

## **Supplemental information**

### **Cardiac protection induced by urocortin-2 enables the regulation of apoptosis and fibrosis after ischemia and reperfusion involving miR-29a modulation**

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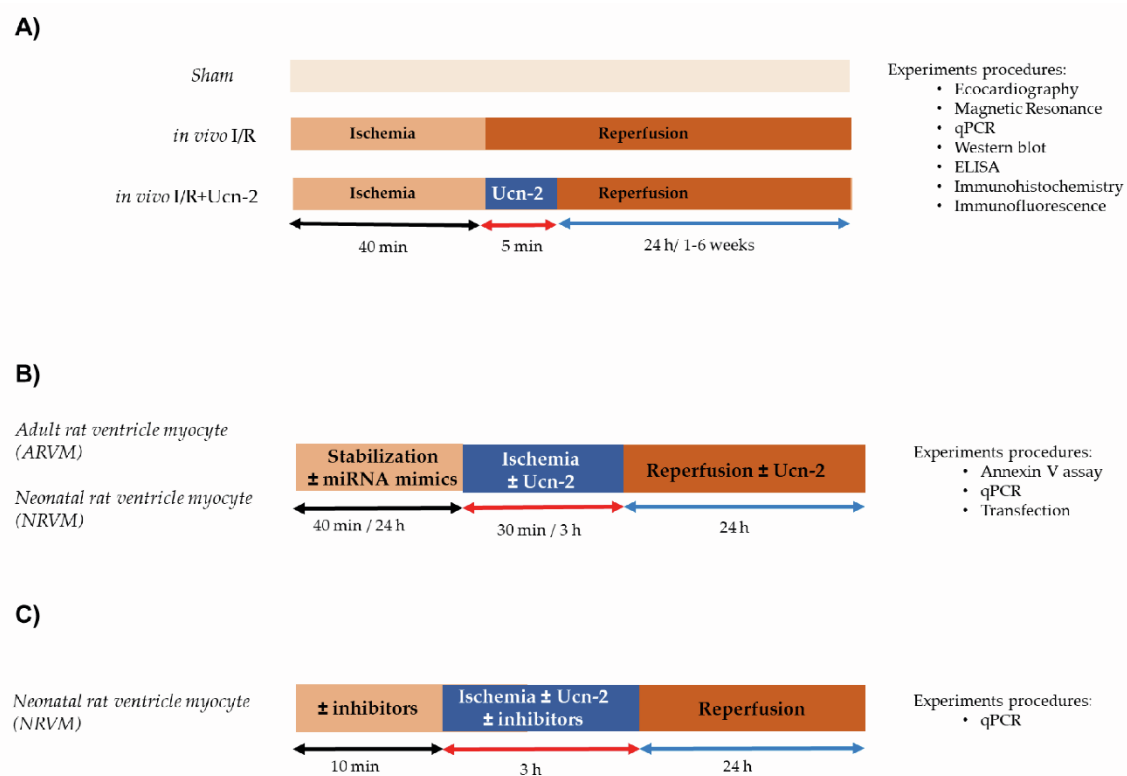
**SUPPLEMENTAL TABLE 1.** Data table show the list of genes from Apoptosis and Survival Tier 1 commercial plate for genes expression in samples taken from control NRVM in I/R, and in NRVM transfected with mimics of miR-29a or miR-251\_1\*. A mix of 4 cultures was added to each pool. Results are expressed in log fold change.

