

**microRNA-130b-3p Attenuates Septic Cardiomyopathy by
Regulating the AMPK/mTOR Signaling Pathways and Directly
Targeting ACSL4 against Ferroptosis**

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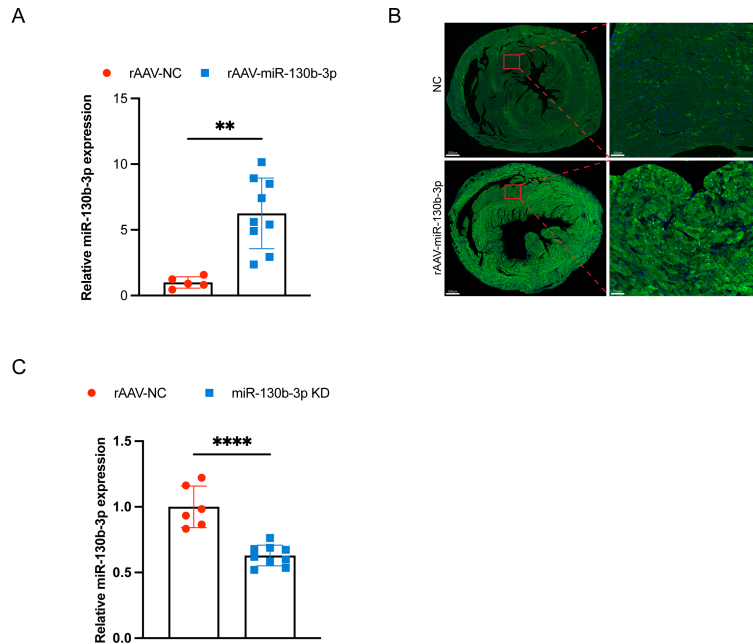
