

Figure S1

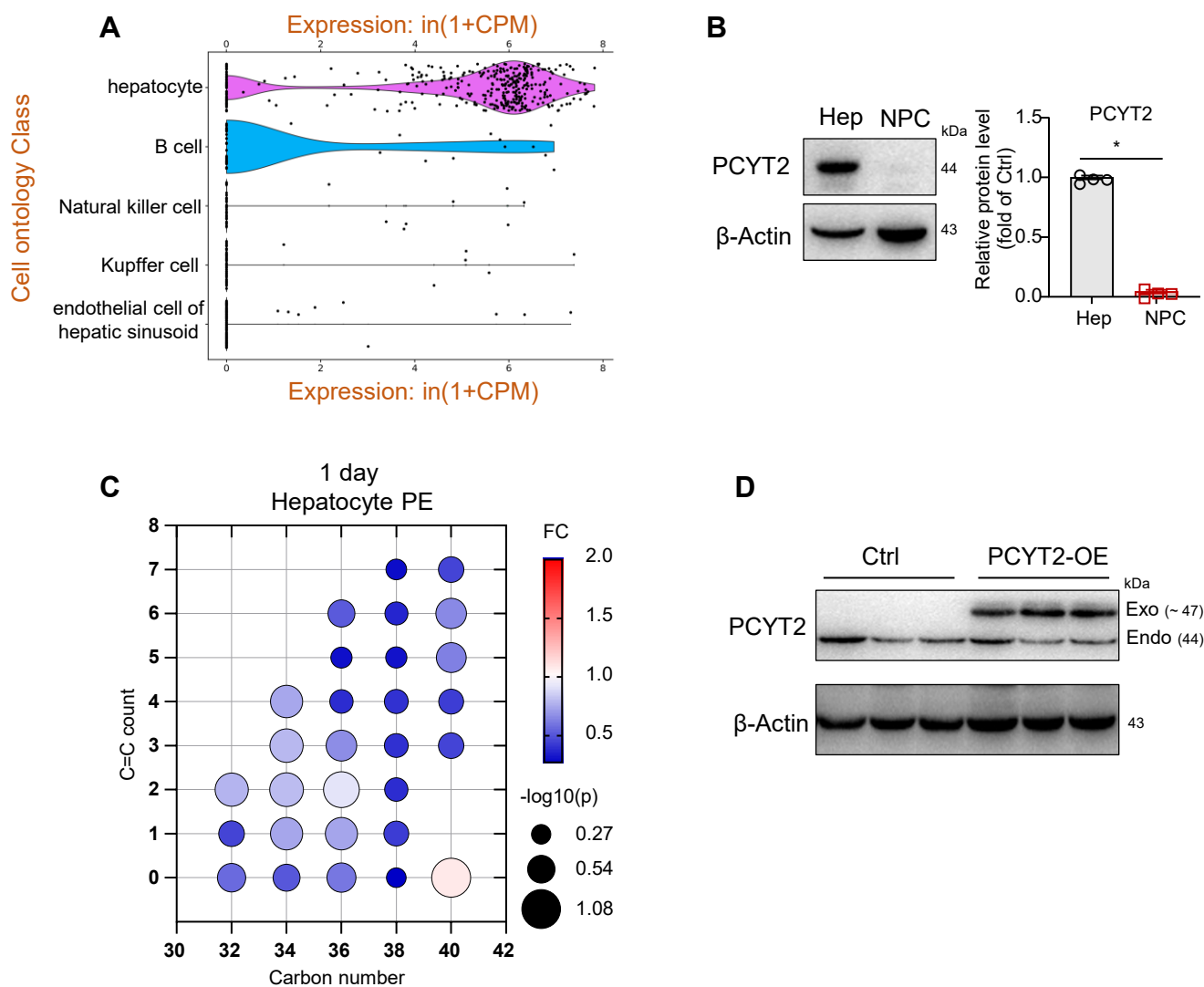


Figure S1. *Pcyt2* is expressed mainly in hepatocytes in the mouse liver and was overexpressed by PCYT2-expressing AAVs. (A) The single-cell sequencing database Tabula Muris shows the expression pattern of *Pcyt2* in diverse liver cells. (B) Hepatocytes and non-parenchymal cells (NPC) were isolated from mouse livers. Western blot analysis of the protein levels of PCYT2. $n = 4$ mice per group. (C) Bubble diagram of PE levels in isolated hepatocytes at 1 day after surgery. $n = 8$ mice per group. (D) Eight-week-old mice were administered AAV-*Pcyt2*-flags bearing the TBG promoter or control AAV through tail vein injection. Western blot analysis of the protein levels of PCYT2 in the liver. β -Actin was used as the internal control. $n = 6$ mice per group. Data present the mean $\pm$ SEM. * $p < 0.05$. Exo, exogenous; Endo, endogenous.