Genes	Condition	log FC	Genes	Condition	log FC	Genes	Condition	log FC
<i>Cycs</i>	I/R	0,784	<i>Bcl2l1</i>	I/R + miR-29a mimic	0,708	<i>Hsp90aa</i>	I/R + miR-451_1* mimic	0,488
<i>Bcl2l1</i>	I/R	0,689	<i>Ngf</i>	I/R + miR-29a mimic	0,481	<i>Myc</i>	I/R + miR-451_1* mimic	0,303
<i>Nfkb1</i>	I/R	0,547	<i>Myc</i>	I/R + miR-29a mimic	0,382	<i>Ngf</i>	I/R + miR-451_1* mimic	0,293
<i>Mdm2</i>	I/R	0,449	<i>Mdm2</i>	I/R + miR-29a mimic	0,329	<i>Nfkb1</i>	I/R + miR-451_1* mimic	0,282
<i>Myc</i>	I/R	0,444	<i>Hsp90aa</i>	I/R + miR-29a mimic	0,300	<i>Abl1</i>	I/R + miR-451_1* mimic	0,169
<i>Cd44</i>	I/R	0,444	<i>Nfkb1</i>	I/R + miR-29a mimic	0,150	<i>Tnfrs1b</i>	I/R + miR-451_1* mimic	0,159
<i>Ngf</i>	I/R	0,422	<i>Cycs</i>	I/R + miR-29a mimic	0,143	<i>Tnfrsf10b</i>	I/R + miR-451_1* mimic	0,153
<i>Hgf</i>	I/R	0,417	<i>Egf2</i>	I/R + miR-29a mimic	0,136	<i>Rb1</i>	I/R + miR-451_1* mimic	0,152
<i>Hsp90aa</i>	I/R	0,345	<i>Vegfa</i>	I/R + miR-29a mimic	0,101	<i>Clu</i>	I/R + miR-451_1* mimic	0,145
<i>Mapk8</i>	I/R	0,329	<i>Tnfrsf10b</i>	I/R + miR-29a mimic	0,098	<i>Hgf</i>	I/R + miR-451_1* mimic	0,116
<i>Dlc1</i>	I/R	0,315	<i>Hmgb1</i>	I/R + miR-29a mimic	0,097	<i>Cdkn1a</i>	I/R + miR-451_1* mimic	0,112
<i>Hmox1</i>	I/R	0,312	<i>Csap8</i>	I/R + miR-29a mimic	0,097	<i>Bcl2l1</i>	I/R + miR-451_1* mimic	0,103
<i>S100a9</i>	I/R	0,300	<i>Abl1</i>	I/R + miR-29a mimic	0,049	<i>Lcn2</i>	I/R + miR-451_1* mimic	0,096
<i>Abl1</i>	I/R	0,294	<i>Tnf</i>	I/R + miR-29a mimic	0,049	<i>Pi3cg</i>	I/R + miR-451_1* mimic	0,090
<i>Casp3</i>	I/R	0,277	<i>Hmox1</i>	I/R + miR-29a mimic	0,043	<i>Fgfr2</i>	I/R + miR-451_1* mimic	0,090
<i>Foxo1</i>	I/R	0,245	<i>Pten</i>	I/R + miR-29a mimic	0,040	<i>Cycs</i>	I/R + miR-451_1* mimic	0,078
<i>Hspd1</i>	I/R	0,241	<i>Tgfb1</i>	I/R + miR-29a mimic	0,037	<i>Casp1</i>	I/R + miR-451_1* mimic	0,060
