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Supplemental information

**miR-146a inhibits mitochondrial
dysfunction and myocardial infarction
by targeting cyclophilin D**

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Supplementary Materials

Table S1. Primers used for genotyping

Mouse allele	Primer	Sequence
miR-146a ^{f/f}	Forward	5'-TACTGTGCGCTCTGTCTCCA-3'
	Reverse	5'-GCAGCCTGAAGTACGTAGCA-3'
<i>Ppij</i> ^{flox/flox}	Forward	5'-TTCTCACCAGTGCATAGGGCTCTG-3'
	Reverse	5'-GCTTTGTTATCCCAGCTGGCGC-3'
MHC-Cre	Forward	5'-ATGACAGACAGATCCCTCCTATCTCC-3'
	Reverse	5'-CTCATCACTCGTTGCATCATCGAC-3'

Table S2. Primers used for qRT-PCR

Gene	Primer	Sequence
<i>Vdac1</i>	Forward	5'-GCCGCCACATCCTCTGA-3'
	Reverse	5'-AGGCCGTA CT CAGTCCATCT-3'
<i>Ant</i>	Forward	5'-AGCGTGAGTTCCATGGTCTG-3'
	Reverse	5'-GACTCCGAAGTAGGCAGCTC-3'
<i>Ppif</i>	Forward	5'-GCGGTATTCAGCTGAGTTGT-3'
	Reverse	5'-GGAGGACTTCGAGGTTGTGT-3'
<i>U6</i>	Forward	5'-ATTGGAACGATACAGAGAAGATT-3'
	Reverse	5'-GGAACGCTTCACGAATTTG-3'
<i>12S rRNA</i>	Forward	5'-AAACTGCTCGCCAGA AACT-3'
	Reverse	5'-TAGGCTGAGCAAGAGGTGGT-3'
<i>Gapdh</i>	Forward	5'-TGTGAACGGATTTGGCCGTA-3'
	Reverse	5'-GATGGTGATGGGTTTCCCGT-3'

Table S3. Microarray analysis of miRNAs induced by ischemic reperfusion in the mitochondria of mice hearts

miRNA	Probe SetID	Signal intensity		Fold change (I/R vs. Sham)
		Sham	I/R	
Upregulated				
miR-150	mmu-miR-150-star_st	428.079	890.627	2.1805
miR-210	mmu-miR-210_st	319.002	1093.851	3.2289
miR-338	mmu-miR-338_st	400.903	960.347	2.2954
miR-92a	mmu-miR-92a-star_st	132.450	399.391	3.1154
miR-696	mmu-miR-669a_st	397.012	972.884	2.5505
miR-532	mmu-miR-532_st	223.051	604.854	2.6117
miR-771	mmu-miR-771-star_st	329.889	689.341	2.2896
miR-450-3p	mmu-miR-450-3p_st	250.858	703.472	2.9042
miR-345-3p	mmu-miR-345-3p_st	141.883	284.888	2.1079
miR-762	mmu-miR-762-star_st	466.012	1024.292	2.2979
Downregulated				
miR-535-5p	mmu-miR-535-3p_st	438.660	182.172	0.4352
miR-330	mmu-miR-330_st	287.856	142.443	0.4748
miR-146a	mmu-miR-146a-star_st	617.733	126.881	0.1953
miR-181a	mmu-miR-181a_st	127.779	58.773	0.4099
miR-34a	mmu-miR-34a-star_st	216.802	74.857	0.3152