Figure S2

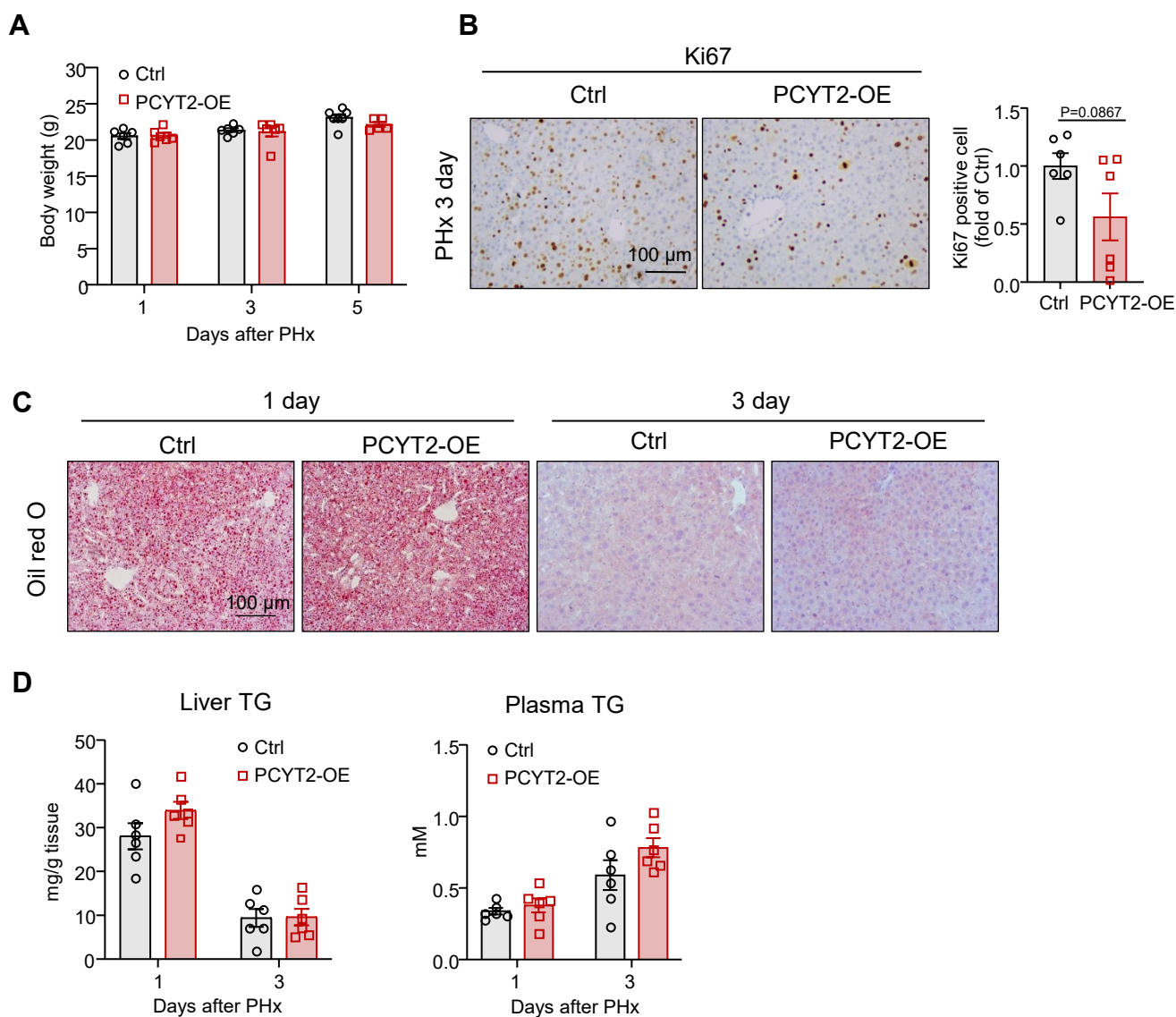


Figure S2. Effects of PCYT2 overexpression on Ki67-positive cell counts and lipid accumulation in the liver after 70% partial hepatectomy (PHx). Eight-week-old mice were administered AAV-PCYT2-flags bearing the TBG promoter or control AAV through tail vein injection. After 10 days, the mice were subjected to 70% PHx, and the tissues were harvested for analysis at the indicated time points. **(A)** Body weights of the mice. **(B)** Immunohistochemical staining of Ki67 in liver sections from mice at 3 days (scale bar = 100 μm). **(C)** Oil red O staining of the liver (scale bar = 100 μm). **(D)** Plasma and liver triglyceride (TG) contents. n = 5–7 mice per group. Data present the mean ± SEM. *p < 0.05. TG, triglyceride.

