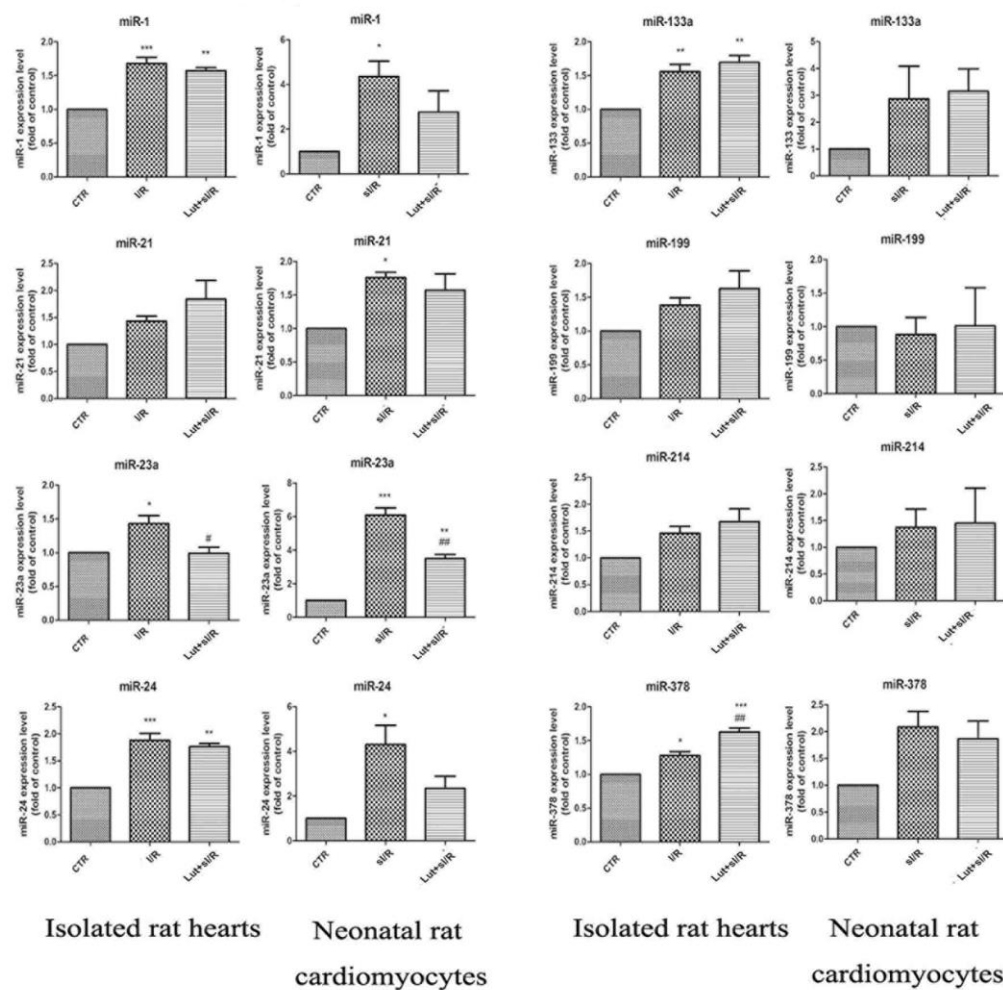
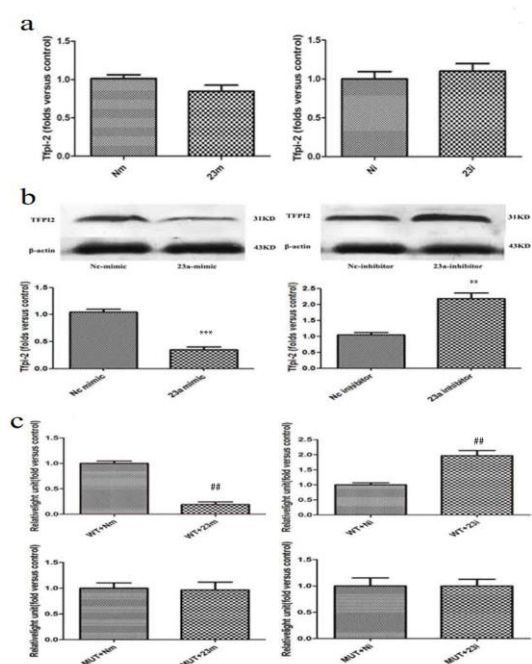