I/R, ischemic reperfusion

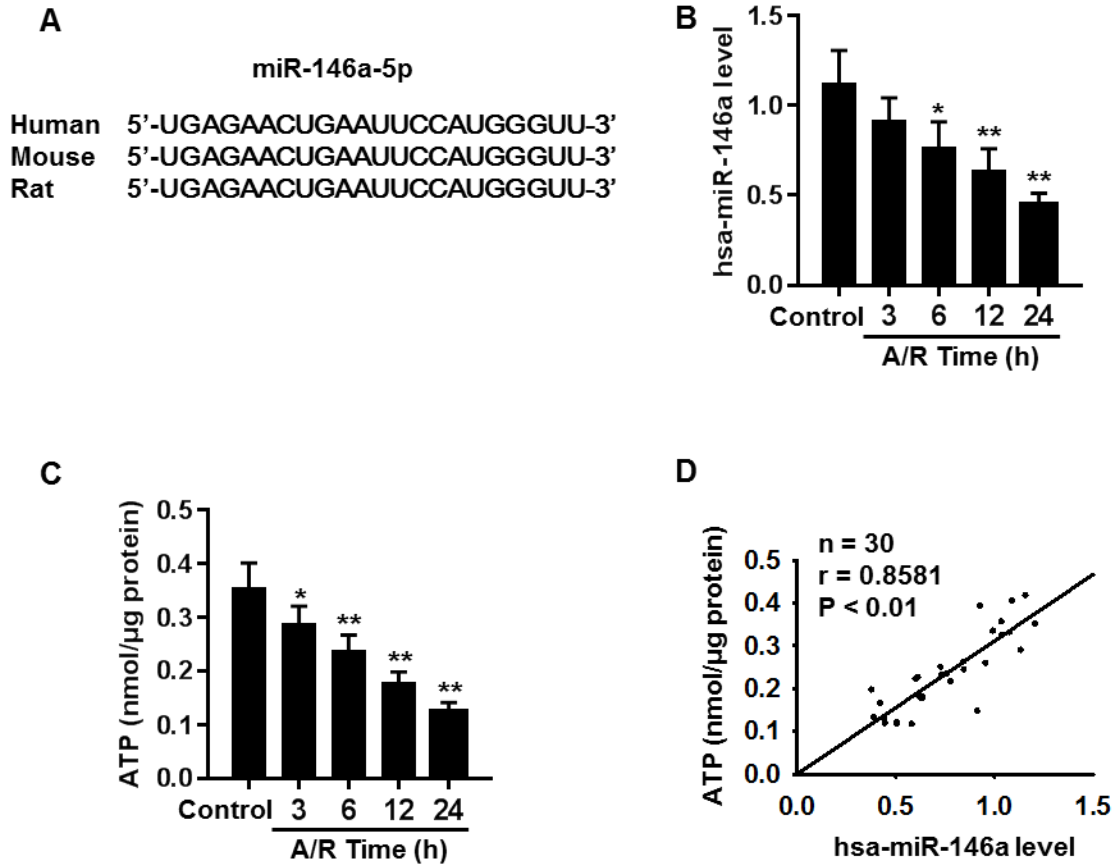


Figure S1 Mitochondrial miR-146a level is associated with mitochondrial dysfunction during cardiac I/R injury. (A) The mature miR-146a-5p strand is conserved between mice and humans. (B and C) Mitochondrial miR-146a level (B) and cellular ATP concentration (C) determined following A/R treatment. Anoxia induced by exposure to 95% N₂ and 5% CO₂ for 24 h, followed by reoxygenation with 95% air and 5% CO₂ for 3, 6, 12 or 24 h. **P* < 0.05, ***P* < 0.01 vs. control, *n* = 6. (D) miR-146a level positively correlates with ATP concentration.

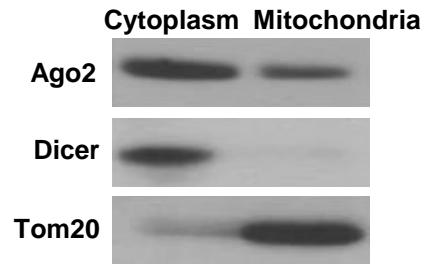


Figure S2 Ago2 and Dicer distribution in mitochondrial and cytosolic fractions. Western blot shows the presence of Ago 2, and absence of Dicer in the mitochondria. Mitochondrial marker protein Tom20 was used as an internal control. $n = 4$.