Figure S3

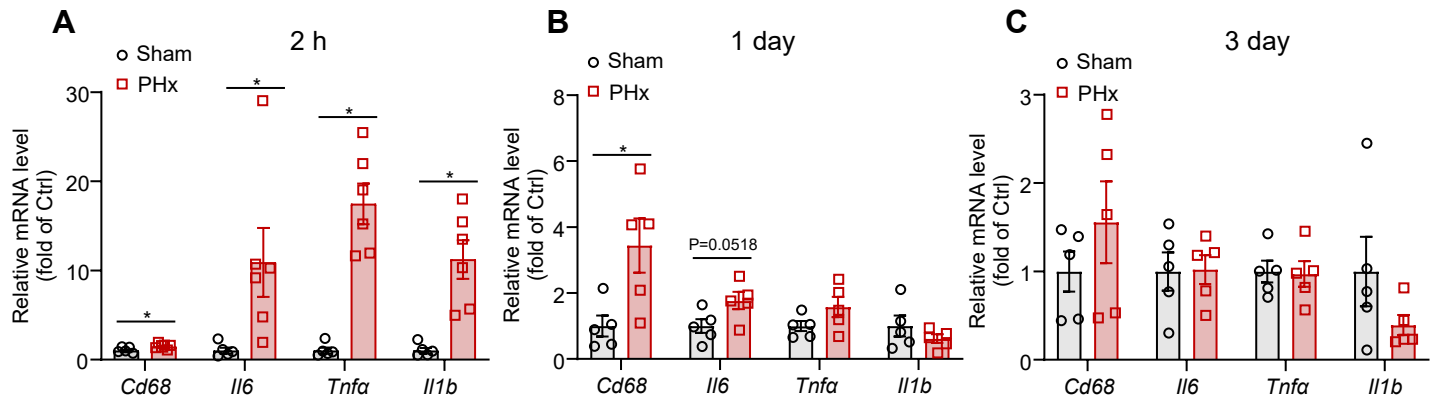


Figure S3. The expression of inflammatory factors in the liver after partial hepatectomy (PHx). Eight-week-old mice were subjected to 70% PHx or sham surgery. qPCR analysis of the mRNA levels of inflammatory factors in mouse livers at 2 h (A), 1 day (B) or 3 days postoperatively (C). $n = 5-6$ mice per group. Data present the mean $\pm$ SEM. $*p < 0.05$.

Figure S4

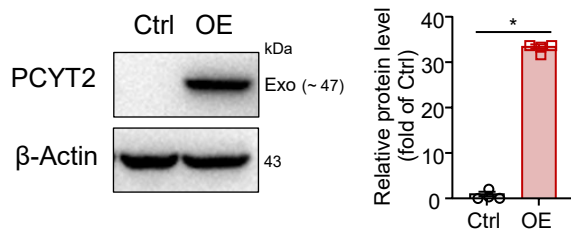


Figure S4. PCYT2 was overexpressed in primary mouse hepatocytes via adenovirus.

Primary mouse hepatocytes were treated with Ad-PCYT2 or Ad-Ctrl for 48 h. Western blot analysis of the protein levels of PCYT2. β-Actin was used as the internal control. n = 4 independent experiments. Data present the mean $\pm$ SEM. *p < 0.05. OE, PCYT2 overexpression. Exo, exogenous.

