Figure S1. H/R increases miR-141 expression in H9C2. H9C2 cardiomyocytes were challenged with hypoxia for 3 h and subsequently reoxygenated (H/R). Cells maintained under normoxic conditions for relevant durations served as controls (N/R). Cellular levels of miR-141 were measured using reverse transcription-quantitative PCR. (A) miR-141 levels in N/R group and H/R group. (B) Effects of a SOD mimetic (Mitotempo) targeting mitochondrial superoxide on miR-141 levels assessed at 6 h after reoxygenation. The cardiomyocytes were pretreated with 20 μM Mitotempo for 1 h prior to the challenge with H/R or N/R. The results are expressed as the mean ± standard error of the mean; n=3. *P<0.01 vs. N/R; $^{\#}$P<0.01 vs. H/R without Mitotempo. H/R, hypoxia/reoxygenation; N/R, normoxia/reoxygenation; SOD, superoxide dismutase.

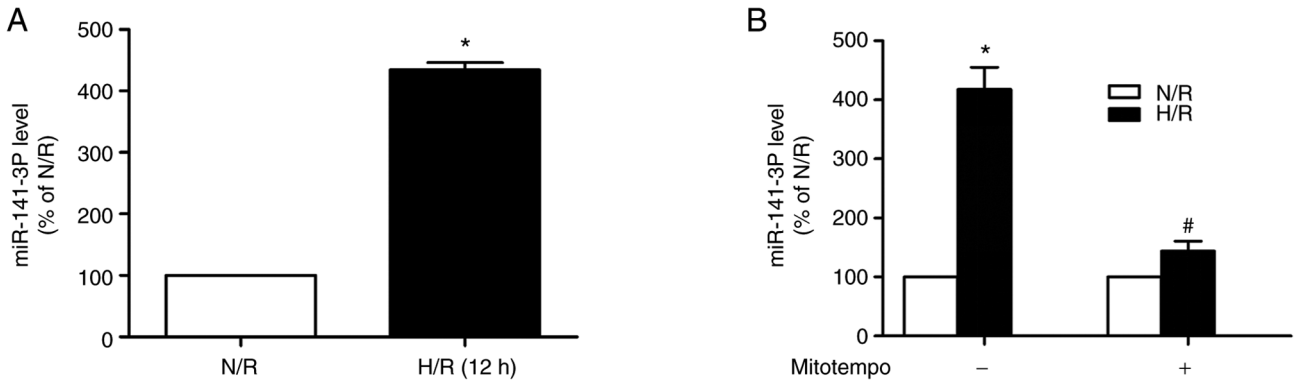
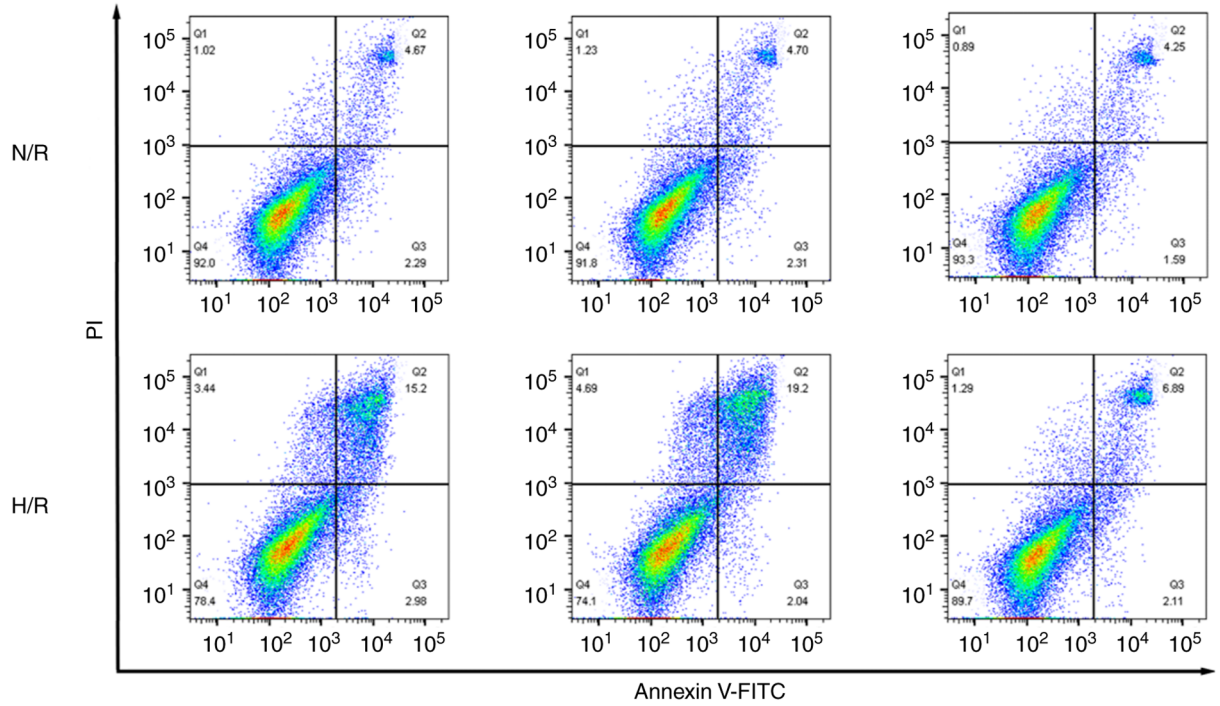