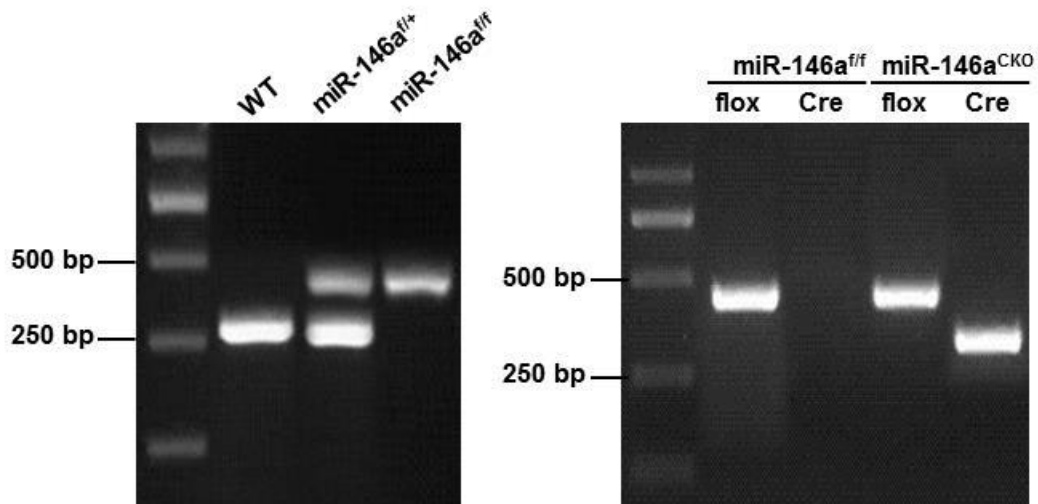


Figure S3 Genotyping analysis of miR-146a cardiomyocyte-specific knockout mice. Examples of DNA band profiles for wild-type (WT), miR-146^{f/+}, miR-146a^{f/f}, and miR-146a^{f/f}/MHC-Cre mice (miR-146a^{CKO}) from PCR analysis of tail biopsies.