Figure S5

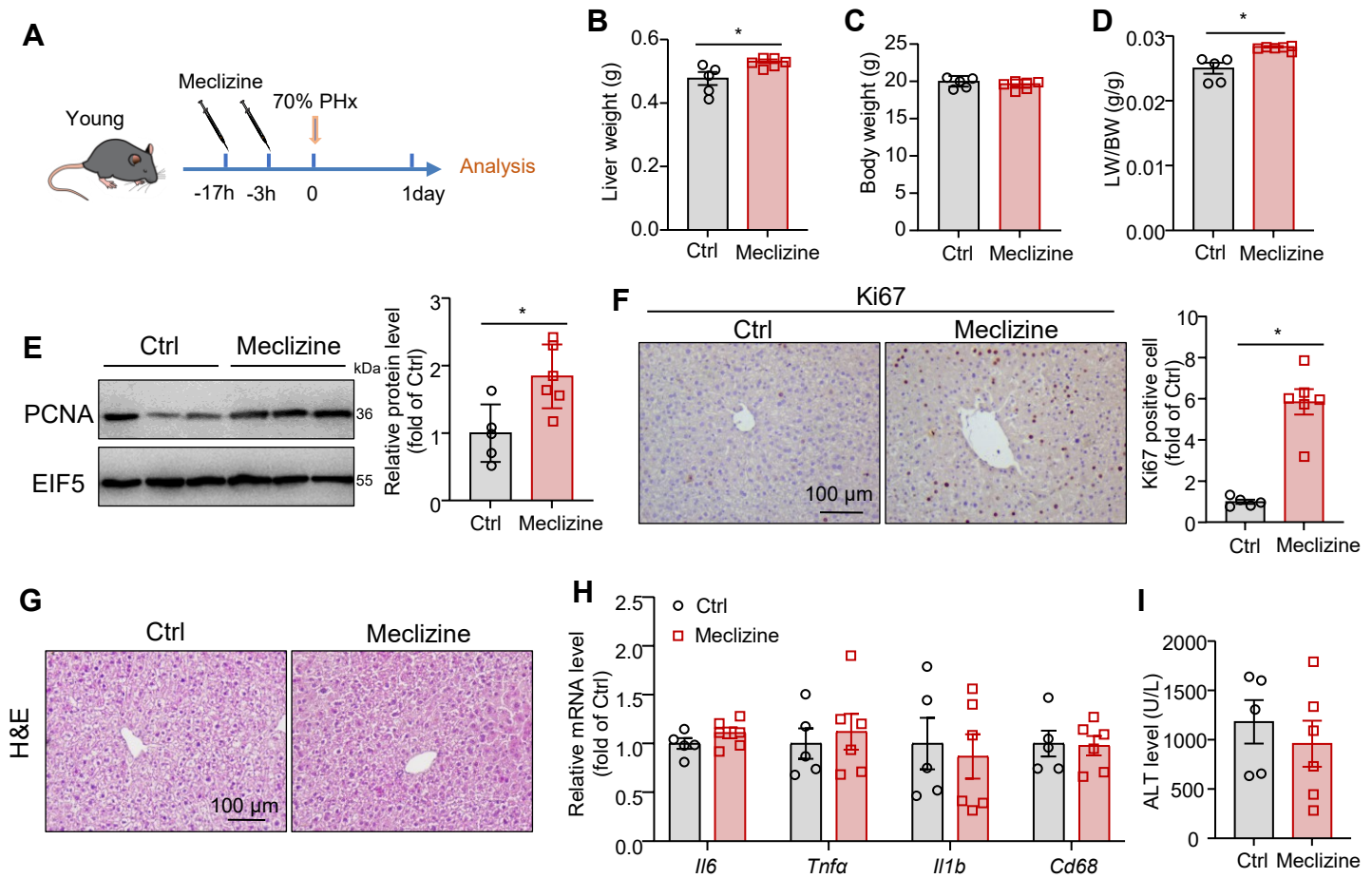


Figure S5. Inhibition of PCYT2 by meclizine promoted liver regeneration in eight-week-old mice. Eight-week-old mice were administered 60 mg/kg meclizine or vehicle 17 h and 3 h before 70% PHx, and then the tissues were harvested for analysis at 1 day after surgery (**A**). (**B**) Liver weights, (**C**) body weights, (**D**) and liver-to-body weight ratios of the mice. (**E**) Western blot analysis of the protein levels of PCNA in the liver. EIF5 was used as the internal control. (**F**) Immunohistochemical staining of Ki67 in liver sections (scale bar = 100 μ m) and quantification of Ki67-positive cells. (**G**) H&E staining of liver sections (scale bar = 100 μ m). (**H**) qPCR analysis of the mRNA levels of inflammatory factors in the liver. (**I**) Plasma ALT levels. $n = 5$ in the control group, $n = 6$ in the meclizine group. Data present the mean $\pm$ SEM. * $p < 0.05$. LW/BW, liver-to-body weight ratios.

