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Supplemental information

Aberrant HSF1 signaling activation underlies metformin amelioration of myocardial infarction in mice

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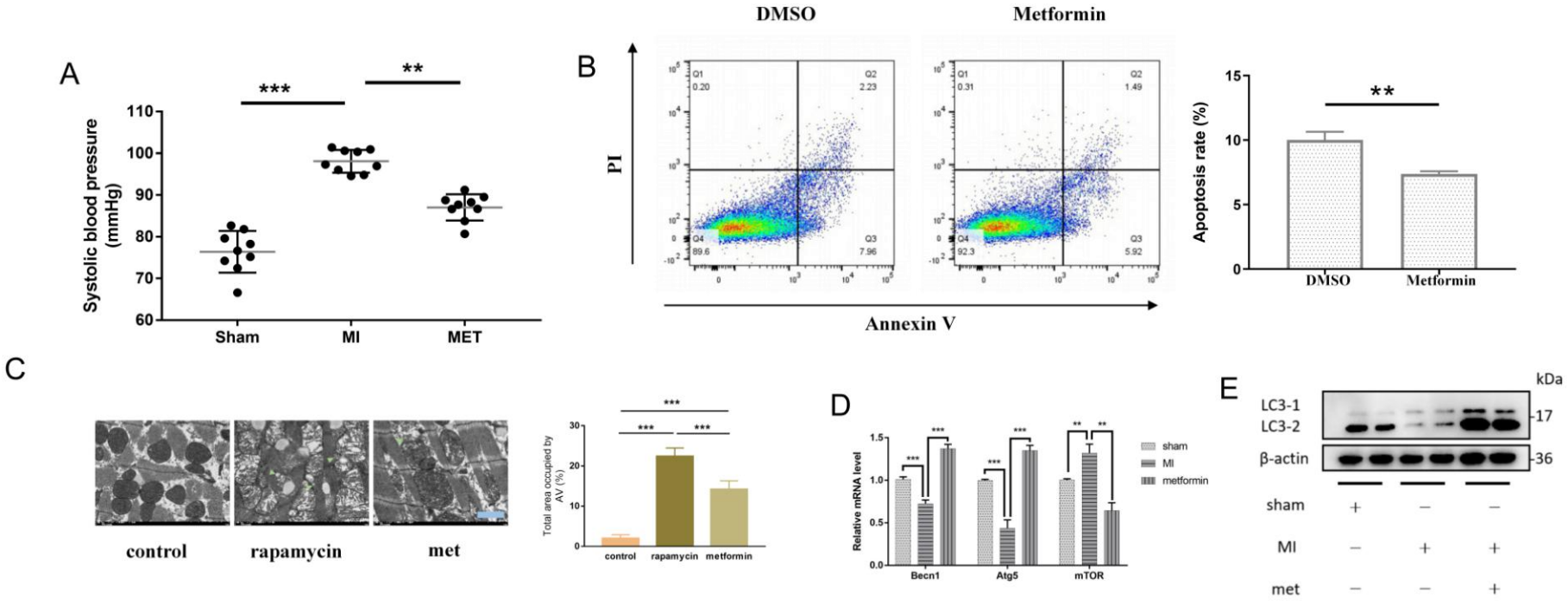


supplementary figure 1

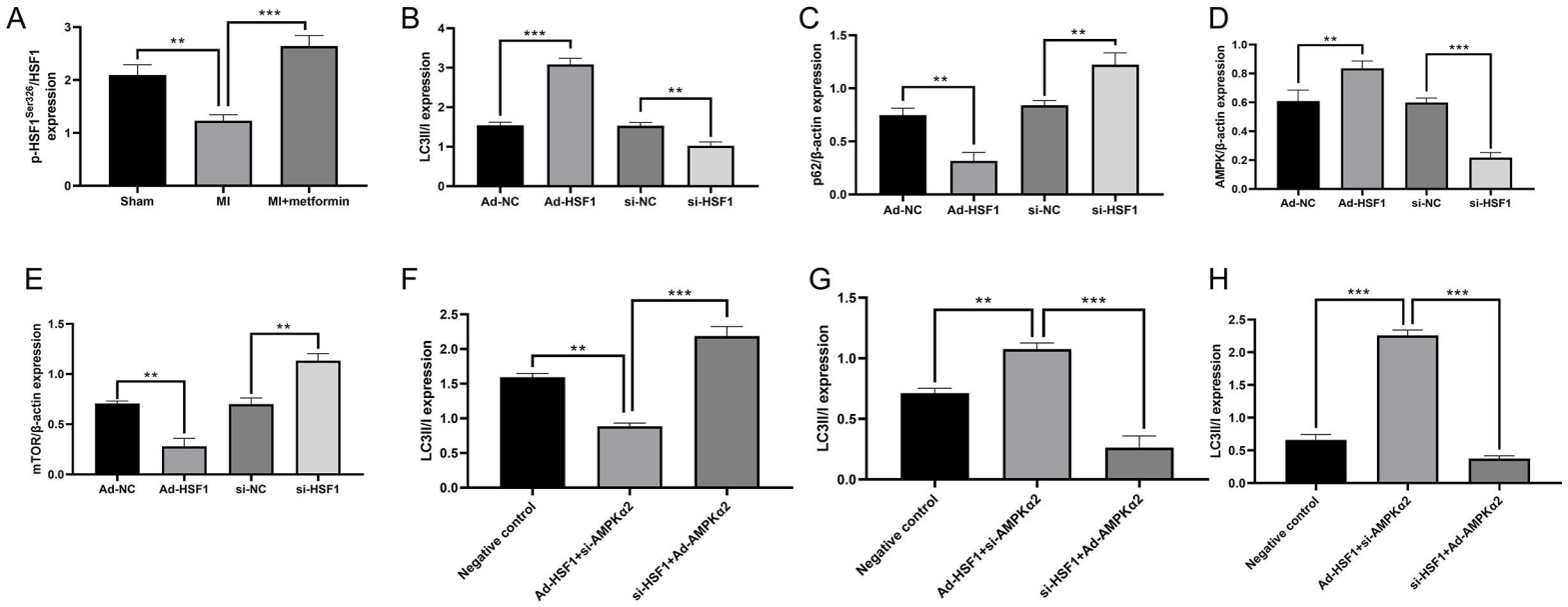
(A) 14 days after the operation, the systolic blood pressure of the mice was compared between the sham operation group, the operation control group and the metformin treatment group. (n=9; SD; ** p <0.01 and *** p <0.001)