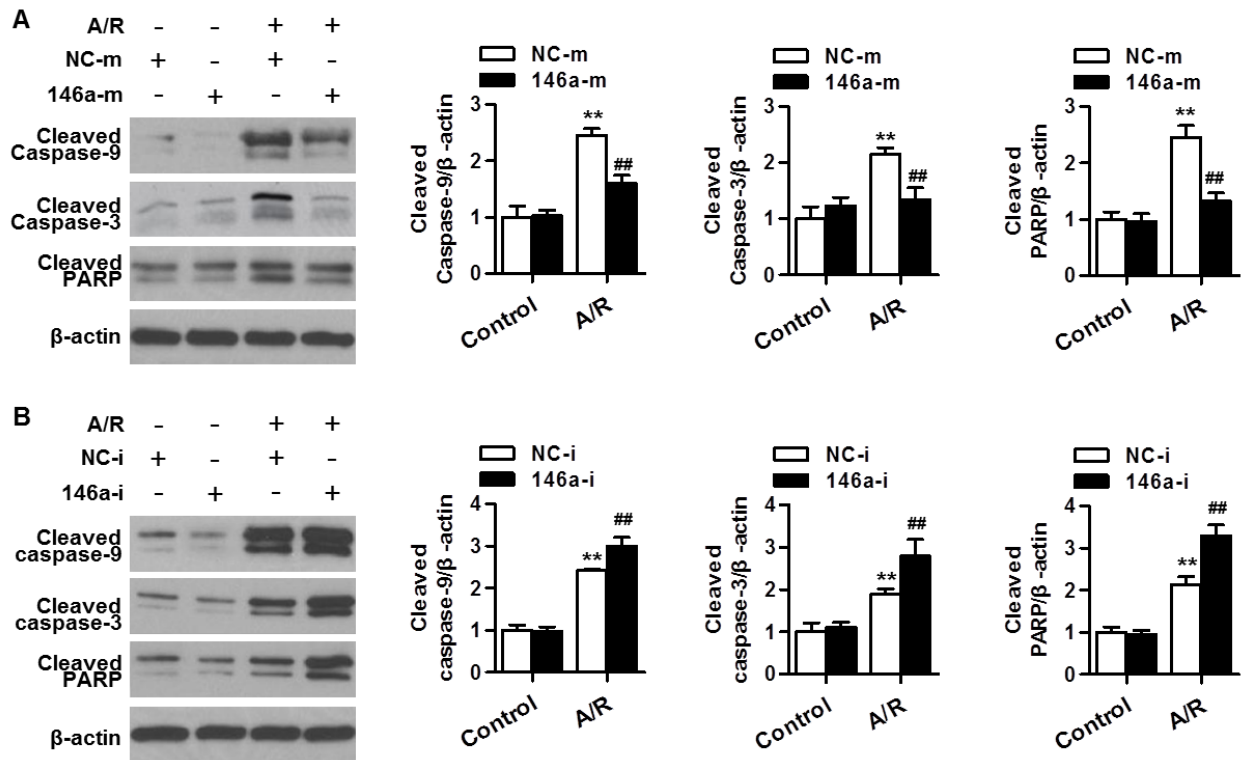


Figure S4 miR-146a regulates activation of caspases induced by A/R stimulation. (A and B) Cardiomyocytes transfected with miR-146a mimic (146a-m, 50 nmol/L, A), miR-146a inhibitor (146a-i, 50 nmol/L, B), or their corresponding negative controls (NC-m or NC-i) 24 h before A/R treatment for 48 h. Western blot analysis of cleaved caspase-9, cleaved caspase-3, and cleaved PARP. $**P < 0.01$ vs. NC-m control or NC-i control; $^{##}P < 0.01$ vs. NC-m A/R or NC-i A/R, $n = 6$.

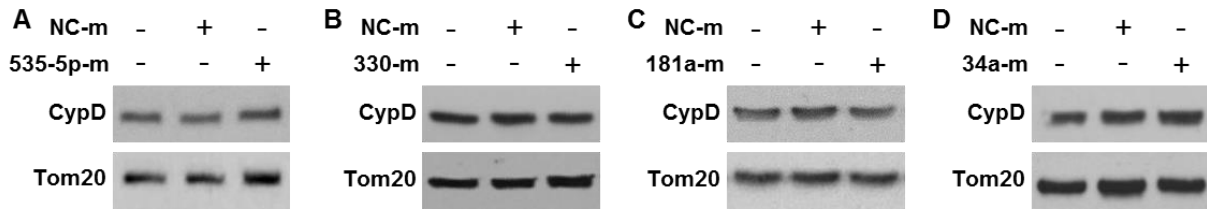


Figure S5 Overexpression of miR-535-5p, miR-330, miR-181a-m or miR-34a does not affect cyclophilin D expression. (A-D) Cardiomyocytes transfected with miR-535-5p mimics, miR-330 mimics, miR-181a mimics, or miR-34a mimics for 48 h. Expression of cyclophilin D was determined by western blot ($n = 4$).

