

Supporting Information

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Malic Enzyme 1 as a Novel Anti-Ferroptotic Regulator in Hepatic Ischemia/Reperfusion Injury

*Xuexian Fang**, *Jiawei Zhang*, *You Li*, *Yijing Song*, *Yingying Yu*, *Zhaoxian Cai*, *Fuzhi Lian*, *Jun Yang*, *Junxia Min* and *Fudi Wang**

Supporting Information for

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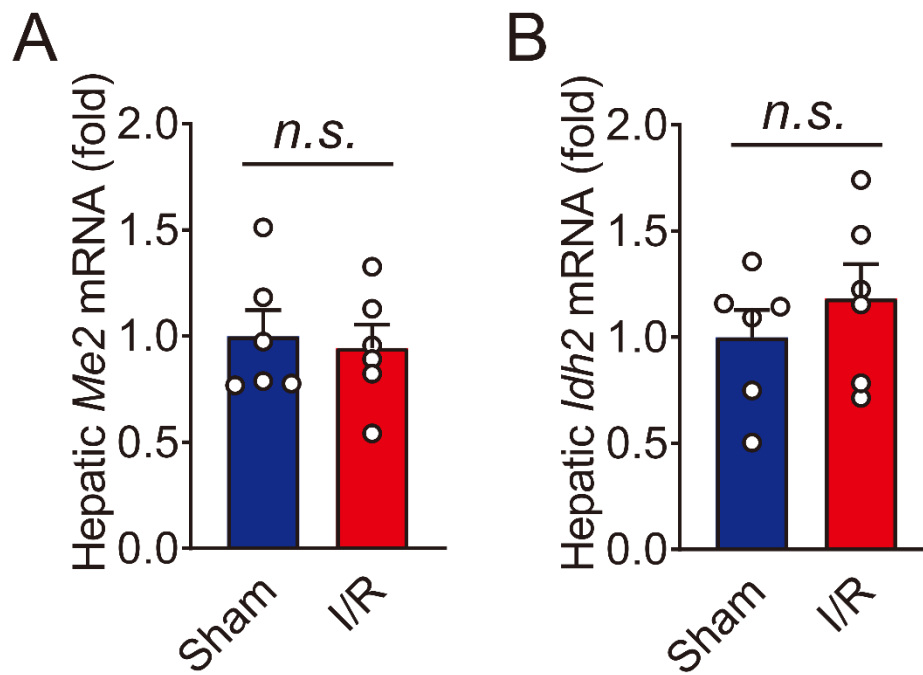


Figure S1. Hepatic *Me2* (A) and *Idh2* (B) mRNA of were measured in mice with sham or I/R injury. Significance was calculated by Student's *t*-test; n.s.=not significant.