Figure S2. Role of miR-141 in H/R-induced cardiomyocyte apoptosis. HL-1 cardiomyocytes were transfected with a miR-141 mimic, miR-141 inhibitor, or miR con. Subsequently, the cells were challenged with H/R, and 12 h following reoxygenation, the indices of apoptosis were evaluated using FACS analyses with Annexin-V/propidium iodide. These figures are from representative experiments carried out at least three independent tests. The viable cells, early apoptotic and necrotic/secondary necrotic cells were represented by the lower left quadrant (Annexin-V^-/PI^-), lower right (Annexin-V^+/PI^-) and upper (Annexin-V^+/PI^+) quadrant, respectively. H/R, hypoxia/reoxygenation; miR con, negative control.



miR con	+	–	–
miR-141 mimic	–	+	–
miR-141 inhibitor	–	–	+

Figure S3. The role of miR-141 in H/R-induced H9C2 cardiomyocyte viability and death. H9C2 cardiomyocytes were transfected with a miR-141 mimic, miR-141 inhibitor, or their negative controls (miR con). Subsequently, the cells were challenged with H/R and 12 h after reoxygenation indices of viability and plasma membrane disruption were assessed. (A) The viability of cells was assessed using a CCK-8 assay. (B and C) LDH and CK-MB release from cells with ruptured membranes. The results are presented as the mean ± standard error of the mean; n=3. $^{*}P<0.05$ vs. N/R + miR con, $^{\#}P<0.05$ vs. H/R + miR con. H/R, hypoxia/reoxygenation; miR con, miR control; CCK-8, cell counting kit-8 assay; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB.