Figure S6

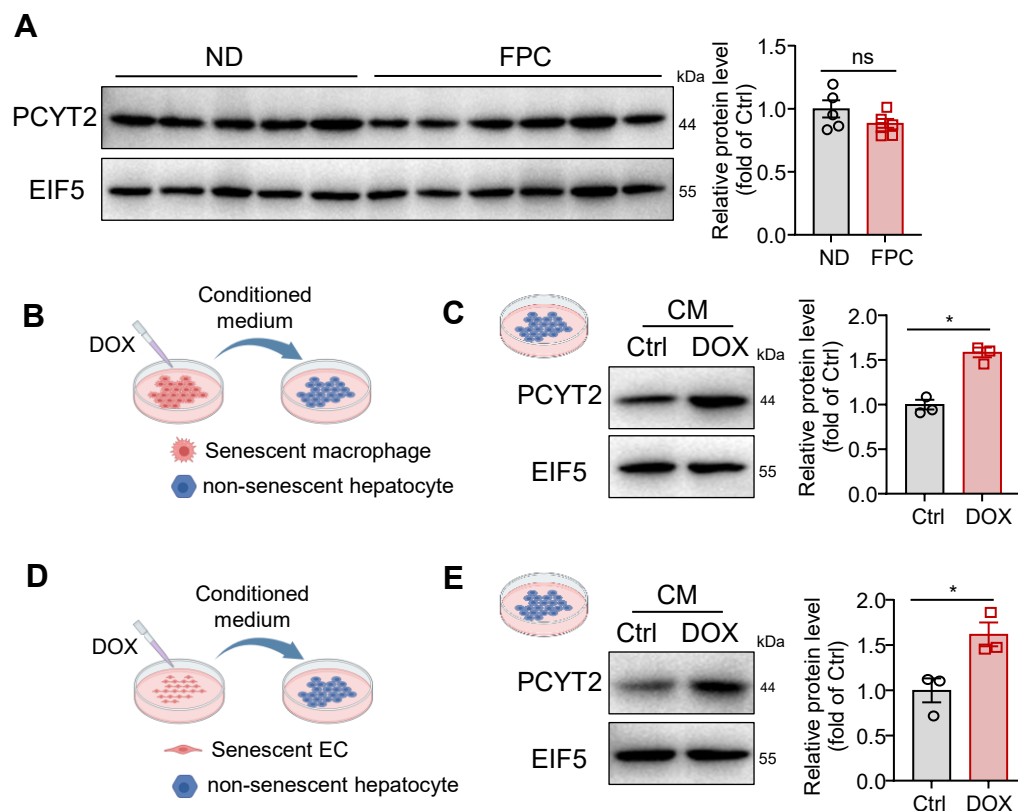


Figure S6. The protein level of PCYT2 in MASH mouse livers and in hepatocytes treated with conditioned medium from senescent cells. (A) Eight-week-old mice were fed a diet rich in fructose, palmitate, and cholesterol (FPC) or a normal diet (ND) for 24 weeks. Western blot analysis of the protein levels of PCYT2. EIF5 was used as the internal control. $n = 5-6$ mice per group. (B–C) Macrophages (RAW264.7 cells) were treated with doxorubicin (DOX) to induce senescence. The conditioned medium was collected to treat non-senescent HepG2 cells. The protein levels of PCYT2 in non-senescent hepatocytes treated with the conditioned medium; EIF5 was used as the internal control. (D–E) Endothelial cells (EA.hy926 cells) were treated with DOX to induce senescence. The conditioned medium was collected to treat non-senescent HepG2 cells. The protein levels of PCYT2 in non-senescent hepatocytes treated with conditioned medium; EIF5 was used as the internal control. (C and E) $n = 3$ independent experiments. Data present the mean $\pm$ SEM. $*p < 0.05$. EC, Endothelial cells.