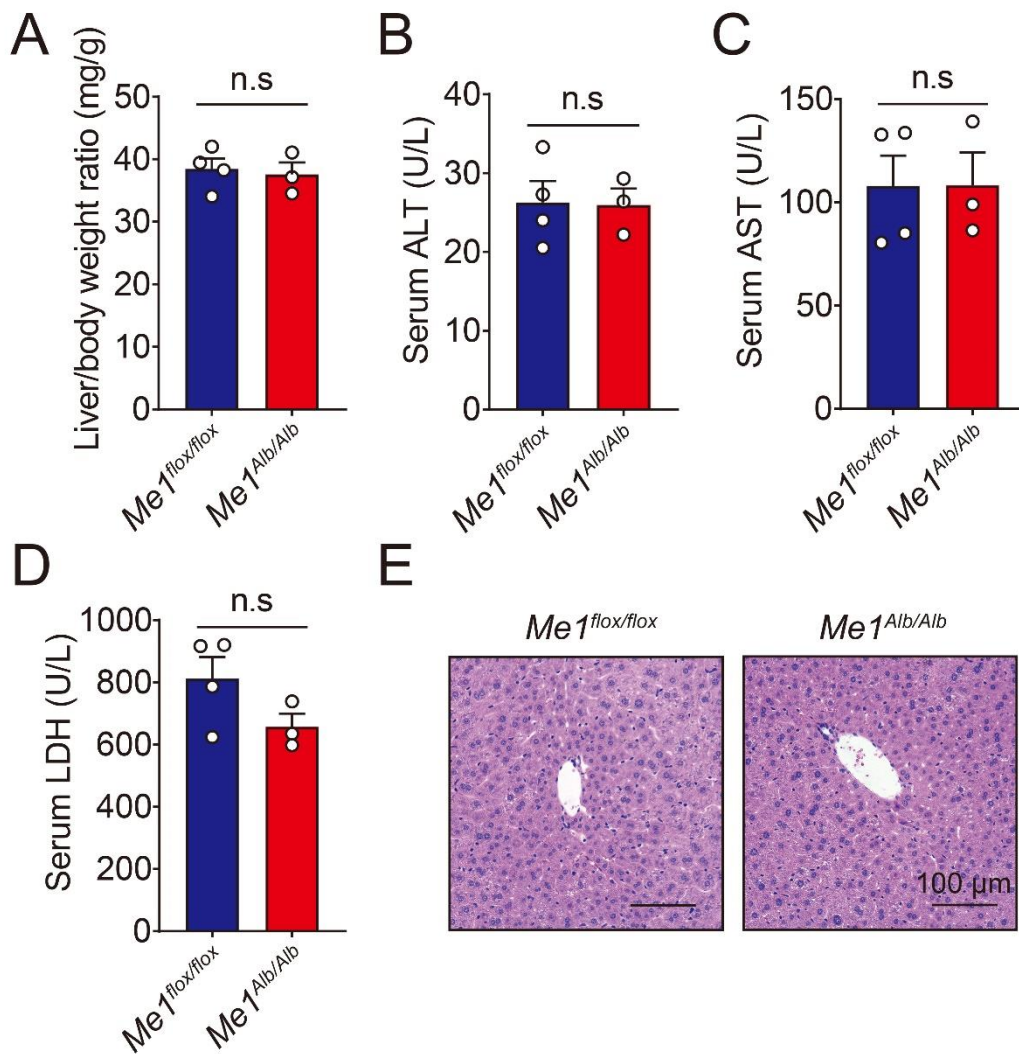


Figure S2. Hepatocyte-specific deletion of *Me1* is not sufficient to induce liver injury in mice. (A-C) Serum levels of ALT (A), AST (B), and LDH (C) were measured in $Me1^{flox/flox}$ and $Me1^{Alb/Alb}$ mice. (D) Representative H&E-stained liver sections from $Me1^{flox/flox}$ and $Me1^{Alb/Alb}$ mice. Significance was calculated by Student's *t*-test; n.s.=not significant.

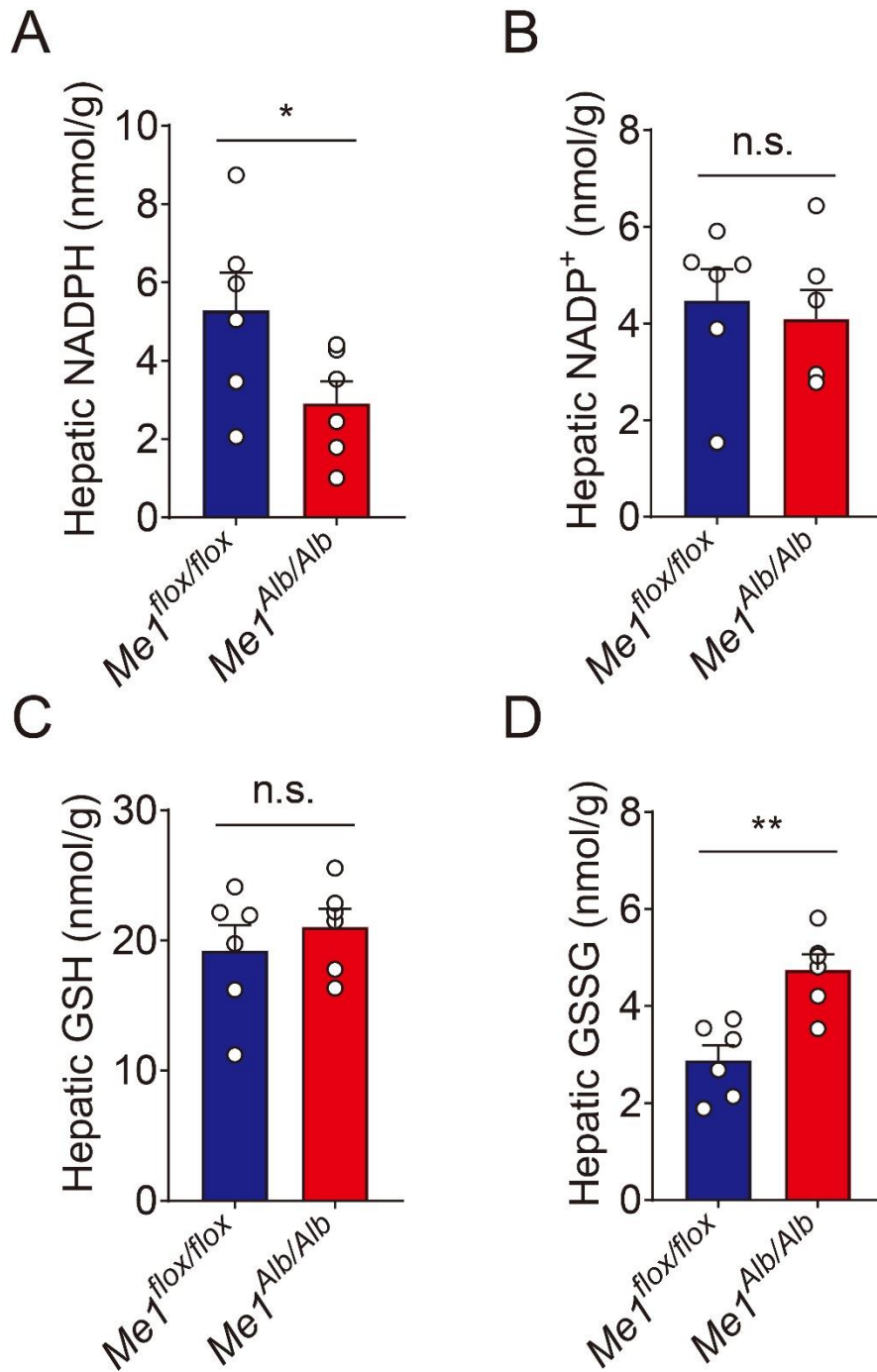


Figure S3. Hepatic levels of NADPH (A), NADP⁺ (B), GSH (C), and GSSG (D) were measured in *Me1^{flox/flox}* and *Me1^{Alb/Alb}* mice subjected to I/R injury. Significance was calculated by Student's *t*-test; **P*<0.05, ***P*<0.01, ****P*<0.001, n.s.=not significant.