<i>Akt2</i>	I/R	0,224	<i>Mcl1</i>	I/R + miR-29a mimic	0,027	<i>Akt3</i>	I/R + miR-451_1* mimic	0,046
<i>Csap8</i>	I/R	0,208	<i>Fgfr2</i>	I/R + miR-29a mimic	0,016	<i>Cflar</i>	I/R + miR-451_1* mimic	0,043
<i>Pi3cg</i>	I/R	0,203	<i>Dlc1</i>	I/R + miR-29a mimic	0,002	<i>Cd4</i>	I/R + miR-451_1* mimic	0,039
<i>Aifm1</i>	I/R	0,191	<i>Tnfrsf10a</i>	I/R + miR-29a mimic	-0,015	<i>Csap8</i>	I/R + miR-451_1* mimic	0,039
<i>Apaf1</i>	I/R	0,181	<i>Il1b</i>	I/R + miR-29a mimic	-0,024	<i>Raf1</i>	I/R + miR-451_1* mimic	0,038
<i>Rps6kb1</i>	I/R	0,179	<i>Hspa5</i>	I/R + miR-29a mimic	-0,038	<i>Src</i>	I/R + miR-451_1* mimic	0,033
<i>Fgfr2</i>	I/R	0,173	<i>Clu</i>	I/R + miR-29a mimic	-0,043	<i>Cd44</i>	I/R + miR-451_1* mimic	0,033
<i>Akt1</i>	I/R	0,168	<i>Fas</i>	I/R + miR-29a mimic	-0,050	<i>Apaf1</i>	I/R + miR-451_1* mimic	0,023
<i>Hmgb1</i>	I/R	0,163	<i>Stat1</i>	I/R + miR-29a mimic	-0,066	<i>Pten</i>	I/R + miR-451_1* mimic	0,010
<i>Hspa5</i>	I/R	0,155	<i>Sod2</i>	I/R + miR-29a mimic	-0,088	<i>Tp53</i>	I/R + miR-451_1* mimic	0,002
<i>Tnfrs1b</i>	I/R	0,152	<i>Rb1</i>	I/R + miR-29a mimic	-0,096	<i>Igfbp3</i>	I/R + miR-451_1* mimic	-0,009
<i>Akt3</i>	I/R	0,140	<i>Akt1</i>	I/R + miR-29a mimic	-0,097	<i>Il17a</i>	I/R + miR-451_1* mimic	-0,016
<i>Ctnb1</i>	I/R	0,138	<i>Mapk14</i>	I/R + miR-29a mimic	-0,099	<i>Hspd1</i>	I/R + miR-451_1* mimic	-0,016
<i>Fas</i>	I/R	0,132	<i>Il17a</i>	I/R + miR-29a mimic	-0,102	<i>Bax</i>	I/R + miR-451_1* mimic	-0,017
<i>Egf2</i>	I/R	0,129	<i>Aifm1</i>	I/R + miR-29a mimic	-0,113	<i>Atm</i>	I/R + miR-451_1* mimic	-0,027
<i>Vegfa</i>	I/R	0,124	<i>Csap9</i>	I/R + miR-29a mimic	-0,114	<i>Akt1</i>	I/R + miR-451_1* mimic	-0,037
<i>Mapk14</i>	I/R	0,118	<i>Raf1</i>	I/R + miR-29a mimic	-0,122	<i>Hmox1</i>	I/R + miR-451_1* mimic	-0,040
<i>Mcl1</i>	I/R	0,113	<i>Casp3</i>	I/R + miR-29a mimic	-0,132	<i>Il1b</i>	I/R + miR-451_1* mimic	-0,040