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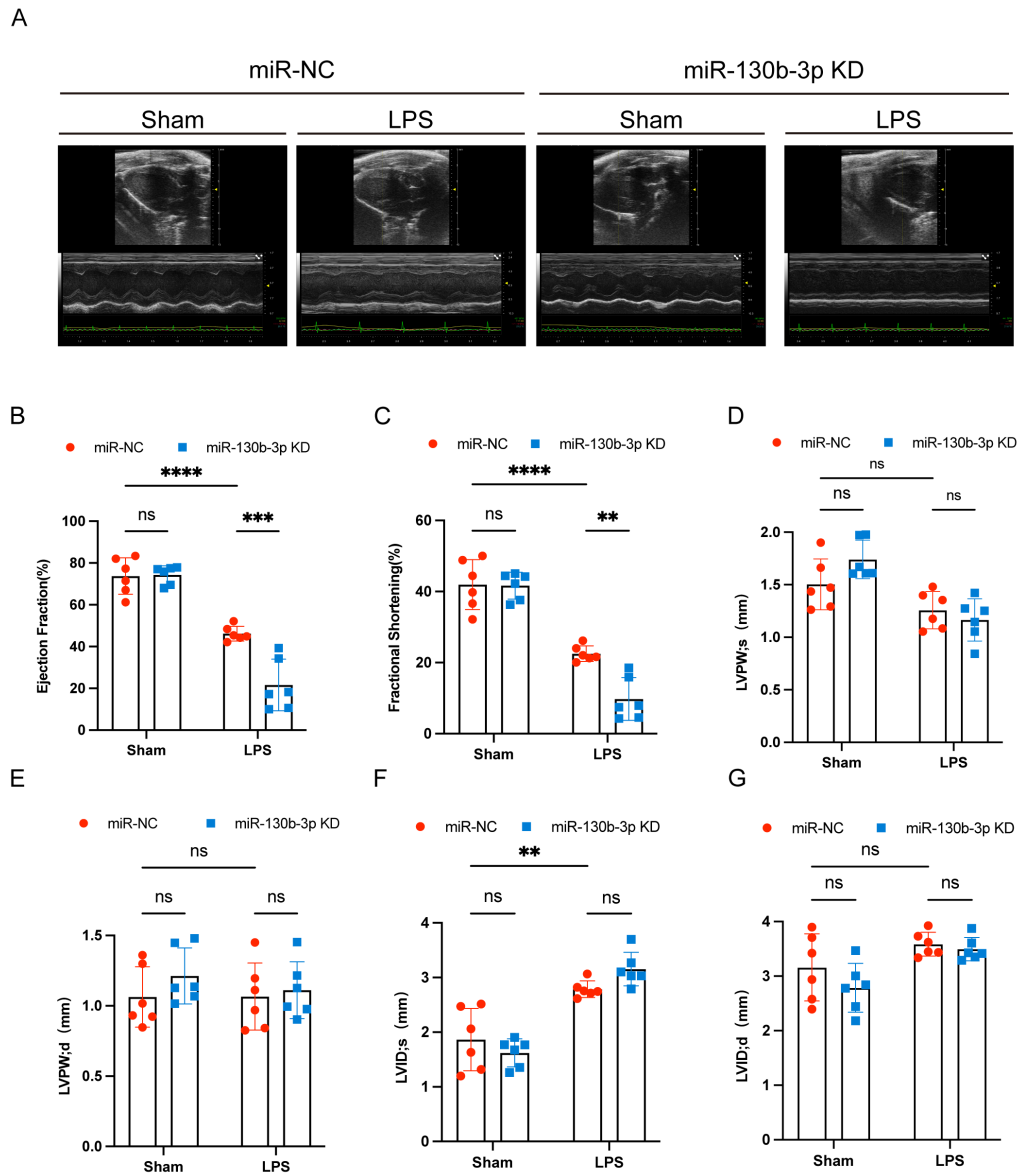
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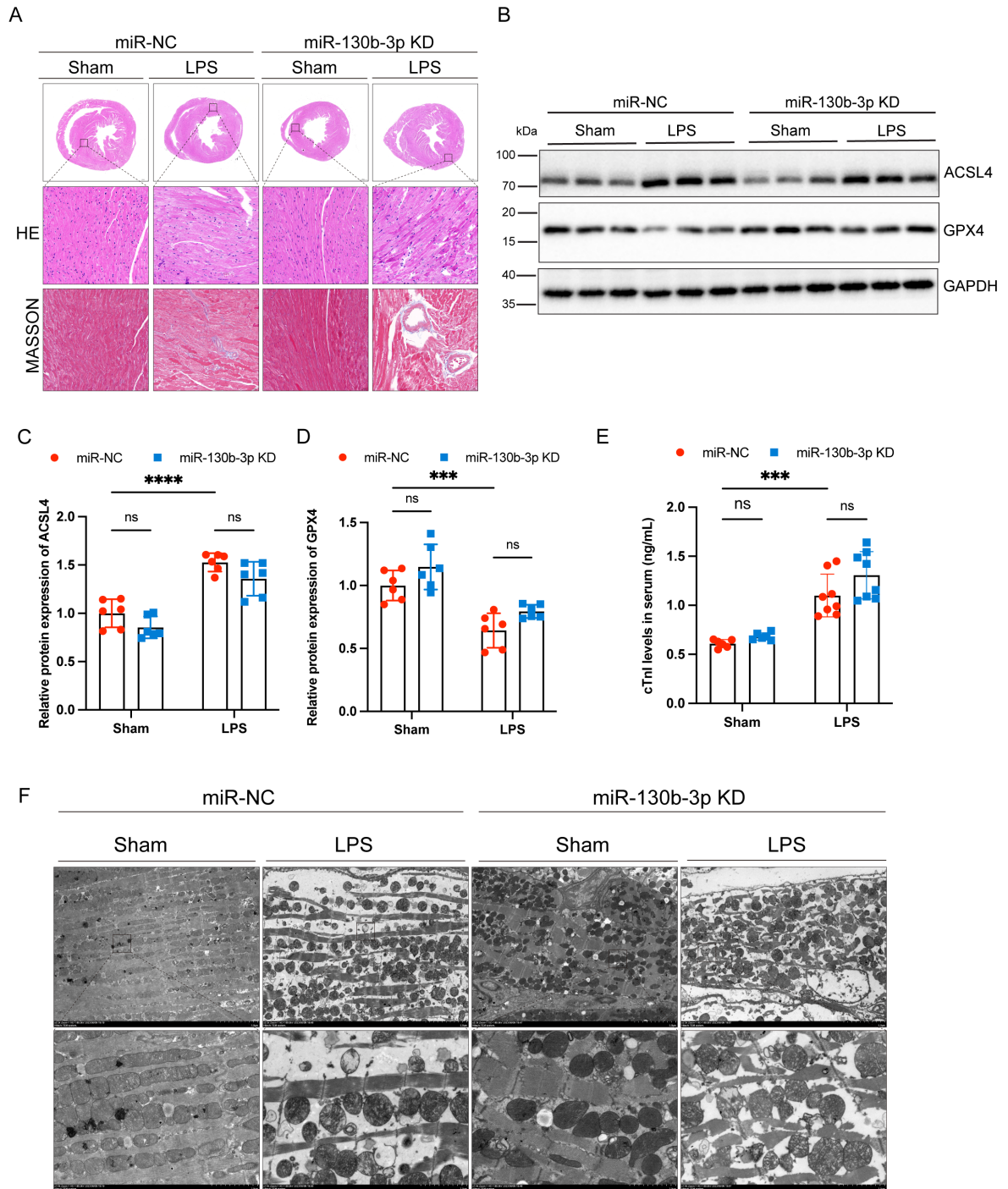
Supplementary information