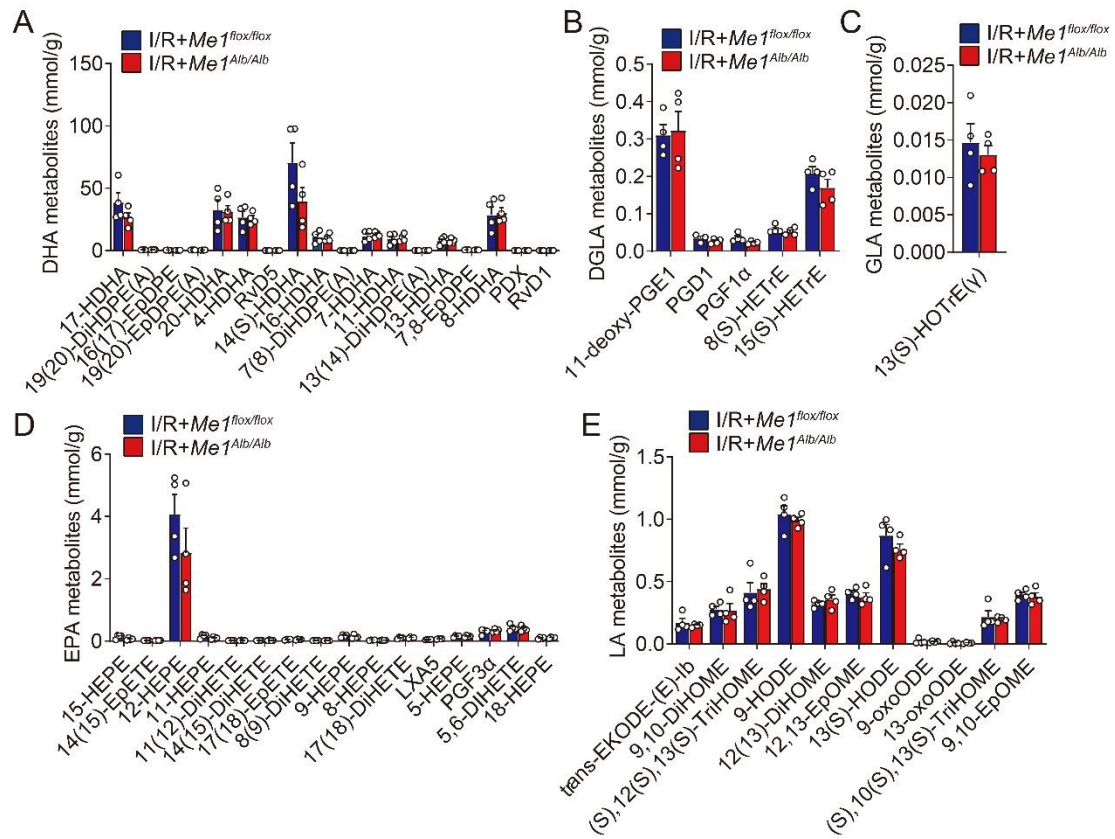


Figure S4. Hepatic docosahexaenoic acid (DHA) metabolites (A), dihomo- γ -linolenic acid (DGLA) metabolites (B), γ -linolenic acid (GLA) metabolites (C), eicosapentaenoic acid (EPA) metabolites (D), linoleic acid (LA) metabolites (E) in *Me1^{flox/flox}* and *Me1^{Alb/Alb}* mice subjected to I/R injury. Significance was calculated by Student's *t*-test.