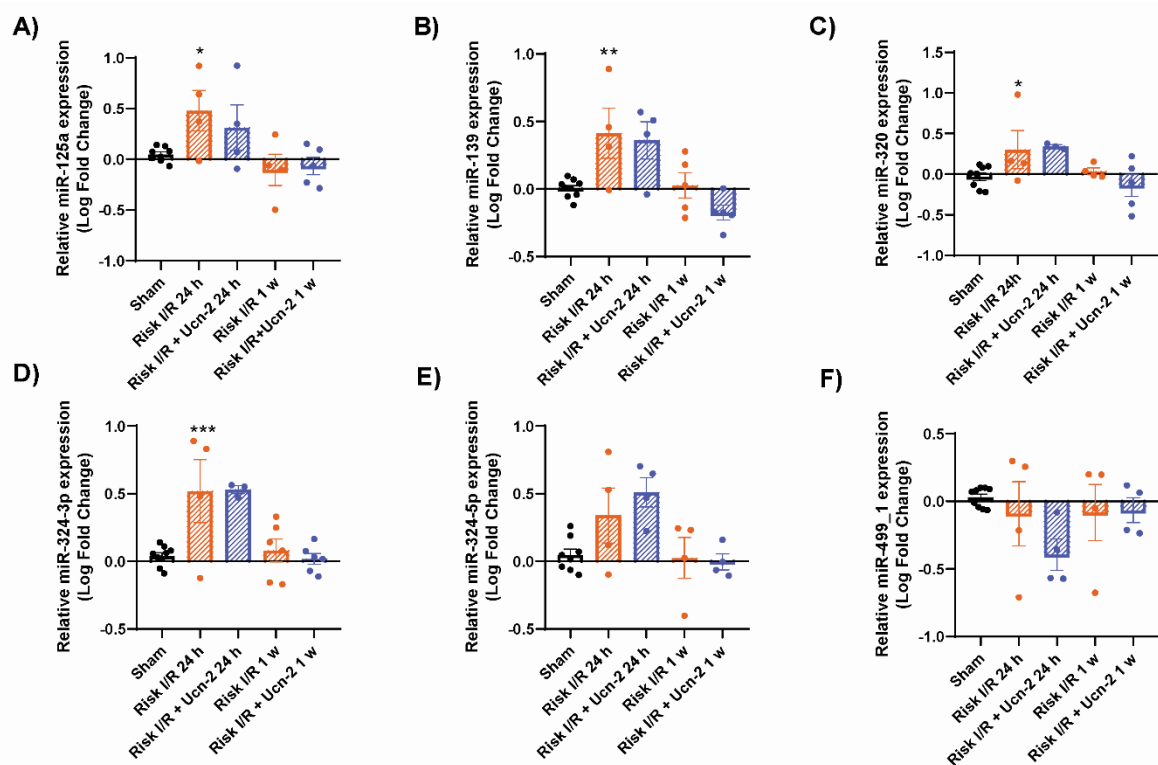
<i>Tnf</i>	I/R	0,106	<i>Casp1</i>	I/R + miR-29a mimic	-0,141	<i>Vegfa</i>	I/R + miR-451_1* mimic	-0,045
<i>Mapk3</i>	I/R	0,102	<i>Cflar</i>	I/R + miR-29a mimic	-0,143	<i>Aifm1</i>	I/R + miR-451_1* mimic	-0,046
<i>Pten</i>	I/R	0,096	<i>Akt3</i>	I/R + miR-29a mimic	-0,155	<i>Sod2</i>	I/R + miR-451_1* mimic	-0,052
<i>Lcn2</i>	I/R	0,095	<i>Tp53</i>	I/R + miR-29a mimic	-0,160	<i>Hmgb1</i>	I/R + miR-451_1* mimic	-0,059
<i>Il1b</i>	I/R	0,092	<i>Cdkn1a</i>	I/R + miR-29a mimic	-0,165	<i>Fas</i>	I/R + miR-451_1* mimic	-0,065
<i>Raf1</i>	I/R	0,089	<i>Lcn2</i>	I/R + miR-29a mimic	-0,169	<i>Dlc1</i>	I/R + miR-451_1* mimic	-0,089
<i>Clu</i>	I/R	0,079	<i>Src</i>	I/R + miR-29a mimic	-0,173	<i>Rps6kb1</i>	I/R + miR-451_1* mimic	-0,096
<i>Tnfrsf10a</i>	I/R	0,063	<i>Pi3cg</i>	I/R + miR-29a mimic	-0,175	<i>Apc</i>	I/R + miR-451_1* mimic	-0,104
<i>Cdkn1a</i>	I/R	0,054	<i>Mapk8</i>	I/R + miR-29a mimic	-0,192	<i>Mdm2</i>	I/R + miR-451_1* mimic	-0,106
<i>Sod2</i>	I/R	0,050	<i>Apaf1</i>	I/R + miR-29a mimic	-0,196	<i>Ifng</i>	I/R + miR-451_1* mimic	-0,108
<i>Cd4</i>	I/R	0,045	<i>Hras</i>	I/R + miR-29a mimic	-0,217	<i>S100a9</i>	I/R + miR-451_1* mimic	-0,119
<i>Birc5</i>	I/R	0,042	<i>Akt2</i>	I/R + miR-29a mimic	-0,218	<i>Tnfrsf10a</i>	I/R + miR-451_1* mimic	-0,121
<i>Il17a</i>	I/R	0,039	<i>Tnfrs1b</i>	I/R + miR-29a mimic	-0,223	<i>Foxo1</i>	I/R + miR-451_1* mimic	-0,127
<i>Tgfb1</i>	I/R	0,026	<i>Cd44</i>	I/R + miR-29a mimic	-0,226	<i>Sfrp1</i>	I/R + miR-451_1* mimic	-0,128
<i>Sfrp1</i>	I/R	0,024	<i>Pik3ca</i>	I/R + miR-29a mimic	-0,226	<i>Mapk3</i>	I/R + miR-451_1* mimic	-0,136
<i>Cav1</i>	I/R	0,018	<i>Bax</i>	I/R + miR-29a mimic	-0,244	<i>Akt2</i>	I/R + miR-451_1* mimic	-0,148
<i>Pik3ca</i>	I/R	0,017	<i>Ctnb1</i>	I/R + miR-29a mimic	-0,258	<i>Stat1</i>	I/R + miR-451_1* mimic	-0,152
