

SUPPLEMENTAL MATERIALS

All primers used in this study

Stem-loop RT-PCR primers for miR-223-5p

Stem-loop RT primer:

5'-*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC CAACTC*-3'

Forward: 5'-GCAGAG cgt gta ttt gac aag -3'

Reverse: 5' - GTGCAGGGTCCGAGGT-3'

Stem-loop RT-PCR primers for miR-223-3p

Stem-loop RT primer:

5'-*GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC TGGGGT*-3'

Forward: 5'-GCAGAGtgt cag ttt gtc aaat

Reverse: 5' -GTGCAGGGTCCGAGGT-3'

RT-PCR primers for U6

RT and reverse primer, 5'-GTGCAGGGTCCGAGGT-3';

Forward primer, 5'-CTCGCTTCGGCAGCACA-3').

RT-PCR primers for TNF- α

Forward: 5'-AAACCACCAAGTGGAGGAGC-3'

Reverse: 5'-ACAAGGTACAACCCATCGGC-3'

RT-PCR primers for IL-1 β

Forward: 5'-CGTGGACCTTCCAGGATGAG-3'

Reverse: 5'-CATCTCGGAGCCTGTAGTGC-3'

RT-PCR primers for GAPDH

Forward: 5'-TTGTGTCCGTCGTGGATCTGA-3'

Reverse: 5'-CCTGCTTCACCACCTTCTTGA-3'

Genotyping primers for pre-miR-223 transgenic mice. Expected PCR product size of transgene α MHC-pre-miR-223 is 400bp.

Forward: 5'-CACATAGAAGCCTAGCCCACAC-3'

Reverse: 5'-GTAAGCTTCTACTCTGATGTTC-3'

Genotyping primers for the internal control: endogenous mouse Oct2 (organic cation transporter, member 2). Expected PCR product size of Oct2 is 196bp.

Oct forward primer: 5'-TCTTAGCTCTGCTCTCCGGT-3'

Oct Reverse primer: 5'-CACTGGCTGAGGAAGGAGAC-3'

Genotyping primers for pre-miR-223 knockout mice. Expected PCR product size for KO, 500bp; for heterozygote, 500bp and 392bp; for wild-type, 392bp.

11189 (Forward primer for wild type): 5'-CAGTGTCACGCTCCGTGTAT-3'

oIMR6101 (Forward primer for knockout): 5'-CTTCCTCGTGCTTTACGGTATCG-3'

Supplemental Figure S1

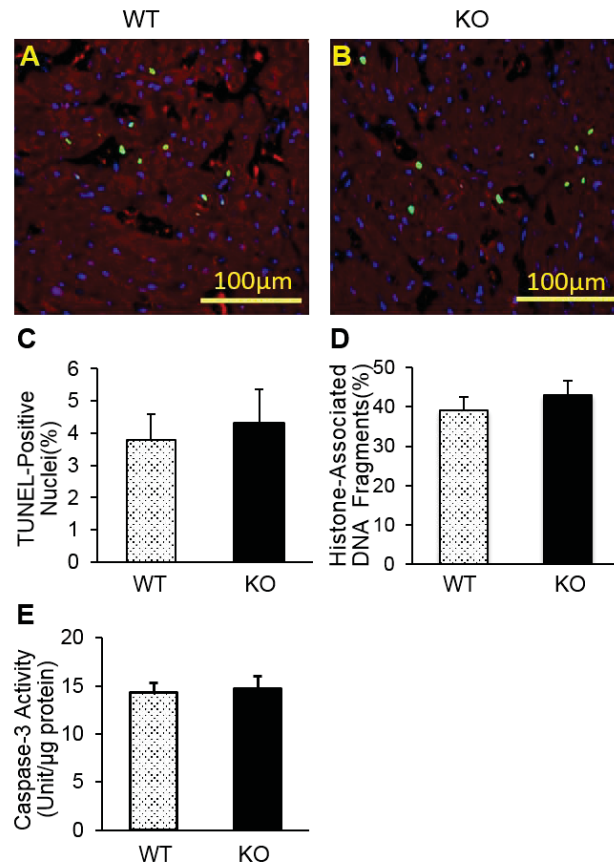


Figure S1: Ablation of pre-miR-223 did not increase I/R-induced myocardial apoptosis, compared to WT controls. (A/B) Representative images and (C) quantitative results of the TUNEL-staining assay in WT and KO hearts after *ex vivo* I/R (45min/1h). Green: TUNEL-positive nuclei; red: α -Actin; blue: DAPI. Scale bar 100 μ m. (D) The degree of histone-associated DNA fragmentation, and (E) Caspase-3 activity both were similar between WT and KO hearts subjected to *ex vivo* I/R. $P > 0.05$ vs. WT controls after t- test (n=6 hearts per group).