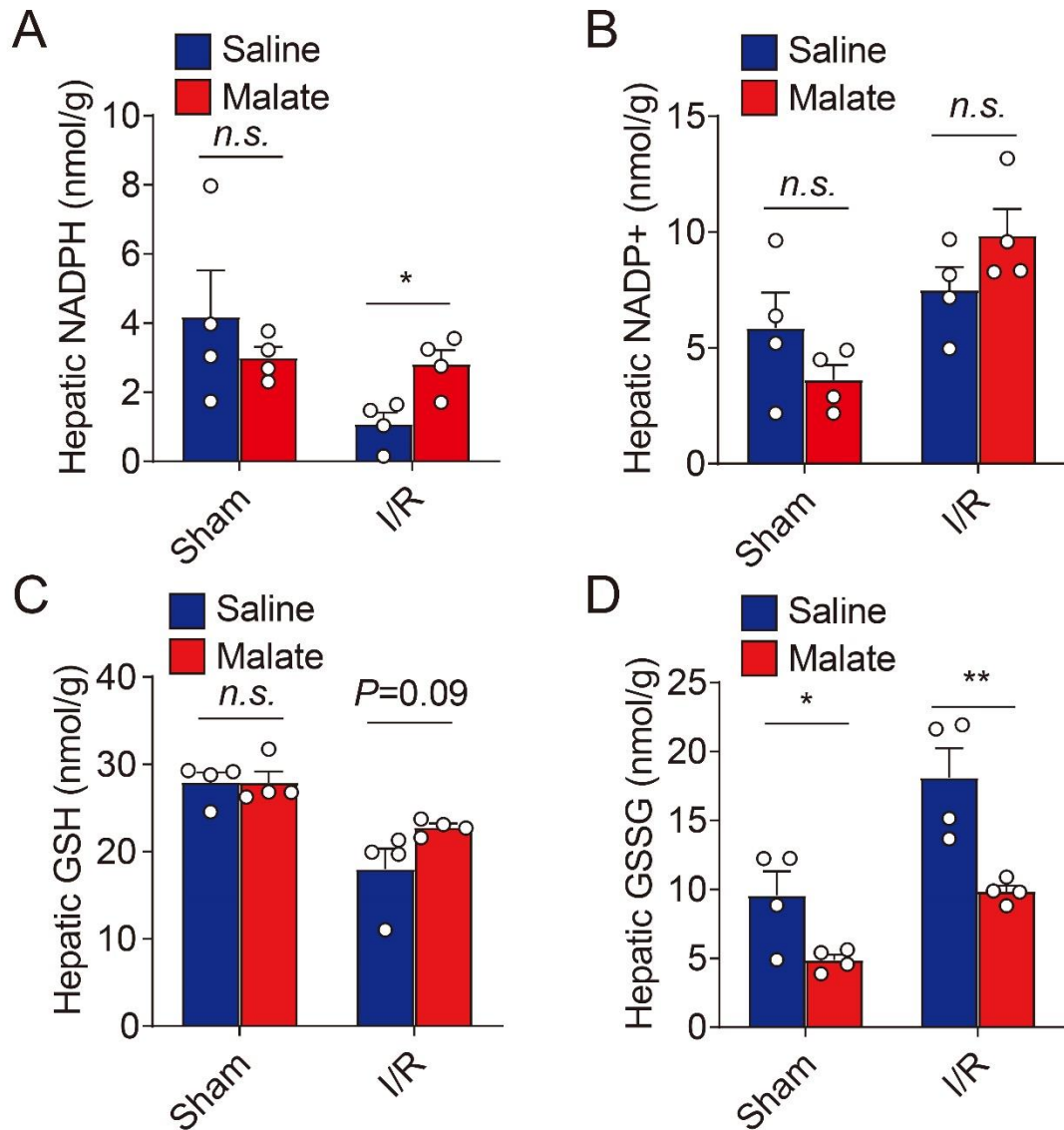


Figure S5. Hepatic levels of NADPH (A), NADP⁺ (B), GSH (C), and GSSG (D) were measured in sham- or I/R-treated mice with or without malate supplementation. Significance was calculated by Student's *t*-test; **P*<0.05, ***P*<0.01, ****P*<0.001, n.s.=not significant.

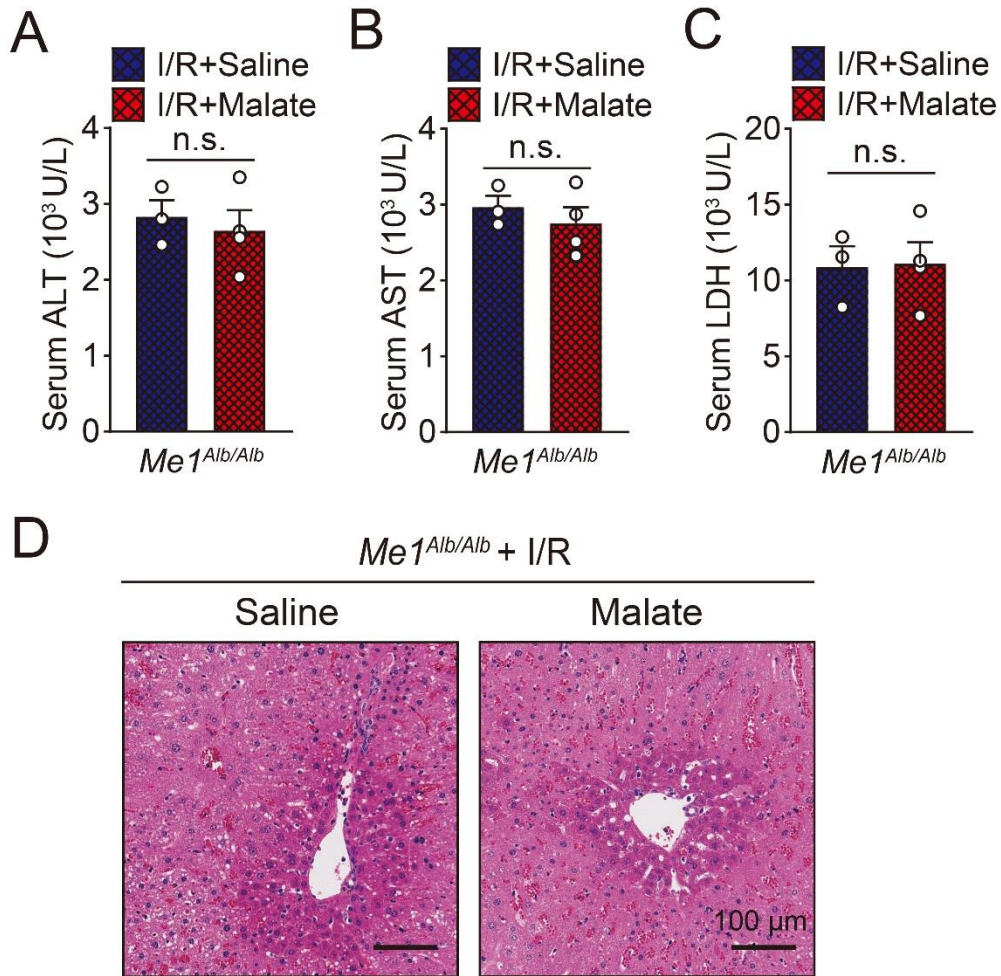


Figure S6. Effect of malate supplementation on *Me1^{Alb/Alb}* mice subjected to I/R injury. (A-C) Serum levels of ALT (A), AST (B), and LDH (C) were measured in I/R-treated *Me1Alb/Alb* mice with or without malate supplementation. (D) Representative H&E-stained liver sections from indicated mice. Significance was calculated by Student's *t*-test; n.s.=not significant.