<i>Src</i>	I/R	0,016	<i>Cav1</i>	I/R + miR-29a mimic	-0,264	<i>Casp3</i>	I/R + miR-451_1* mimic	-0,155
<i>Hspa</i>	I/R	0,012	<i>Hspd1</i>	I/R + miR-29a mimic	-0,271	<i>Hspa</i>	I/R + miR-451_1* mimic	-0,160
<i>Rb1</i>	I/R	0,009	<i>Birc5</i>	I/R + miR-29a mimic	-0,278	<i>Mapk1</i>	I/R + miR-451_1* mimic	-0,165
<i>Igfbp3</i>	I/R	0,004	<i>Hspa</i>	I/R + miR-29a mimic	-0,278	<i>Mapk8</i>	I/R + miR-451_1* mimic	-0,166
<i>Atm</i>	I/R	-0,001	<i>Cdkn2a</i>	I/R + miR-29a mimic	-0,292	<i>Hspa5</i>	I/R + miR-451_1* mimic	-0,173
<i>Ifng</i>	I/R	-0,018	<i>Igfbp7</i>	I/R + miR-29a mimic	-0,308	<i>Tnf</i>	I/R + miR-451_1* mimic	-0,226
<i>Cflar</i>	I/R	-0,020	<i>Ifng</i>	I/R + miR-29a mimic	-0,334	<i>Csap9</i>	I/R + miR-451_1* mimic	-0,226
<i>Apc</i>	I/R	-0,028	<i>Rps6kb1</i>	I/R + miR-29a mimic	-0,339	<i>Xiap</i>	I/R + miR-451_1* mimic	-0,232
<i>Cdkn2a</i>	I/R	-0,030	<i>Atm</i>	I/R + miR-29a mimic	-0,349	<i>Bad</i>	I/R + miR-451_1* mimic	-0,236
<i>Stat1</i>	I/R	-0,058	<i>S100a9</i>	I/R + miR-29a mimic	-0,351	<i>Mcl1</i>	I/R + miR-451_1* mimic	-0,253
<i>Tp53</i>	I/R	-0,059	<i>Foxo1</i>	I/R + miR-29a mimic	-0,352	<i>Pik3ca</i>	I/R + miR-451_1* mimic	-0,269
<i>Nfkb1a</i>	I/R	-0,082	<i>Mapk3</i>	I/R + miR-29a mimic	-0,366	<i>Hras</i>	I/R + miR-451_1* mimic	-0,286
<i>Bax</i>	I/R	-0,094	<i>Nfkb1a</i>	I/R + miR-29a mimic	-0,409	<i>Tgfb1</i>	I/R + miR-451_1* mimic	-0,288
<i>Igfbp7</i>	I/R	-0,101	<i>Xiap</i>	I/R + miR-29a mimic	-0,429	<i>Bak1</i>	I/R + miR-451_1* mimic	-0,292
<i>Bcl2</i>	I/R	-0,140	<i>Sfrp1</i>	I/R + miR-29a mimic	-0,463	<i>Birc5</i>	I/R + miR-451_1* mimic	-0,302
<i>Bad</i>	I/R	-0,176	<i>Mapk1</i>	I/R + miR-29a mimic	-0,490	<i>Fgf2</i>	I/R + miR-451_1* mimic	-0,316
<i>Xiap</i>	I/R	-0,184	<i>Cd4</i>	I/R + miR-29a mimic	-0,491	<i>Cdkn2a</i>	I/R + miR-451_1* mimic	-0,317