Figure S7

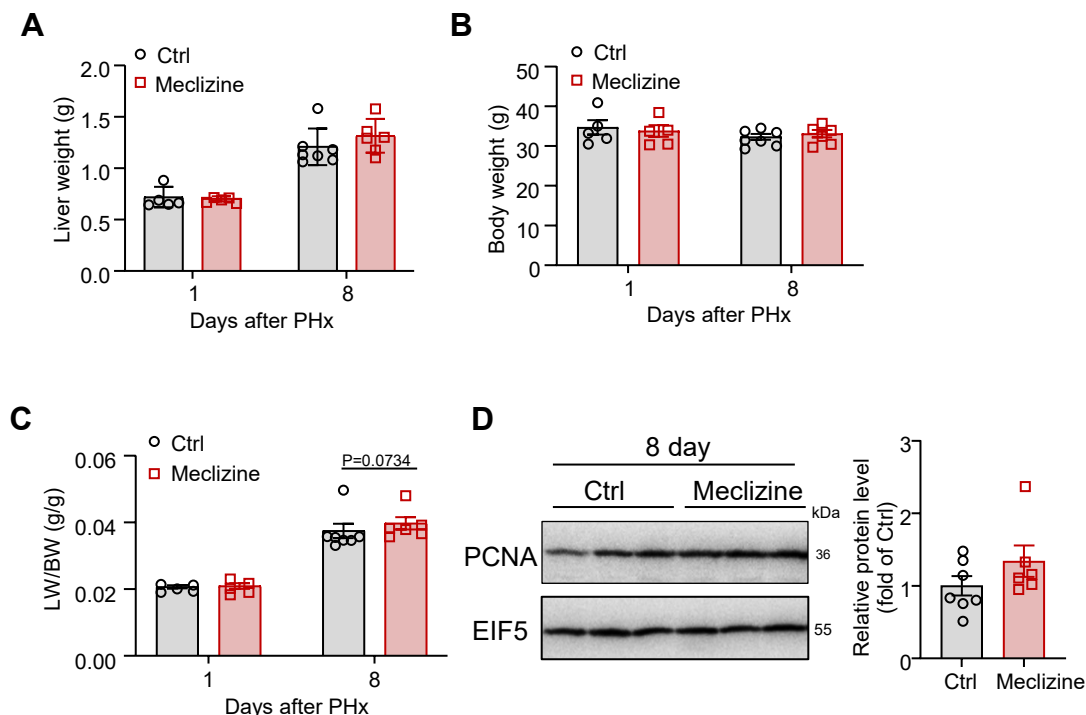


Figure S7. Liver weights of meclizine-treated middle-aged mice after 70% partial hepatectomy (PHx). Ten-month-old mice were administered 60 mg/kg meclizine or vehicle 17 h and 3 h before 70% PHx, and then the tissues were harvested for analysis at 1 day (n = 5 mice per group) or 8 days (n = 7 mice in the control group; n = 6 mice in the meclizine group) postoperatively. **(A)** Liver weights, **(B)** body weights, **(C)** and liver-to-body weight ratios of the mice. **(D)** Western blot analysis of the protein levels of PCNA in mouse livers at 8 days after surgery. EIF5 was used as the internal control. Data present the mean $\pm$ SEM. * $p < 0.05$. LW/BW, liver-to-body weight ratios.

A

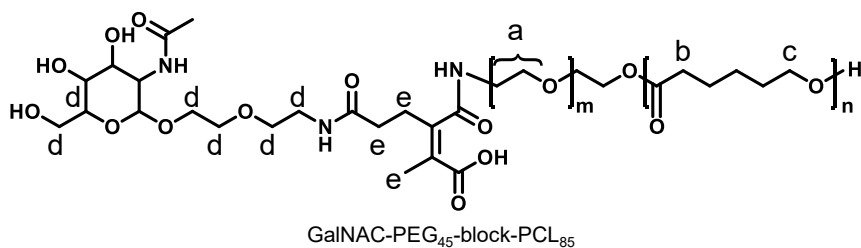


Figure S9

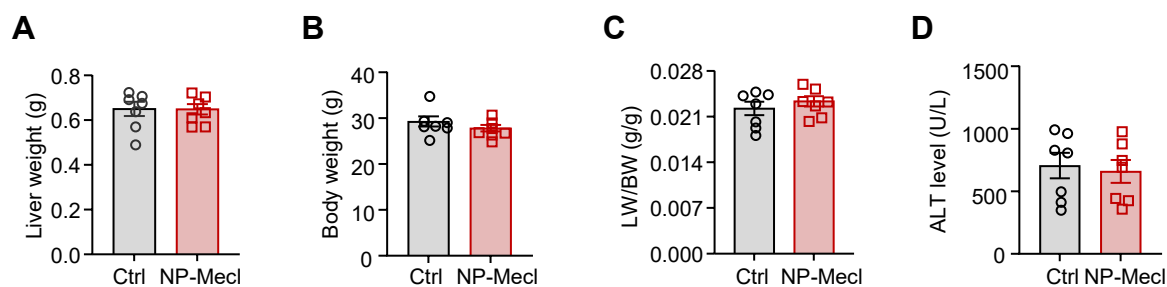


Figure S9. Liver weights of NP-mecizine-treated middle-aged mice after 70% partial hepatectomy (PHx). Ten-month-old mice were administered 150 μ g NP-mecizine (containing 25 μ g mecizine) or PBS 17 h and 6 h before 70% PHx, and then the tissues were harvested for analysis 1 day postoperatively (n = 7 mice per group). **(A)** Liver weights, **(B)** body weights, **(C)** and liver-to-body weight ratios. **(D)** Plasma ALT levels. Data present the mean $\pm$ SEM. *p < 0.05. LW/BW, liver-to-body weight ratios; NP-Mecl, NP-mecizine.