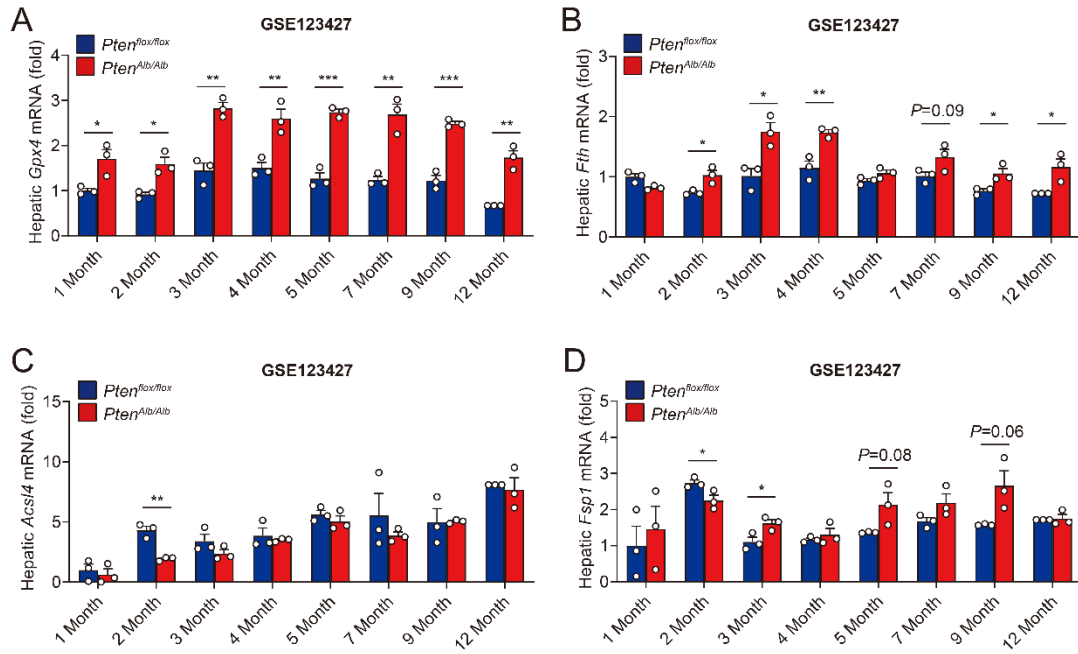


Figure S7. Hepatic expression of *Gpx4* (A), *Fth* (B), *Acsl4* (C), and *Fsp1* (D) were measured in $Pten^{flox/flox}$ and $Pten^{Alb/Alb}$ mice subjected to I/R injury at different months old. Significance was calculated by Student's *t*-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s.=not significant.

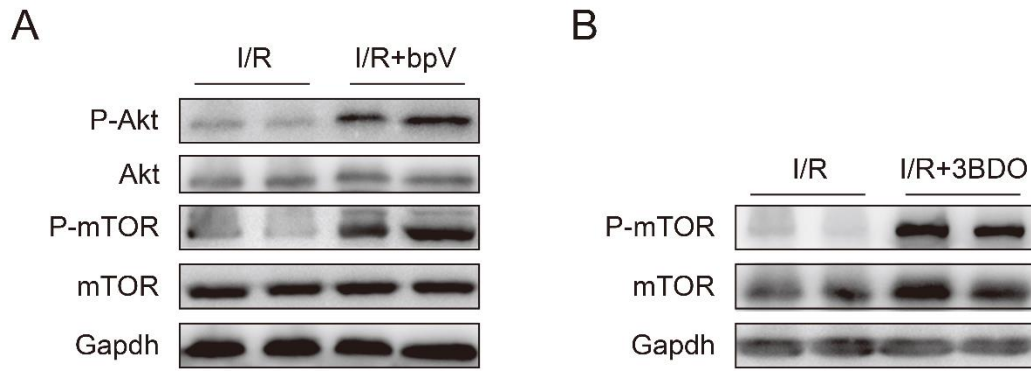


Figure S8. Validation of PTEN inhibitor (bpV) and mTOR activator (3BDO). (A) Immunoblots of hepatic P-Akt, Akt, P-mTOR, and mTOR were measured in I/R-treated mice with or without PTEN inhibitor bpV injection. (B) Immunoblots of hepatic P-mTOR, and mTOR were measured in I/R-treated mice with or without mTOR activator 3BDO injection.