<i>Hras</i>	I/R	-0,188	<i>Apc</i>	I/R + miR-29a mimic	-0,513	<i>Bcl2</i>	I/R + miR-451_1* mimic	-0,325
<i>Casp1</i>	I/R	-0,229	<i>Igfbp3</i>	I/R + miR-29a mimic	-0,526	<i>Ctnb1</i>	I/R + miR-451_1* mimic	-0,360
<i>Csap9</i>	I/R	-0,235	<i>Hgf</i>	I/R + miR-29a mimic	-0,547	<i>Nfkb1a</i>	I/R + miR-451_1* mimic	-0,412
<i>Bak1</i>	I/R	-0,256	<i>Bcl2</i>	I/R + miR-29a mimic	-0,563	<i>Igfbp7</i>	I/R + miR-451_1* mimic	-0,417
<i>Tnfrs1a</i>	I/R	-0,268	<i>Tnfrs1a</i>	I/R + miR-29a mimic	-0,732	<i>Cav1</i>	I/R + miR-451_1* mimic	-0,430
<i>Mapk1</i>	I/R	-0,351	<i>Bak1</i>	I/R + miR-29a mimic	-0,741	<i>Tnfrs1a</i>	I/R + miR-451_1* mimic	-0,524
<i>Fadd</i>	I/R	-0,412	<i>Fadd</i>	I/R + miR-29a mimic	-0,876	<i>Fadd</i>	I/R + miR-451_1* mimic	-0,553
<i>Tnfrsf10b</i>	I/R	-0,815	<i>Bad</i>	I/R + miR-29a mimic	-1,061	<i>Mapk14</i>	I/R + miR-451_1* mimic	-0,623

**SUPPLEMENTAL TABLE 2.** Primers designed for genes RT- PCR purchased from Merck-Sigma-Aldrich (USA)

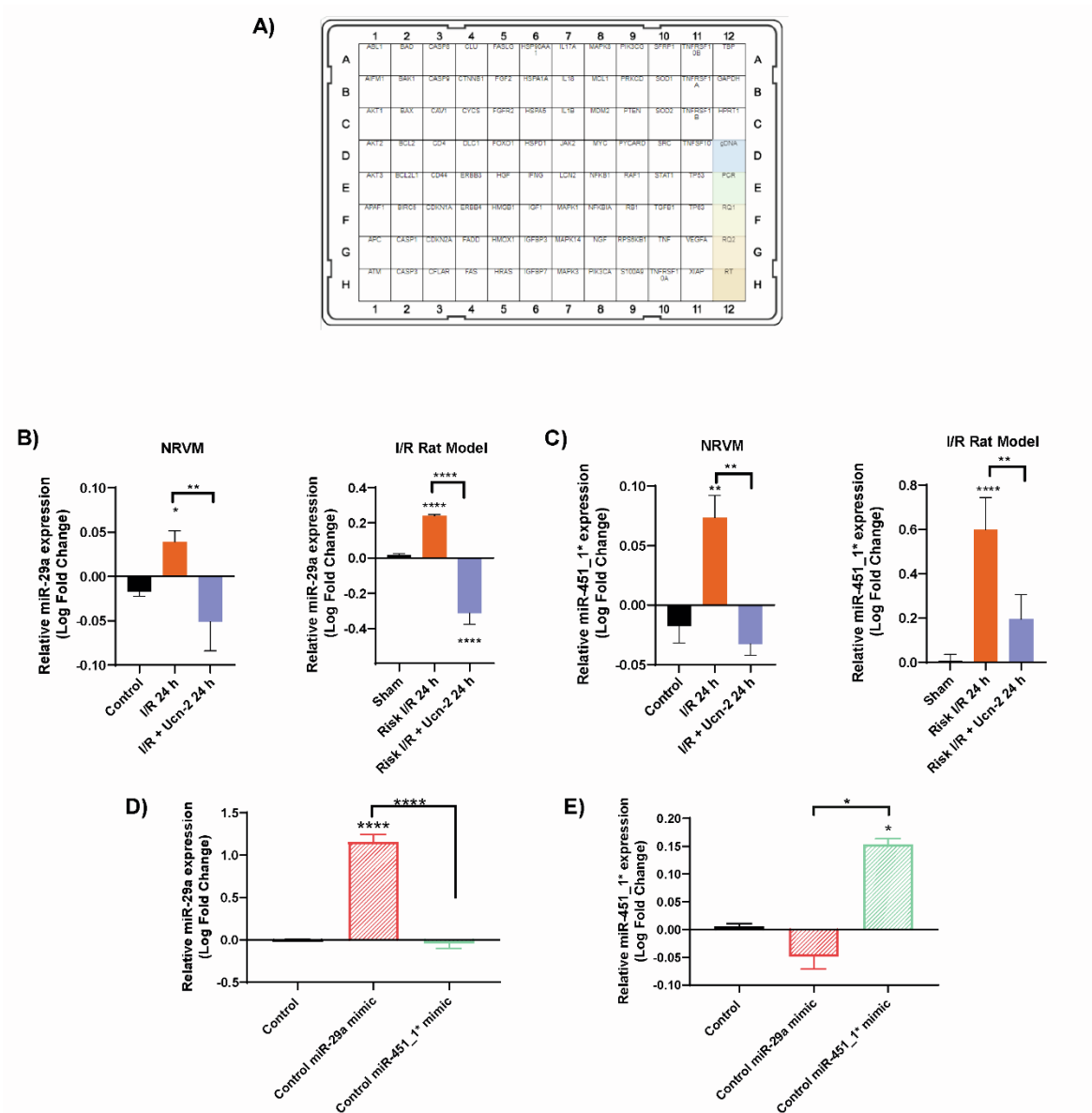
<i>β-actin Fw</i> (5'-TTCTTCCTGGGTATGGAAT-3')	<i>β-actin Rv</i> (5'-TTCTTCCTGGGTATGGAAT-3')
<i>Bcl-2 Fw</i> (5'-TTCGGGATGGAGTAAACTGG-3')	<i>Bcl-2 Rv</i> (5'-AAGGCTCTAGGTGGTCATTCAG-3')
<i>Cyts Fw</i> (5'-CAAAGTGTGCCAGTGCCA-3')	<i>Cyts Rv</i> (5'-CAGCAGCCTGGCCTGTCTTC-3')
<i>Apaf-1 Fw</i> (5'-TCCAGCGGCAAGGACACAGACG-3')	<i>Apaf-1 Rv</i> (5'-CAAACCGCGTGCAAAGATTCTGCA-3')
<i>Mapk-8 Fw</i> (5'-TGCCATCATGAGCAGAAAGCAAAC3')	<i>Mapk-8 Rv</i> (5'-TCTGATTCTGAAATGGCCGGCTG-3')
<i>Hmox-1 Fw</i> (5'-AGGCTAAGACCGCCTTCCT-3')	<i>Hmox-1 Rv</i> (5'-TGTGTTCTCTGTCAGCATCA-3')
<i>Aifm-1 Fw</i> (5'-GAGAAACAGAGAAGAGCCA-3')	<i>Aifm-1 Rv</i> (5'-GTCACGTCCCTTCCTGCT-3')
<i>Col-I Fw</i> (5'-TTCACCTACAGCACGCTTGT-3')	<i>Col-I Rv</i> (5'-TTGGGATGGAGGGAGTTTAC-3')
<i>Col-III Fw</i> (5'-GGTCACTTTCCTGGTTGACGA-3')	<i>Col-III Rv</i> (5'-GGTCACTTTCCTGGTTGACGA-3')
<i>Tgf-β1 Fw</i> (5'-ATTCTGGCGTTACCTTGG-3')	<i>Tgf-β1 Rv</i> (5'-AGCCCTGTATTCCGTCTCCT-3')