Figure S10

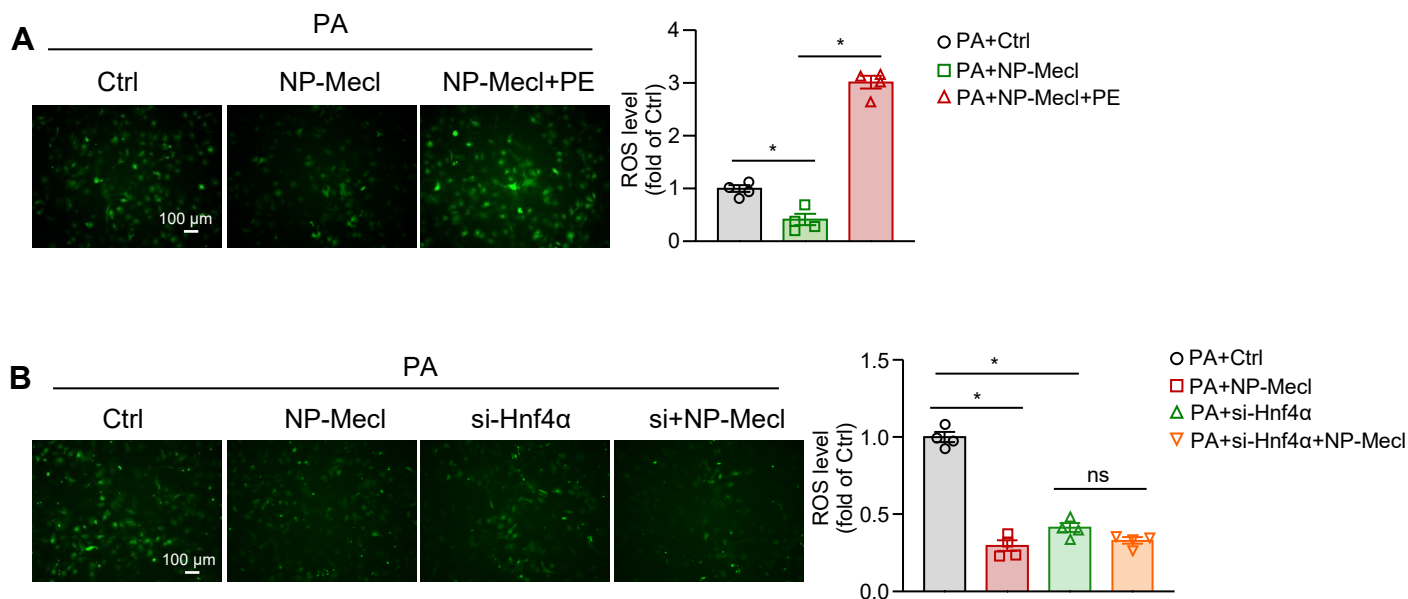


Figure S10. Influence of PE or HNF4 α knockdown on the NP-mecizine-mediated ROS reduction in fatty acid-overloaded hepatocytes. (A) Primary mouse hepatocytes were treated with 3.75 μ g/mL NP-mecizine (containing 1 μ M mecizine) for 17 h, and then the hepatocytes were co-treated with PA (100 μ M) and PE (10 μ M) for another 24 h. **(B)** Primary mouse hepatocytes were transfected with si-Hnf4 α or si-NC; 24 h later, the hepatocytes were treated with 3.75 μ g/mL NP-mecizine (containing 1 μ M mecizine) for 17 h, and then the hepatocytes were co-treated with PA (100 μ M) for another 24 h. (A and B) Representative images and quantitative analysis of ROS in hepatocytes (scale bar = 100 μ m). n = 4 independent experiments. Data present the mean $\pm$ SEM. *p < 0.05. NP-Mecl, NP-mecizine.