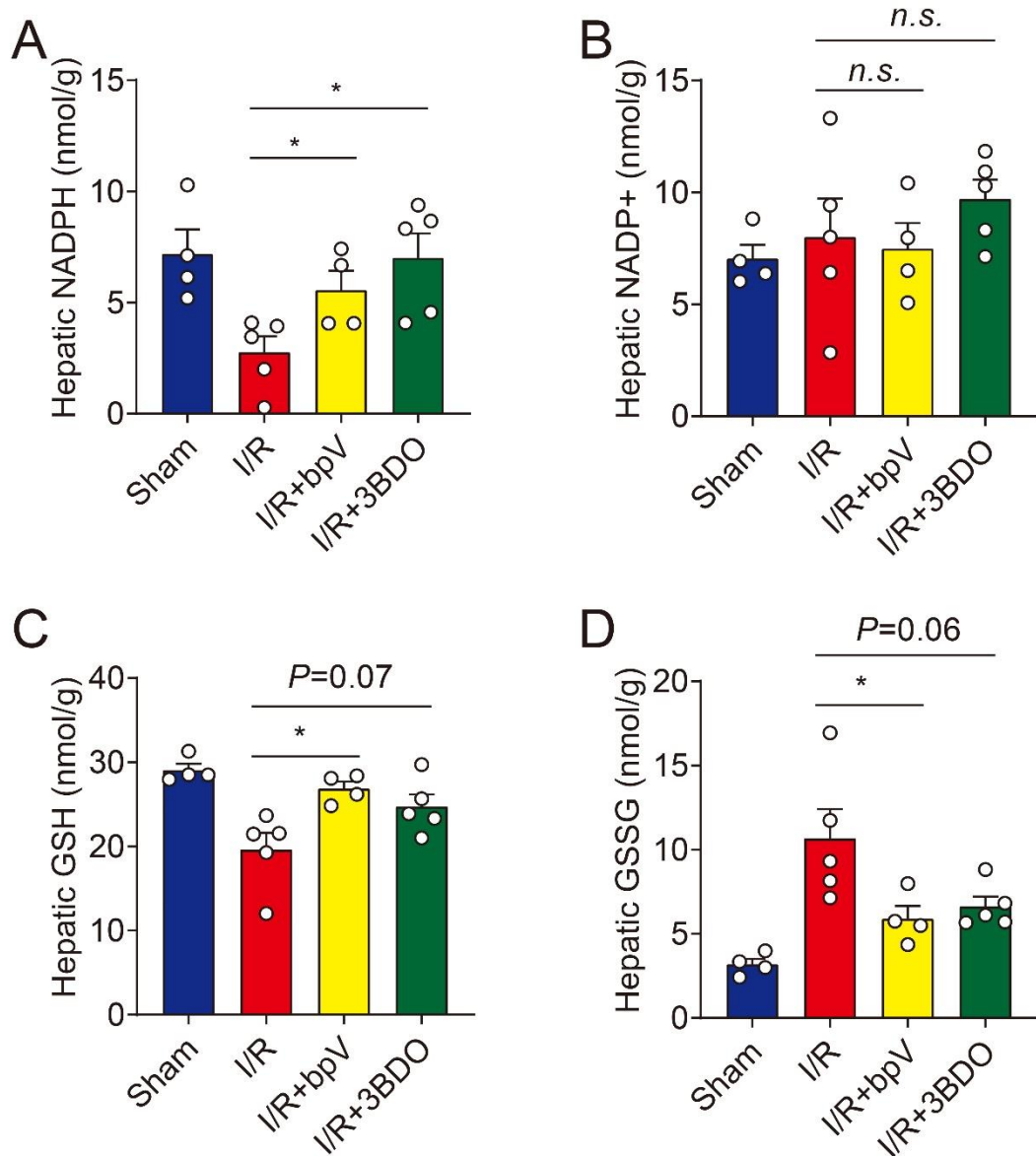


Figure S9. Hepatic levels of NADPH (A), NADP⁺ (B), GSH (C), and GSSG (D) were measured in I/R-treated mice with bpV or 3BDO injection. Significance was calculated by Student's *t*-test; **P*<0.05, n.s.=not significant.

