Supplemental information

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

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Table S1. Primers used for genotyping

| Mouse allele   | Primer | Sequence                      |
|---------------|--------|-------------------------------|
| miR-146a<sup>ff</sup> | Forward | 5'-TACTGTGCGCTCTGCTCCA-3'     |
|               | Reverse| 5'-GCAGCCTGAAGTACGTAAGCA-3'   |
| Ppf<sup>flox/flox</sup> | Forward | 5'-TTCTCACCAGTGATAGGGCTCTG-3' |
|               | Reverse| 5'-GGCTTTGTTATCCACGTCGGGGC-3' |
| MHC-Cre       | Forward | 5'-ATGACAGACAGATCCCTCTATCTCC-3' |
|               | Reverse| 5'-CTCATCAGTCGCATCATCGAC-3'   |
| Gene   | Primer | Sequence                                      |
|--------|--------|----------------------------------------------|
| **Vdac1** | Forward | 5’-GCCGCCACATCCTCTCTGA-3’                   |
|        | Reverse | 5’-AGGCCGTACTCAGTCCATCT-3’                  |
| **Ant** | Forward | 5’-AGCGTGAGTTCCATGTTCTG-3’                  |
|        | Reverse | 5’-GACTCCGAAGTAGGAGCCT-3’                   |
| **Ppif** | Forward | 5’-GCGGTATTCAGCTGAGTT-3’                    |
|        | Reverse | 5’-GGAGGACTTCGAGGTGT-3’                     |
| **U6**  | Forward | 5’-ATTGAACGATACACAGAGATT-3’                 |
|        | Reverse | 5’-GGAACGCTTCAGGAATT-3’                     |
| **12S rRNA** | Forward | 5’-AAACTGCTGCCCAGAACA-3’                   |
|        | Reverse | 5’-TGGCTGAGCAAGAGGATT-3’                    |
| **Gapdh** | Forward | 5’-GTGGAACGGATTTGAGGTAC-3’                  |
|        | Reverse | 5’-GATGGTGATGGGTTCCGT-3’                    |
Table S3. Microarray analysis of miRNAs induced by ischemic reperfusion in the mitochondria of mice hearts

| miRNA    | Probe SetID      | Signal intensity | Fold change |
|----------|------------------|------------------|-------------|
|          |                  | Sham  | I/R     | (I/R vs. Sham) |
| 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-146a$^{+/+}$, miR-146a$^{ff}$, and miR-146a$^{ff}$/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*+/+, *Ppif*−/−, and *Ppif*−/−/MHC-Cre mice (*Ppif*CKO) from PCR analysis of tail biopsies.