Supplementary Figure 1: Down-regulation of miRNA-23a confers protection against I/R injury by upregulating Tfp2 following luteolin

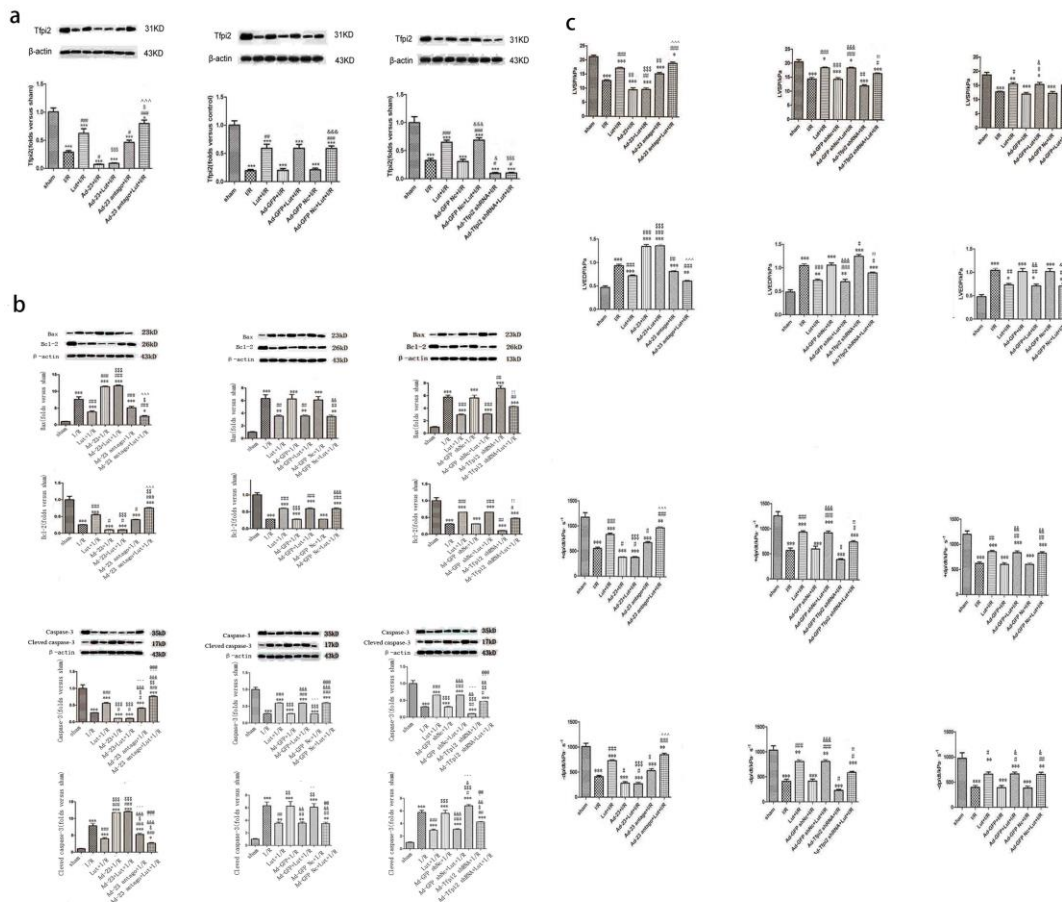
pretreatment ($n = 3$). (A) MTT detected the toxicity of luteolin in NRCs and H9c2 cells models. (B) LDH detection in NRCs and H9c2 cells sI/R models. (C) TUNEL assay detected the apoptosis rate in NRCs and H9c2 cells sI/R models. (D) Cleaved caspase-3 was detected in NRCs and H9c2 cells sI/R models. (E) MiRNA-23a was detected in NRCs and H9c2 cells sI/R models. (F) The ratio of TUNEL positive cells was detected in the 23m group (miRNA-23a mimic) and 23i group (miRNA-23a inhibitor) after sI/R injury. (G) Cleaved caspase-3 was detected in the 23m group and 23i group after sI/R injury. (H) Over-expression of miRNA-23a abolished the protective effects of luteolin on the ratio of TUNEL positive cells. (I) Over-expression of miRNA-23a abolished the effects of luteolin on cleaved caspase-3. (J) TFPI2 protein expression was detected in NRCs and H9c2 cells sI/R models. (K) TFPI2 protein expression was detected after its knock-down by si-RNAs. (L) The activity of cleaved caspase-3 was partly abolished by TFPI2 knock-down. (M) TFPI2 and cleaved caspase-3 proteins were detected under miRNA-23a over-expression in H9c2 cells after sI/R. (N) The effect of miR-23a and *Tfpi2* on the infarct size of I/R injury by TTC *in vivo*. *indicates comparison with control group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; # indicates comparison with sI/R group, $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$; @ indicates comparison with Nm group, $@P < 0.05$, $@@P < 0.01$, $@@@P < 0.001$; % indicates comparison with Ni group $\%P < 0.05$, $\%\%P < 0.01$, $\%\%\%P < 0.001$; \$ indicates comparison with Lut + I/R group, $\$P < 0.05$, $\$\$P < 0.01$, $\$\$\$P < 0.001$; ^ indicates comparison with Ad-23a antago + I/R group, $^P < 0.05$, $^^P < 0.01$, $^^^P < 0.001$; & indicates comparison with Ad-GFP shNc + I/R group, $\&P < 0.05$, $\&\&P < 0.01$, $\&\&\&P < 0.001$; ! indicates comparison with Ad-Tfpi2 shRNA + I/R group, $^!P < 0.05$, $!!P < 0.01$, $!!!P < 0.001$. LDH: Lactate dehydrogenase; miRNA: MicroRNA; NRCs: Neonatal rat cardiomyocytes.