Supplementary Figure S1 (A) qRT-PCR analysis of miR-130b-3p in the myocardium of mice treated with rAAV-miR-NC or rAAV-miR-130b-3p. rAAV-miR-NC group n=5, rAAV-miR-130b-3p group n=9. (B) Representative immunofluorescence staining image of mice hearts treated with rAAV-miR-130b-3p, n=5 per group. (C) qRT-PCR analysis of miR-130b-3p in the myocardium of mice treated with rAAV-miR-NC or rAAV-miR-130b-3p Sponge. rAAV-miR-NC group n=6, rAAV-miR-130b-3p Sponge group n=9. Data are expressed as mean \pm SD. **p < 0.01. ****p < 0.0001.

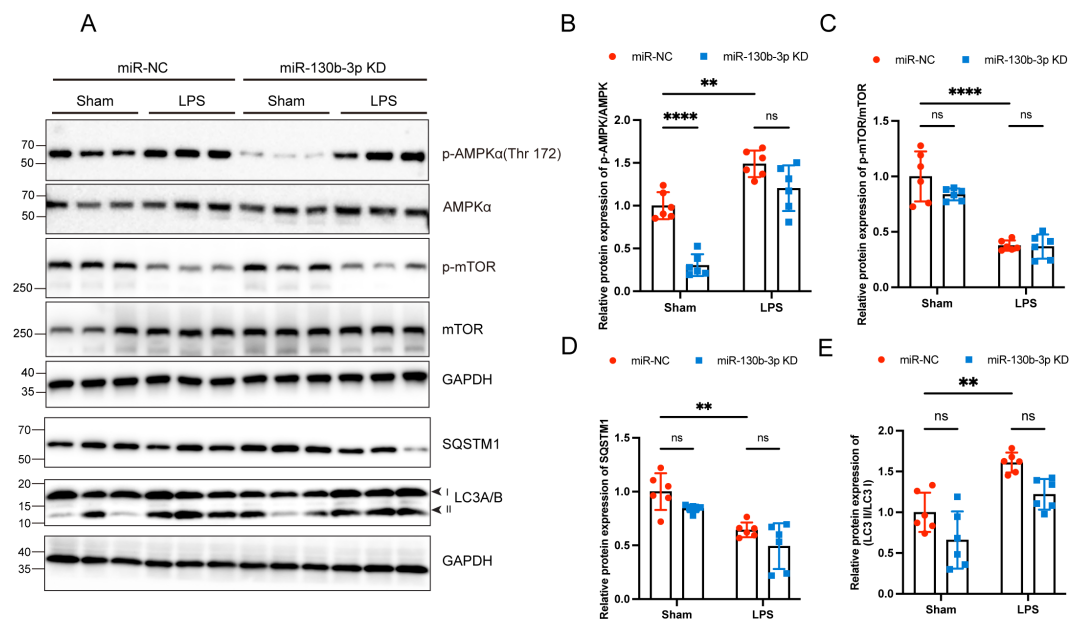


Supplementary Figure S2 | Knocking down miR-130b-3p deteriorated cardiac function in sepsis-induced cardiomyopathy mice. (A) Representative images of echocardiography. (B-G) Echocardiographic analysis of (B) Ejection fraction (EF), (C) Fractional shortening (FS), (D) Left ventricular posterior wall systolic thickness (LVPW_s), (E) Left ventricular posterior wall diastolic thickness (LVPW_d), (F) Left ventricular internal systolic dimension (LVID_s), and (G) Left ventricular internal diastolic dimension (LVID_d). n=6. Data are expressed as mean ± SD. **p < 0.01, ***p < 0.001, ****p < 0.0001; ns: no significant difference.