<i>Tgf-β2 Fw</i> (5'-GCAGAGTTCAGGGTCTTTTCG-3')	<i>Tgf-β2 Rv</i> (5'-GCTGGGTTGGAGATGTTAGG-3')



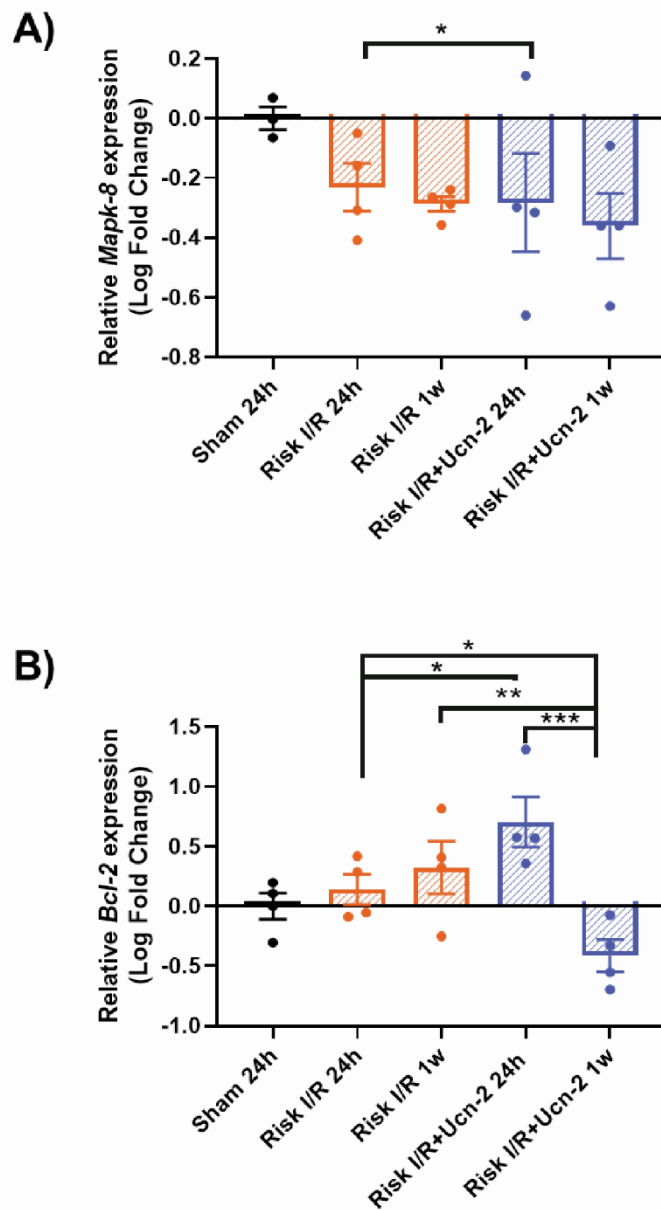
**SUPPLEMENTAL FIGURE 1. Cartoons outlining the experimental protocols of ischemia and reperfusion (I/R) conducted *in vivo* in animal models and *in vitro* in isolated cardiac myocytes. (A) Experiments were performed *in vivo* in 3 experimental group: **Sham** (upper bar), rats subjected to open chest surgery without ligation of the coronary artery. *In vivo* **I/R** (middle bar): rats subjected to transient ischemia produced by left descending coronary artery ligation during 40 min and infusion of the vehicle (0.9% NaCl) through tail vein injection 5 min before reperfusion. *In vivo* **I/R + Ucn-2** (lower bar): Same procedure as in I/R group, but instead of vehicle, Ucn-2 (150 µg/Kg) was infused through the tail vein 5 min before reperfusion. In right, experimental procedures performed using the animal model 24h, 1 or 6 weeks after the intervention. (B) *In vitro* protocol conducted in adult rat ventricle myocyte (ARVM) and in neonatal rat ventricle myocyte (NRVM). Isolated cardiac myocytes were divided in the following groups, **Control** for untreated cardiac myocytes. **I/R** for cells exposed to simulated ischemia solution during 30 min (ARVM) or 3 h (NRVM) and later reoxygenated with freshly control solution. ARVM were transfected with miRNA mimics 24 h before starting the experiments. **I/R + Ucn-2**: same as in I/R, but Ucn-2 (10-30 nM) was added before and during reoxygenation. In right, Experimental procedures performed 24h after. (C) Same protocol as in B. In this condition inhibitors, such as astressin (10 µM, CRF-R2 inhibitor), ESI-05 (10 µM, Epac2 inhibitor), H89 (1 µM inhibitor of PKA) or PD 098059 (5 µM, ERK1/2 inhibitor), were added 10 min before ischemia with 10 nM Ucn-2.