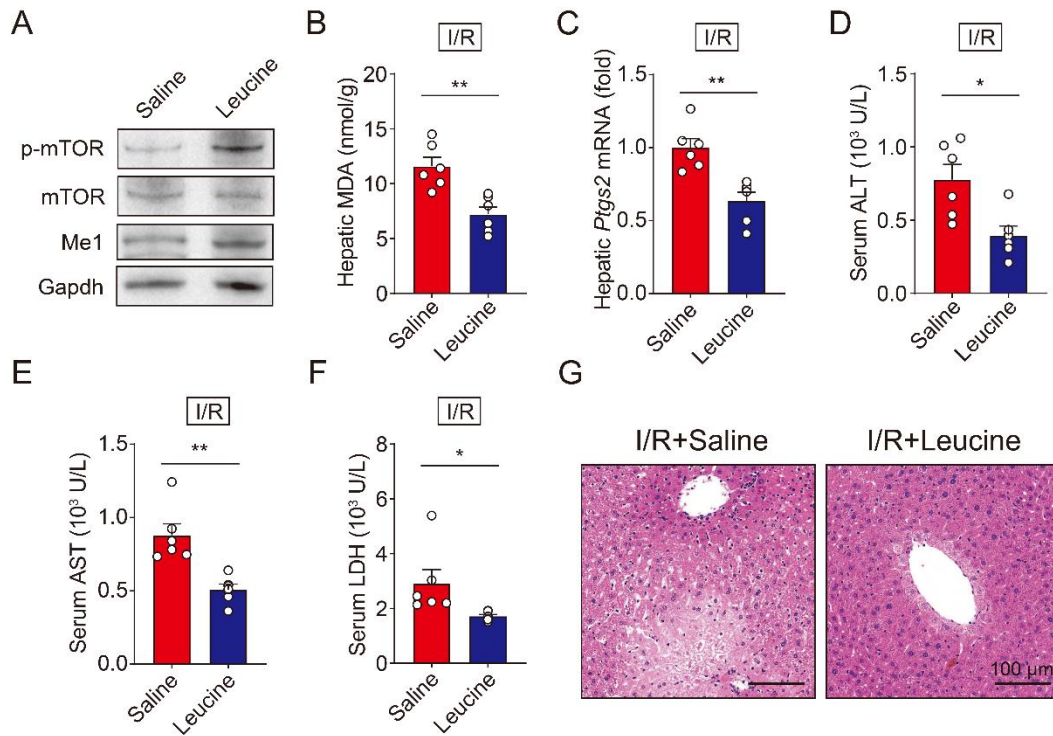


Figure S10. L-leucine supplementation protects against hepatic ferroptosis during I/R injury. (A) Immunoblots of hepatic P-mTOR, mTOR, and Me1 were measured in mice with or without leucine supplementation. (B,C) Hepatic *Ptgs2* mRNA (B) and MDA levels (C) were measured in I/R-treated mice with leucine supplementation. (D-F) Serum levels of ALT (D), AST (E) and LDH (F) were measured in I/R-treated mice with leucine supplementation. (G) Representative H&E-stained liver sections from indicated mice. Significance was calculated by Student's t-test; * $P < 0.05$, ** $P < 0.01$

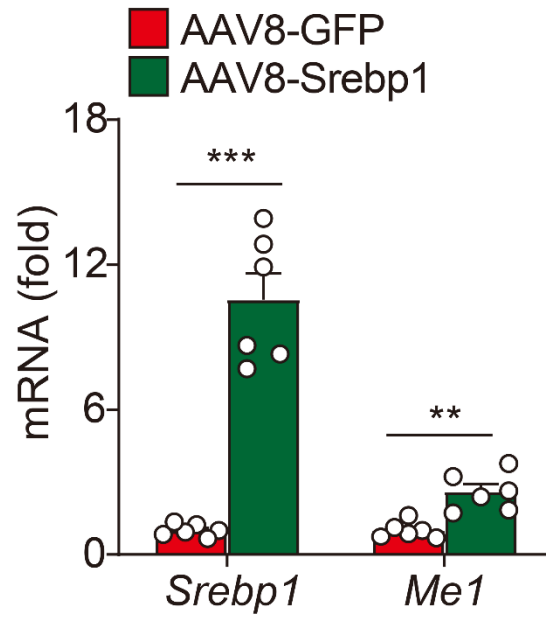


Figure S11. Hepatic mRNA of *Srebp1* and *Me1* were measured in mice treated with AAV8-Srebp1 or AAV8-GFP. Significance was calculated by Student's *t*-test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

