**Online Data Supplement**

*Animals:* Adult outbred ICR mice from Harlan (Indianapolis, Indiana) were used and the guidelines on humane use and care of laboratory animals for biomedical research published by NIH (No. 85-23, revised 1996) were strictly complied for all animal experiments.

*Langendorff Isolated Perfused Heart Preparation:* The methodology of the Langendorff isolated perfused heart has been previously described in detail (1). In brief, the mice were anesthetized with an IP injection of sodium pentobarbital (100 mg/kg containing 33 international units of heparin). The hearts were removed and submerged in ice-cold Krebs-Henseleit (K-H) solution containing heparin. The aorta was secured to a 20 gauge stainless steel blunt needle, and the hearts were perfused at a constant temperature and pressure in a retrograde fashion via the aorta with K-H solution. Following a stabilization period of 30 minutes, I/R injury was produced by subjecting the hearts to 30 minutes of normothermic, no-flow global ischemia followed by reperfusion for 60 minutes. To obtain the developed pressure, a left atrial incision was made to expose the mitral annulas through which a water-filled latex balloon was passed into the left ventricle (LV). The balloon was attached via polyethylene tubing to a Gould pressure transducer that was connected to a PowerLab Acquisition System (ADInstruments 8SP, Australia). The balloon was inflated to adjust the LV end-diastolic pressure (LVEDP) to ~ 10 mmHg. Myocardial ischemic damage was measured using multiple, independent end points of tissue injury. These included infarct size, LV developed pressure (LVDP), LVEDP, rate-pressure product (RPP), heart rate, and coronary flow by timed collection of the perfusate. LVDP was calculated by subtracting LVEDP from the peak systolic pressure. RPP, an index of cardiac work, was calculated by

multiplying LVDP with heart rate. The hearts were not paced.

*Ischemic Preconditioning Protocol in Isolated Mouse Hearts:* The protocol for ischemic preconditioning (IPC) is similar to that described above. However, 4 min. prior to 30 min. of prolonged ischemia, the hearts are subjected to 2 cycles of 30 sec. ischemia followed by 90 sec. reperfusion.

*miRNA extraction, verification and treatment:* miRNAs were extracted from the hearts of different groups, using a miRNA isolation kit from Ambion (Austin, Texas). The isolation method combines the chemical and solid phase extraction techniques to obtain optimal miRNAs. The extracted miRNAs were first treated with DNase to eliminate DNA contamination (DNA-free™, Ambion). Then, the miRNAs were verifed by RT-PCR using miRNA Detection Kit from Ambion. Prior to cardiac injection, miRNAs were incubated with polyamine at 22$^{o}$C for 30 minutes to form miRNA-amine or miRNA inhibitor-amine complexes which facilitate miRNAs' entry into cells (2,3,4). GAPDH RNA was used as control for miR-1, miR-21 and miR-24 and endogenous U1A small nuclear RNA (RNU1A) was used as control for miR-23b and miR-483 and for miR-21 to examine its uptake following injection.

*Injection of miRNA in the heart:* The animals were anesthetized with the injection of pentobarbital (70 mg/kg *ip*), intubated orotracheally and ventilated on a positive-pressure ventilator. The tidal volume was set at 0.2 ml, and the respiratory rate was adjusted to 133 cycles/min. All surgical procedures were carried out under sterile conditions. A left

thoracotomy was performed at the fourth intercostal space, and the heart was exposed by stripping the pericardium. Using 27G needles, three volumes of 10µl each containing 0.7 µg of miRNAs or PBS were injected into the myocardium of the left ventricle. After injection, the air was expelled from the chest. The animals were extubated and then received intramuscular doses of analgesia (buprenex; 0.02 mg/kg; sc) and antibiotic (Gentamicin; 0.7 mg/kg; IM, for 3 days). 48 h after injection, the myocardial infarction protocol was carried out.

*Myocardial Infarction Protocol (in vivo):* The methodology of in vivo myocardial infarction protocol has been previously described in detail (5). The animals were anesthetized with the injection of pentobarbital (70 mg/kg *ip*), intubated orotracheally and ventilated on a positive-pressure ventilator. The tidal volume was set at 0.2 ml, and the respiratory rate was adjusted to 133 cycles/min. All surgical procedures were carried out under sterile conditions. A left thoracotomy was performed at the fourth intercostal space, and the heart was exposed by stripping the pericardium. The left descending coronary artery was then identified and occluded by a 7.0 silk ligature that was placed around it for 30 min. followed by 24 h of reperfusion. At the onset of reperfusion, the air was expelled from the chest. The animals were extubated and then received intramuscular doses of analgesia (buprenex; 0.02 mg/kg; sc) and antibiotic (Gentamicin; 0.7 mg/kg; IM, for 3 days).

*Infarct Size Assessment:* After completion of the infarction protocol, the heart was quickly removed and mounted on a Langendorff apparatus. The coronary arteries were perfused with 0.9% NaCl containing 2.5 mM $CaCl_2$. After the blood was washed out, ~2 ml of 10% Evans

blue dye were injected as a bolus into the aorta until most of the heart turned blue. The heart was perfused with saline to wash out the excess Evans blue. Finally, the heart was removed, frozen, and cut into 6–8 transverse slices from apex to base of equal thickness (~1 mm). The slices were then incubated in a 10% TTC in isotonic phosphate buffer (pH 7.4) at room temperature for 30 min. The areas of infarcted tissue, the risk zone, and the whole left ventricle were determined by computer morphometry using a Bioquant imaging software.

*Data Analysis and Statistics:* All data were normalized by their corresponding control and presented as the group means ± standard error of mean. The difference among experimental groups was compared by unpaired *t* test or one-way ANOVA followed by Student-Newman-Keuls post-hoc test. $P<0.05$ was considered as statistically significant.

## Reference List


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