Supplementary Figure 2: The expression of eight miRNAs following Luteolin pretreatment in PRHs (isolated rat hearts) and in NRCs during I/R injury ($n = 3$). Luteolin pretreatment down-regulated miRNA-23a both in PRHs and in NRCs compared to the I/R or sI/R group. * indicates comparison with control group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; # indicates comparison with I/R or sI/R group, $\#P < 0.05$, $\##P < 0.01$, $\###P < 0.001$. miRNAs: MicroRNAs; NRCs: Neonatal rat cardiomyocytes.



Supplementary Figure 3: Luteolin pretreatment protected the cardiomyocytes against sI/R injury partly by up-regulating TFPI2 via miRNA-23a in NRCs and H9c2 cells ($n = 3$). (A) Over-expression of miRNA-23a had no detectable effect on the mRNA level of TFPI2. (B) MiRNA-23a regulated the protein levels of TFPI2. (C) The luciferase activity was detected when the reporter plasmid was transfected with induced-mutation on the predicted binding site of miRNA-23a (MUT + 23m, MUT + 23i group) in the H9c2 cell. * indicates comparison with Nm or Ni group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; # indicates comparison with WT + Nm or WT + Ni group, $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$. miRNA: MicroRNA; NRCs: Neonatal rat cardiomyocytes.



Supplementary Figure 4: Luteolin pretreatment decreased apoptosis proteins and improved hemodynamic parameters through up-regulation of TFPI2 mediated by miRNA-23a *in vivo* ($n = 3$). (A) Over-expressing miRNA-23a decreased *Tfpi2* and abolished the effects of Luteolin on *Tfpi2* *in vivo*. (B) Luteolin pretreatment decreased apoptosis proteins through up-regulation of TFPI2 mediated by miRNA-23a *in vivo*. (C) Luteolin pretreatment improved hemodynamic parameters through up-regulation of TFPI2 mediated by miRNA-23a *in vivo*. * indicates comparison with control group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; # indicates comparison with sI/R group, $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$; \$ indicates comparison with Luteolin + I/R group, $\$P < 0.05$, $\$\$P < 0.01$, $\$\$\$P < 0.001$; ^ indicates

comparison with Ad-23a antago + I/R group, $^{\wedge}P < 0.05$, $^{\wedge\wedge}P < 0.01$,
 $^{\wedge\wedge\wedge}P < 0.001$; $^{\&}$ indicates comparison with Ad-GFP Nc + I/R group,
 $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, $^{\&\&\&}P < 0.001$; $^{\!}$ indicates comparison with Ad-Tfpi2
shRNA + I/R group, $^{\!}P < 0.05$, $^{\!}P < 0.01$, $^{\!}P < 0.001$. miRNA: MicroRNA.