(B) Basic apoptosis rate of mouse primary cardiomyocytes between control group and metformin treatment group. (n=3;**p<0.01)

(C) Transmission electron microscope representative pictures of the tissues adjacent to the infarcted myocardium in the untreated control group, rapamycin (positive control) group and metformin treatment group. Arrows show autophagosomes. Quantitative statistics of autophagic vesicles per unit area. (n=6; Bar=500nm)

(D) Real-time quantitative PCR was used to detect the mRNA expression levels of autophagy-related genes Becn 1, Atg 5 and mTOR in the myocardial tissue of the sham operation group and the tissues adjacent to the infarction in the operation group and metformin treatment group. (n=3; SD; ** $p < 0.01$ and *** $p < 0.001$)

(E) Western blotting was used to detect the levels of autophagy-related protein LC3II/I in the myocardial tissue of the sham operation group and the tissues adjacent to the infarction in the operation group and metformin treatment group.

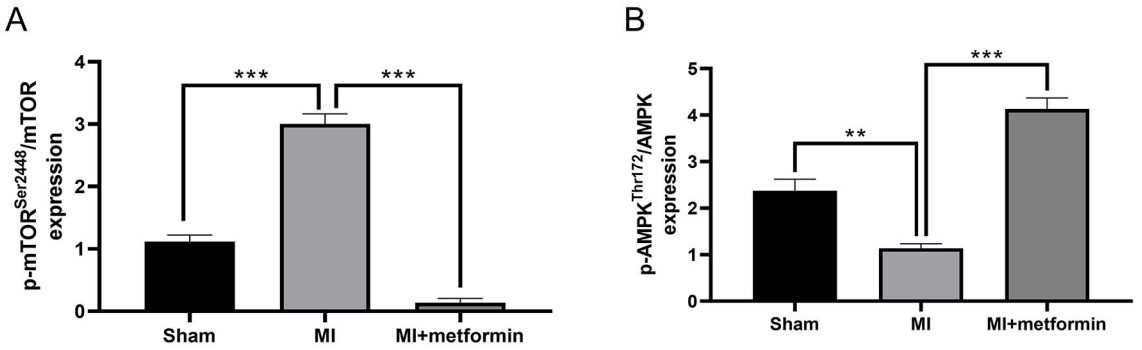


supplementary figure 2

(A-B) Western blot statistical histogram of figure 2A.

(C-E) Western blot statistical histogram of figure 4D.

(F-H) Western blot statistical histogram of figure 4F.



supplementary figure 3

Western blot statistical histogram of figure 6A

Supplementary Table 1: The primer sequence of qPCR

Primer	Forward (5′→3′)	Reverse (5′→3′)
Becn1	ATGGAGGGGTCTAAGGCGTC	TGGGCTGTGGTAAGTAATGGA
Atg5	TGTGCTTCGAGATGTGTGGTT	ACCAACGTCAAATAGCTGACTC
mTOR	CAGTTCGCCAGTGGACTGAAG	GCTGGTCATAGAAGCGAGTAGAC
Hsf1	GGGAAACAGGAGTGTATGGACT	CTTGTTGACAACTTTTTGCTGCT
Prkaa2	AAGATCGGACACTACGTCCTG	TGCCACTTTATGGCCTGTCAA
Mtor	CAGTTCGCCAGTGGACTGAAG	GCTGGTCATAGAAGCGAGTAGAC
Actb	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC

Supplementary Table 2: si-RNA sequence information

gene symbol	Target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)
Hsf1	473-495	CCGAAAAGTAGTCCACATTGAGC	UCAAUGUGGACUACUUUUCGG GAAAAGUAGUCCACAUUGAGC
Prkaa2	216-238	GACAGACTTTTTTATGGTAATGG	AUUACCAUAAAAAAGUCUGUC CAGACUUUUUUAUGGUAAUGG

Supplementary Table 3: SPR experimental method design and parameters

Analysis Project	HSF1-metformin and AMPK-metformin
Buffer	PBST(pH = 7.4，0.1% Tween 20)
Regeneration buffer	Glycine-HCl (pH = 2.0)
Analyte concentration	200nM，400nM，800nM，1600nM，3200nM
Analyte injection speed	0.5μL/s
Binding time	600s
Dissociation time	360s
Regeneration buffer flow rate	2μL/s
Regeneration time	300s
Binding reaction temperature	4℃
Chip selection	Photo-cross-linker SensorCHIP™
Test conditions	Humidity: 11.96 %; Temperature: 4.00 ℃; N_2 atmosphere (1.025 ATMs)
Loading apparatus	Biodot AD-1520 Array Printer
Analytical equipment	Berthold bScreen LB 991