9N	1:1000
PDGF-AA	Abcam, USA	Ab216619	Polyclonal	1:1000
CIDEC	Novus Biologicals, USA	NB100-430SS	Polyclonal	1:1000
β-ACTIN	ZSGB-BIO, China	TA-09	OTI1	1:2000
CIDEA	Proteintech, USA	13170-1-AP	Polyclonal	1:1000
COL1A1	Abcam, USA	Ab34710	Polyclonal	1:1000
p-AKT (S473)	Bioworld Technology, USA	BS4007	Polyclonal	1:1000
p-AKT (T308)	Bioworld Technology, USA	AP0056	Polyclonal	1:1000
AKT	Bioworld Technology, USA	AP0059	Polyclonal	1:1000

p-RhoA (S188)	Abcam, USA	Ab41435	Polyclonal	1:1000
RhoA	Abcam, USA	Ab187027	EPR18134	1:1000
F4/80	Cell Signaling Technology, USA	70076T	D2S9R	1:1000
ATF3	Abcam, USA	Ab207434	EPR19488	1:1000
COL3A1	Proteintech	22734-1-AP	Polyclonal	1:1000
RhoA	Cell Signaling Technology, USA	2117T	67B9	1:1000
RhoB	Cell Signaling Technology, USA	2098T	Polyclonal	1:1000
RhoC	Cell Signaling Technology, USA	3430T	D40E4	1:1000
RAC1/2/3	Cell Signaling Technology, USA	2465T	Polyclonal	1:1000
p-RAC1	Cell Signaling Technology, USA	2461T	Polyclonal	1:1000
CDC42	Cell Signaling Technology, USA	2466T	11A11	1:1000
PI3 Kinase p85	Cell Signaling Technology, USA	4257	19H8	1:1000
Rabbit Anti-	Abcam	Ab6728	Polyclonal	1:5000

Mouse IgG H&L				
Goat Anti-Rabbit IgG H&L	Abcam	Ab6721	Polyclonal	1:5000

Supplementary References

1. Tuo, L. *et al.* PCK1 negatively regulates cell cycle progression and hepatoma cell proliferation via the AMPK/p27Kip1 axis. *J. Exp. Clin. Cancer Res.* **38**, 50 (2019).