**



**SUPPLEMENTAL FIGURE 2.** miRNAs relative expression assessed in infarcted heart's risk zones of I/R and I/R+Ucn-2 animal model at 1 week. (A-F) Bar graphs summarizing relative expression of miR-125a, miR-139, miR-320, miR-324-3p, miR-324-5p and miR-499-5p\_1 in hearts' samples excised from sham, I/R, and I/R+Ucn-2 rats, 24 h and 1 week after the intervention. miRNA expressions were normalized to the endogenous control. Values are mean of Log Fold change  $\pm$  SEM (n = triplicate of 4-6). "\*", "\*\*\*", and "\*\*\*\*" indicate significance at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.



**SUPPLEMENTAL FIGURE 3. Control experiments for the expression of miR-29a and miR-451\_1\*.** (A) Image view of the Apoptosis and Survival Plate Tier 1 used for Prime PCR assay. (B, C) Bar graphs comparing the expression of miR-29a and miR-451\_1\* in rats and NRVM subjected to *in vivo* and *in vitro* protocol of I/R. Samples were processed 24 h after the intervention. (D, E) Bar graphs show miRNAs' expression after NRVM transfection with mimics of miR-29a and miR-451\_1\*. Values are mean of Log Fold change  $\pm$  SEM (n = triplicate of 4-6). "\*", "\*\*", and "\*\*\*\*" indicate significance at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.



**SUPPLEMENTAL FIGURE 4. miRNAs relative expression assessed in risk zones of infarcted hearts.** (A, B) Bar graphs summarizing relative expression of *Mapk-8* and *Bcl-2* in hearts' samples excised from sham, I/R, and I/R+Ucn-2 rats, 24 h and 1 week after the intervention. Gene's expressions are normalized to the endogenous control. Values are mean of Log Fold change  $\pm$  SEM (n = triplicate of 4). "\*", "\*\*", and "\*\*\*" indicate significance at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.