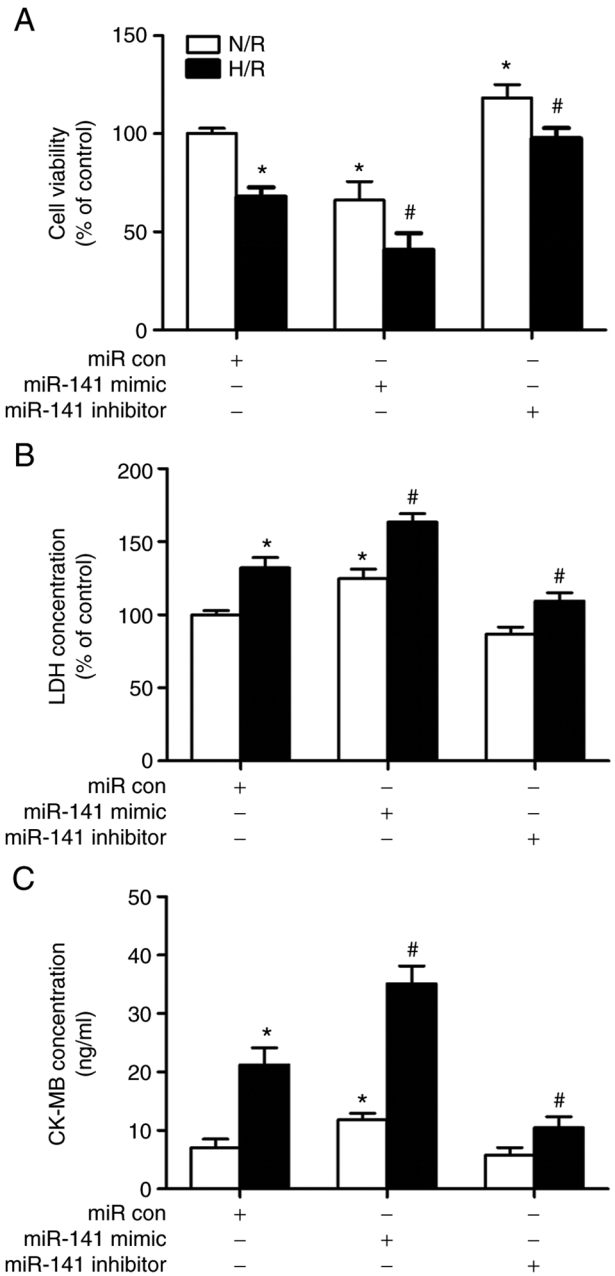


Figure S4. Eestablishment of the miR-141 mimic and inhibitor models. HL-1 cardiomyocytes were transfected with a miR-141 mimic, miR-141 inhibitor, or their negative controls (miR con) respectively. Following transfection, cellular levels of miR-141 were measured using reverse transcription-quantitative PCR. (A) Transfection with miR-141 mimics significantly increased the expression of miR-141. (B) Transfection with miR-141 inhibitor reduced the expression of miR-141. All bar graphs represent the mean ± standard error of the mean; n=3. *P<0.001 vs. NC group (for A); *P<0.05 vs. NC group (for B).

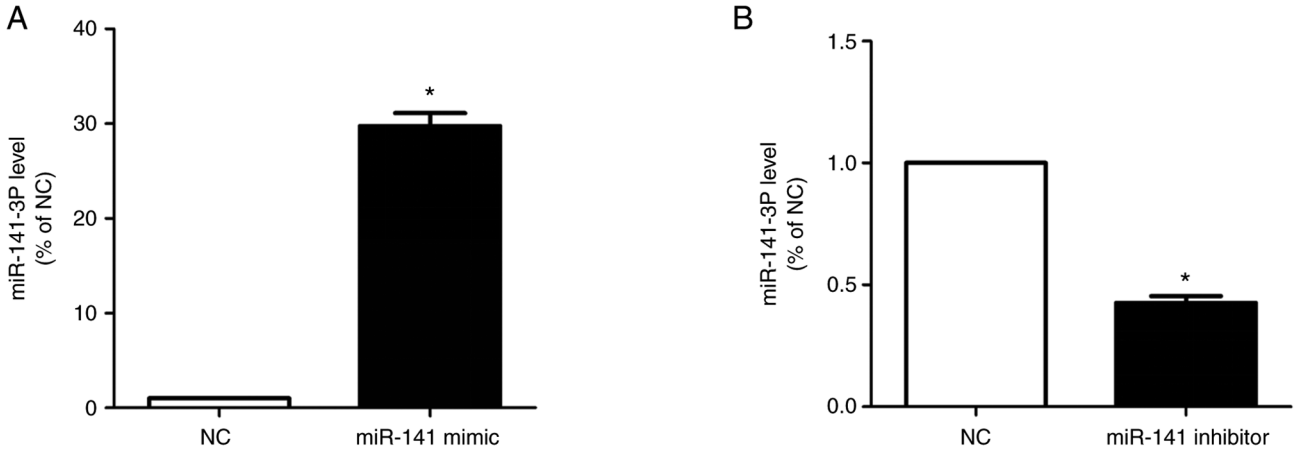


Figure S5. Construction of Sirt1 knockdown cell model. HL-1 cardiomyocytes were transfected with Sirt1 siRNA or control siRNA. After 24 h, the protein expression of Sirt1 in HL-1 cells with or without Sirt1 siRNA was analyzed using western blotting. All bar graphs represent the mean ± standard error of the mean; n=3. *P<0.01 vs. control (open bar).