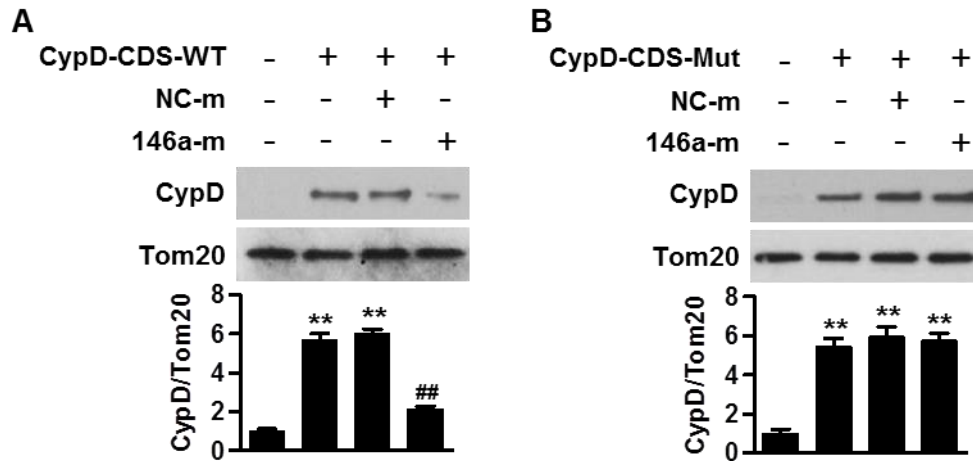


Figure S6 Overexpression of miR-146a does not affect cyclophilin D-CDS-Mut expression. (A and B) Cardiomyocytes co-transfected with the luciferase construct carrying cyclophilin D-CDS-WT (A) or cyclophilin D-CDS-Mut (B) and miR-146a mimic or mimic negative control. Cyclophilin D protein expression analyzed by western blot. ** $P < 0.01$ vs. control; ## $P < 0.01$ vs. cyclophilin D-CDS-WT, $n = 4$.

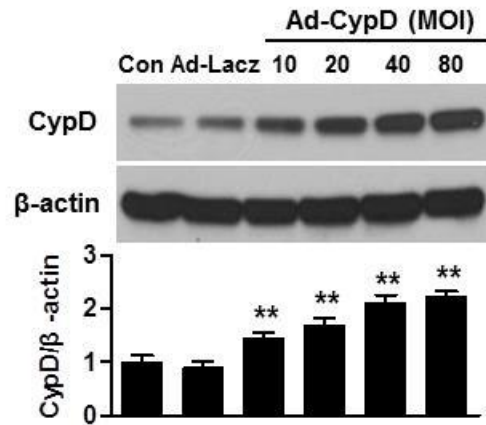


Figure S7 Effect of cyclophilin D adenovirus on cyclophilin D expression in cardiomyocytes. Cardiomyocytes treated with adenoviruses harboring LacZ DNA (Ad-Lacz, at multiplicity of infection [MOI] of 80) or cyclophilin D DNA (Ad-CypD, at MOI of 10, 20, 40, or 80) for 24 h. Cyclophilin D protein expression is shown. ** $P < 0.01$ vs. control, $n = 6$.

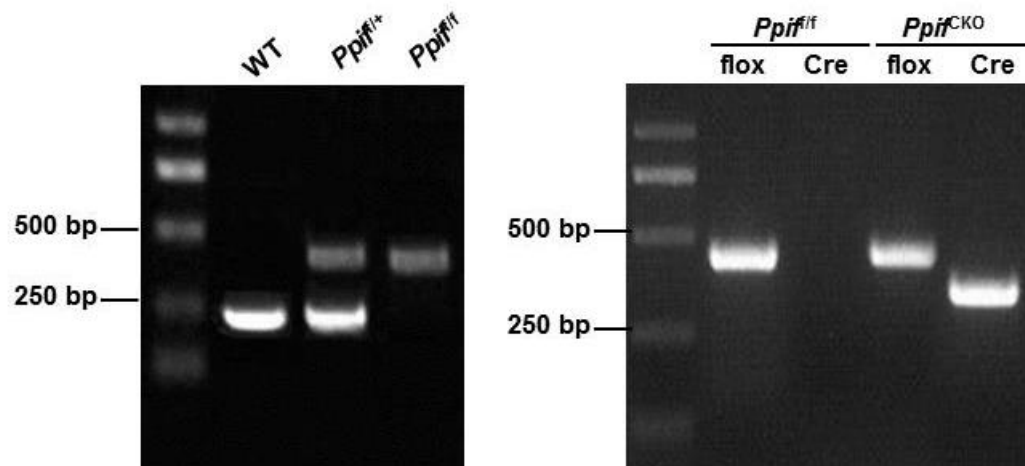


Figure S8 Genotyping analysis of *Ppif* cardiomyocyte-specific knockout mice. Examples of DNA band profiles for wild-type (WT), *Ppif*^{f/+}, *Ppif*^{f/f}, and *Ppif*^{f/f}/MHC-Cre mice (*Ppif*^{CKO}) from PCR analysis of tail biopsies.