Figure S11

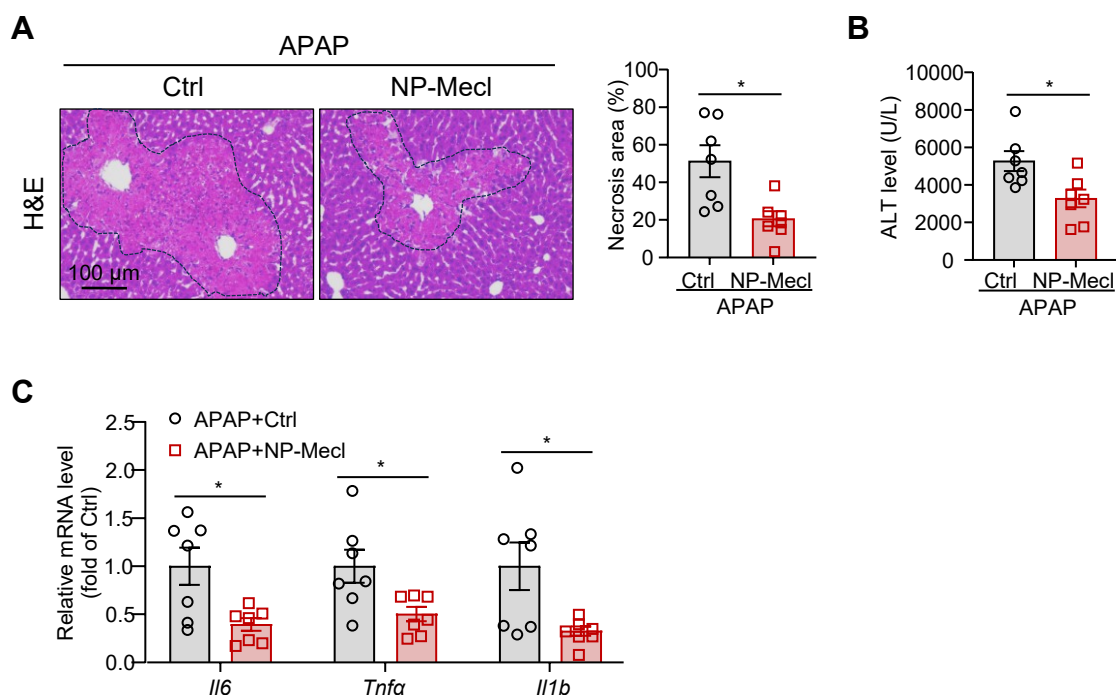


Figure S11. NP-mecizine attenuated acetaminophen (APAP)-induced liver injury.

Eight-week-old mice were administered 180 μ g of NP-mecizine (containing 25 μ g of mecizine) or PBS 17 h and 6 h before being treated with APAP for 24 h. **(A)** H&E staining of liver sections and the quantitative analysis of liver necrosis areas (scale bar = 100 μ m). **(B)** Plasma ALT levels. **(C)** qPCR analysis of the mRNA levels of inflammatory factors in mouse livers. n = 7 mice in the control group. Data present the mean $\pm$ SEM. *p < 0.05. NP-Mecl, NP-mecizine.

Table S1. List of oligonucleotide primer pairs used in qPCR

Target gene	Sense primer	Antisense primer
<i>18S (h/m)</i>	5'-GGAAGGGCACCACCAGGAGT-3'	5'-TGCAGCCCCGGACATCTAAG-3'
<i>Pcyt2 (m)</i>	5'-AGGCTGGGAGGTACAGAGAG-3'	5'-ATCTCCTGGCTGCTGTGATG-3'
<i>Pemt (m)</i>	5'-CTGTGGAGGCTTCGGCAATA-3'	5'-AACCTAGGAATGCAAGGCCC-3'
<i>Cept (m)</i>	5'-CAGCTTCTCTCAGGAACCGT-3'	5'-GCCACAGTACAAAGTGCAGG-3'
<i>Pisd (m)</i>	5'-CTAGTCACTGGGAGGTGTCC-3'	5'-AGCTTACGCCGGAAGAACTC-3'
<i>Etnk1 (m)</i>	5'-GTCAATCAGTTTGCGTTGGCT-3'	5'-CCGCATACCCAAGGAAGTCG-3'
<i>Hnf4a (m)</i>	5'-GGGGTTCCTGCAGATCACAT-3'	5'-TCCCAGAGATGGGAGAGGTG-3'
<i>Il6 (m)</i>	5'-CACTTCACAAGTCGGAGGCT-3'	5'-GCCACTCCTTCTGTGACTCC-3'
<i>Il1b (m)</i>	5'-CTCTGTGACTCATGGGATG-3'	5'-CTTCTTCTTTGGGTATTGC-3'
<i>Tnfa (m)</i>	5'-CTGTGAAGGGAATGGGTGTT-3'	5'-CAGGGAAGAATCTGGAAAGGTC-3'
<i>Cd68 (m)</i>	5'-CCCAAGGAACAGAGGAAG-3'	5'-GTGGCAGGGTTATGAGTG-3'
<i>Hnf4a (h)</i>	5'-TGCGACTCTCCAAAACCTC-3'	5'-ATTGCCCATCGTCAACACCT-3'

m: Mus musculus; h: Homo sapiens