Supplementary Figure S3 | Knocking down miR-130b-3p deteriorated myocardial injury and ferroptosis in sepsis-induced cardiomyopathy mice. (A) Representative H&E staining and Masson trichrome staining images of myocardium from septic mice, $n = 6$. Scale bar, $20 \mu\text{m}$. (B) Representative immunoblots and (C-D) densitometric quantification analysis of the protein expression levels of ACSL4 and GPX4 in cardiac tissue of septic mice, $n=6$. (E) Serum cardiac troponin (cTnI) levels detected by ELISA, Sham+miR-NC, Sham+miR-130b-3p KD, $n = 6$; LPS+ miR-NC, LPS+miR-130b-3p

KD, n=8. (F) Representative images of TEM of myocardium from septic mice, n = 6. Scale bar, 2.0 μm . Data are expressed as mean \pm SD. **p < 0.01, ***p < 0.001, ****p < 0.0001; ns: no significant difference.



Supplementary Figure S4 | Knocking down miR-130b-3p modulates autophagy by activating the AMPK/mTOR signaling pathway to deteriorate ferroptosis in septic cardiomyopathy. (A) The protein expression levels of p-AMPK α , AMPK α , p-mTOR, mTOR, SQSTM1, and LC3 A/B in myocardium from septic mice were detected by Western blot. (B) Densitometric quantification of the protein expression levels of p-AMPK α , AMPK α , p-mTOR, mTOR, SQSTM1, and LC3 A/B in heart tissue of septic mice, n=6. Data are expressed as mean \pm SD. **p < 0.01, ***p < 0.0001; ns: no significant difference.

Supplemental Table S1

The sequences of miRNA mimic, miRNA inhibitor, and small interfering RNA (siRNA)

Gene	Sequences (5'-3')
rno-miR-130b-3p-mimic	sense: CAGUGCAAUGAUGAAAGGGCAU antisense: AUGCCCUUUCAUCAUUGCACUG
rno-NC-mimic	sense: UUCUCCGAACGUGUCACGU antisense: ACGUGACACGUUCGGAGAA
rno-miR-130b-3p -inhibitor	sense: AUGCCCUUUCAUCAUUGCACUG antisense: ACGUGACACGUUCGGAGAA
rno-NC- inhibitor	sense: GCAGAAGUGUGUAGAGCAATT antisense: UUGCUCUACACACUUCUGCTT
Rat- <i>Prkaa1</i> -siRNA#1	sense: CGAGUUGACUGGACAUAAATT antisense: UUAUGUCCAGUCAACUCGTT
Rat- <i>Prkaa1</i> -siRNA#2	sense: GGAUAGUAGGACUUACUUATT antisense: UAAGUAAGUCCUACUAUCCTT
Rat- <i>Prkaa1</i> -siRNA#3	sense: UUCUCCGAACGUGUCACGUTT antisense: ACGUGACACGUUCGGAGAATT
NC-siRNA	

Supplemental Table S2 The primer sequences used for quantitative real-time PCR

Gene(miRNA)	Primer (5'-3')
rno-miR-130b-3p	Forward: GCGCAGTGCAATGATGAAA
	Reverse: AGTGCAGGGTCCGAGGTATT
	RT Primer:
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACATGCCC
mmu-miR-130b-3p	Forward: GCGCAGTGCAATGATGAAA
	Reverse: AGTGCAGGGTCCGAGGTATT
	RT Primer:
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACATGCCC
U6	Forward: CTCGCTTCGGCAGCACA
	Reverse: AACGCTTCACGAATTTGCGT
	RT Primer:
	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACAAAATATGGA
Rattus <i>Acsl4</i>	Forward: CTCACTGCACTGGGACTGAA
	Reverse: ATAATGCCGCCTTCAGTTTG
Rattus <i>Prkaal</i>	Forward: TTGCGTGTGCGAAGGAAGAACC
	Reverse: CTGTGGAGTAGCAGTCCCTGATTTG
Rattus <i>Gapdh</i>	Forward: GACATGCCGCCTGGAGAAAC
	Reverse: AGCCCAGGATGCCCTTTAGT
Mus <i>Gapdh</i>	Forward: AAGAGGCTAAGACCGCCTTC
	Reverse: GCATAAATTCCCCTGCCCAC