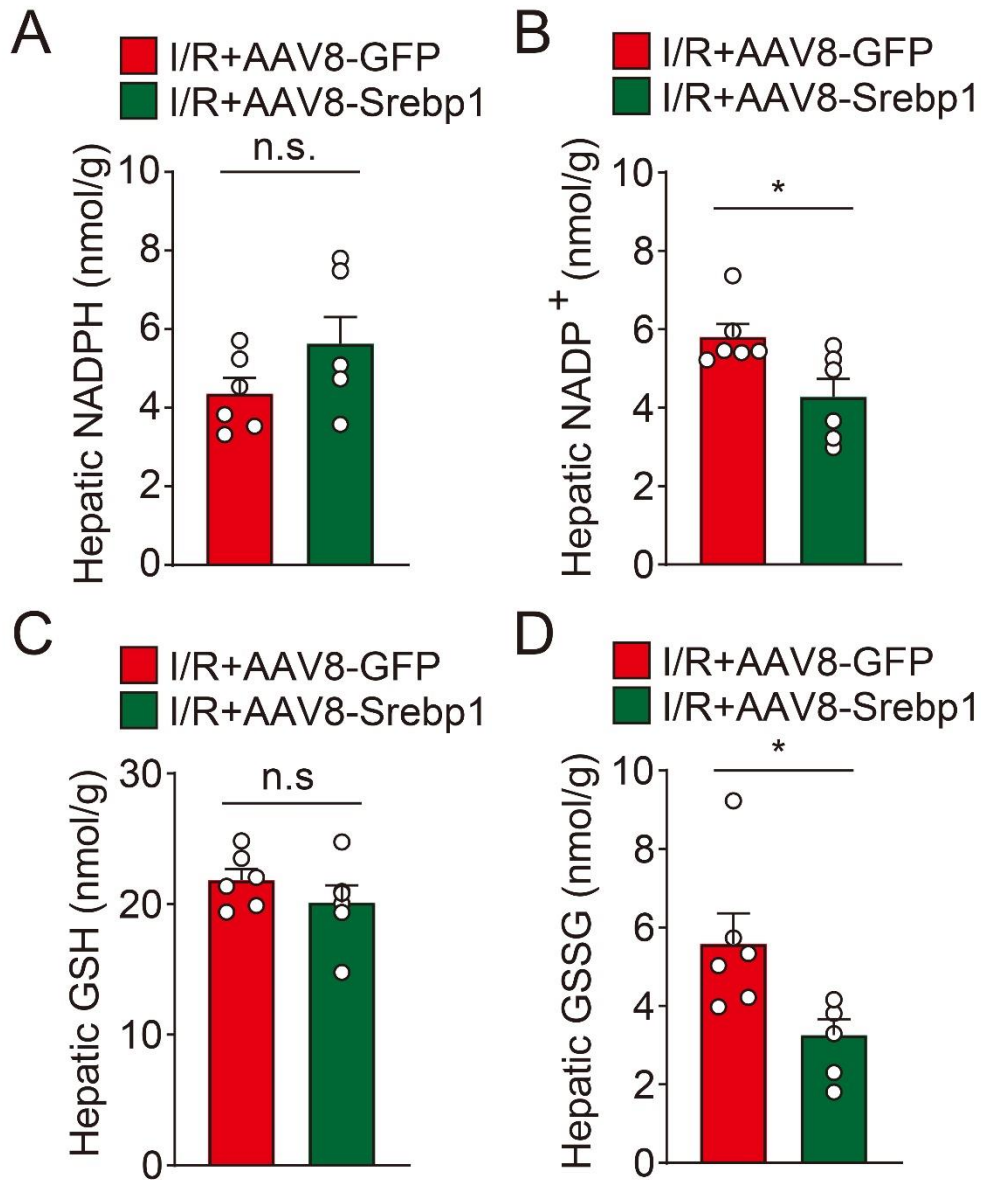


Figure S12. Hepatic levels of NADPH (A), NADP⁺ (B), GSH (C), and GSSG (D) were measured in I/R-treated mice with or without AAV-mediated *Srebp1* overexpression. Significance was calculated by Student's *t*-test; **P*<0.05, n.s.=not significant.

Table S1. Primers used for genotyping and real-time PCR analysis.

Gene	Primer sequence (5'→3')
Genotyping	
<i>Me1</i> forward	TAGCCGCACGCTGATGATAG
<i>Me1</i> reverse	GCAGCTGTCAGACTAGCCAA
<i>Alb-Cre</i> common	TGGCAAACATACGCAAGGG
<i>Alb-Cre</i> mutant	CGGCAAACGGACAGAAGCA
<i>Alb-Cre</i> mutant	GGCAATGGTTCCTCTCTGCT
Real-time PCR	
<i>Gapdh</i> forward	ATCATCCCTGCATCCACT
<i>Gapdh</i> reverse	ATCCACGACGGACACATT
<i>Ptgs2</i> forward	CTGCGCCTTTTCAAGGATGG
<i>Ptgs2</i> reverse	GGGGATACACCTCTCCACCA
<i>Me1</i> forward	GTCGTGCATCTCTCACAGAAG
<i>Me1</i> reverse	TGAGGGCAGTTGGTTTTATCTTT
<i>Me2</i> forward	TACCACTCCTTGTACCTTGACC
<i>Me2</i> reverse	TCTTGTAACGTAAACGCCATTCC
<i>G6pd</i> forward	TCAGACAGGCTTTAACCGCAT
<i>G6pd</i> reverse	CCATTCCAGATAGGGCCAAAGA
<i>6pgd</i> forward	TACAGACACGAGATGCTGCC
<i>6pgd</i> reverse	TGAGCCCCAAAGTAATCCCG
<i>Idh1</i> forward	GGTTATGGCTCCCTTGGCAT
<i>Idh1</i> reverse	CCCTTTCTGGTACATGCGGT
<i>Idh2</i> forward	GGAGAAGCCGGTAGTGGAGAT
<i>Idh2</i> reverse	GGTCTGGTCACGGTTTGGAA
<i>Mthfd1</i> forward	GGGAATCCTGAACGGGAAACT
<i>Mthfd1</i> reverse	TGAGTGGCTTTGATCCCAATC
<i>Mthfd2</i> forward	AGTGCGAAATGAAGCCGTTG
<i>Mthfd2</i> reverse	GACTGGCGGGATTGTCACC

<i>Aldh1l1</i> forward	CAGGAGGTTTACTGCCAGCTA
<i>Aldh1l1</i> reverse	CACGTTGAGTTCTGCACCCA
<i>Aldh1l2</i> forward	ACCAGCCGGGTTTATTTCAA
<i>Aldh1l2</i> reverse	ACTCCCACTACTCGGTGGC

Table S2. Antibodies used in this study.

Antibody	Application	Description	Source	Catalog no.
Primary Antibodies				
anti-Me1	WB/IHC	Rabbit Polyclonal	Proteintech	16619-1-AP
anti-Pten	WB	Rabbit Monoclonal	Cell Signaling	9559
anti-mTOR	WB	Rabbit Monoclonal	Cell Signaling	2983
anti-P-mTOR	WB	Rabbit Monoclonal	Cell Signaling	5536
anti-Akt	WB	Rabbit Monoclonal	Cell Signaling	4691
anti-P-Akt	WB	Rabbit Monoclonal	Cell Signaling	4060
anti-S6K1	WB	Rabbit Monoclonal	Cell Signaling	2708
anti-P-S6K1	WB	Rabbit Polyclonal	Cell Signaling	9208
anti-Srebp1	WB/ChIP	Mouse Monoclonal	Santa Cruz	sc-13551
anti-Gapdh	WB	Mouse Monoclonal	Proteintech	60004-1-Ig
Secondary Antibodies				
anti-mouse IgG	WB	Goat	Proteintech	SA00001-1
anti-rabbit IgG	WB	Goat	Proteintech	SA00001-2

IHC, immunohistochemistry; WB, western blotting.