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# Inhibition of lncRNA XIST Improves Myocardial I/R Injury by Targeting miR-133a through Inhibition of Autophagy and Regulation of SOCS2

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**The objective of this study was to investigate the role of lncRNA XIST and its relationship with miR-133a in myocardial I/R injury. H9C2 cells treated by hypoxia/reoxygenation (H/R) were used to establish an *in vitro* I/R model. The small interfering RNA (siRNA) for XIST and miR-133 mimics, inhibitor, and suppressor of cytokine signaling (SOCS2) recombinant plasmids were used to transfect the cells. Cell apoptosis was determined by flow cytometry analysis, and cell viability was used for 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, Thiazolyl Blue Tetrazolium Bromide (MTT) assay. The dual-luciferase reporter assay was performed to confirm binding between XIST and miR-133a, as well as miR-133a and SOCS2. To inhibit or overexpress XIST, miR-133a, or SOCS2 in I/R mice, we used recombinant lentivirus vectors and adenovirus vectors for tail vein injection. The expression of XIST, miR-133a, and SOCS2 was determined by quantitative real-time PCR, and LC3 I/II and Beclin1 was determined by western blotting. The expression of XIST and SOCS2 was significantly upregulated, whereas the miR-133a level was remarkably downregulated in both H/R H9C2 cells and I/R mice myocardial tissues. In both H/R H9C2 cells and I/R mice, the inhibition of XIST led to decreased apoptosis and autophagy, and inhibition of miR-133a reversed these effects. Similarly, overexpression of miR-133a resulted in reduced apoptosis and autophagy, which were reversed by overexpression of SOCS2. The inhibition of XIST and overexpression of miR-133a also promote cell viability of H/R cells. The dual-luciferase reporter assay significantly showed that XIST directly targeted on miR-133a, and miR-133a directly targeted on SOCS2. The inhibition of XIST could improve myocardial I/R injury by regulation of the miR-133a/SOCS2 axis and inhibition of autophagy.**

## INTRODUCTION

Because nowadays reperfusion is still the only effective therapeutic method to rescue acute myocardial infraction, myocardial I/R injury is one of the clinical challenges in the treatment of ischemic myocardial infraction.[1,2] Numerous studies have found that I/R injury is associated with complex bioprocesses and molecular mechanisms, such as cell apoptosis, autophagy, intracellular calcium overload, inflammatory response, oxygen free radical release, and vascular endothelial cell injury.[3–5] However, the underlying mechanisms for myocardial I/R injury still need further illumination.

The relationship between long non-coding RNAs (lncRNAs) and myocardial I/R injury has been reported in several research studies. It was reported that lncRNA MALAT1 was upregulated in myocardial I/R injury, and inhibition of MALAT1 could improve I/R injury through regulation of miR-145.[6] In a recent study, Wu et al.[7] found that 2,292 lncRNAs were observed to be upregulated and 1,848 lncRNAs downregulated in myocardial I/R injury patients. Among the lncRNAs, XIST is a new-found lncRNA that shows biofunctions in several diseases, such as liver cancer and bladder cancer.[8,9] In 2019, it was reported that XIST was upregulated in H/R cells and could promote cell apoptosis of H/R cells by regulation of miR-130a.[10] However, no study focused on the relationship between XIST and autophagy in myocardial I/R injury, and deeper insights for XIST in myocardial I/R injury are still unclear.

miR-133a has been reported to play important roles in many diseases such as breast cancer.[11] In a recent study it was also found that miR-133a could improve myocardial I/R injury through targeting DAPK2.[12] However, no study reported the interaction between XIST and miR-133a in myocardial I/R injury. In the present research, we used both *in vitro* and *in vivo* models to investigate the role of XIST in myocardial I/R injury and its relationship with miR-133a. Results showed that lncRNA XIST could directly target miR-133a, and the inhibition of XIST resulted in improvement of myocardial I/R injury through regulation of SOCS2 and inhibition of autophagy. These results might give deeper insights for molecular mechanisms of myocardial I/R injury.

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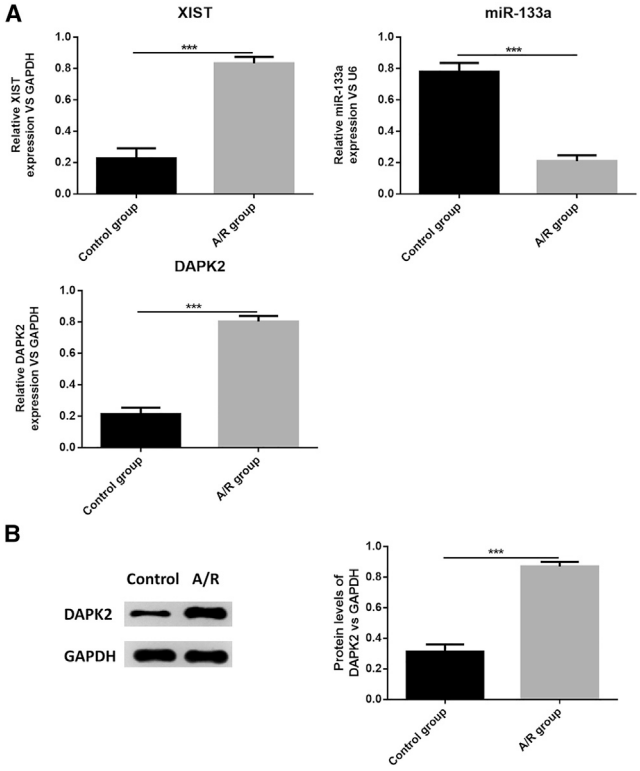


**Figure 1. XIST and SOCS2 Were Upregulated and miR-133a Was Downregulated in H/R H9C2 Cells**

(A) Expression of XIST, miR-133a, and DAPK2 in A/R and control cells by qRT-PCR. (B) Protein level of DAPK2 in A/R and control cells by western blotting. ***$p < 0.001$.

## RESULTS

### XIST and SOCS2 Were Upregulated and miR-133a Was Downregulated in H/R H9C2 Cells

First, the expression of XIST, miR-133a, and SOCS2 in H/R and the control H9C2 cells were determined by qRT-PCR. As shown in Figure 1A, the expression of XIST and SOCS2 was significantly upregulated, whereas the miR-133a level was remarkably downregulated in H/R H9C2 cells. The western blot assay also showed the protein level of SOCS2 was upregulated in H/R H9C2 cells (Figure 1B). All of these results indicated that XIST, miR-133a, and SOCS2 might be associated with the H/R process of myocardial cells.

### Inhibition of XIST Promoted Cell Viability and Inhibited Apoptosis and Autophagy through Regulation of miR-133a

To further investigate the role of XIST and miR-133a in H/R H9C2 cells, both XIST and miR-133a were suppressed by si-XIST and miR-133a inhibitor, respectively. Results showed that when transfected with si-XIST or miR-133a inhibitor, the expression of XIST and miR-133a was remarkably decreased (Figure 2A), suggesting the successful knockdown of the two genes. Then MTT assay was used to determine the cell viability of different groups of cells. It was observed that when transfected with si-XIST in H/R cells, the cell viability was significantly enhanced and the cell apoptosis was remarkably inhibited compared with the si-NC group (Figures 2B–2D). However, inhibition of miR-133a dramatically reversed the effects of si-XIST. Similarly, downregulation of XIST significantly inhibited the LC3 II/I level and the Beclin1 level (Figures 2E and 2F). However, co-transfection with miR-133a inhibitor remarkably reversed these effects. All of these results indicated that silence of XIST could promote cell viability and inhibit cell apoptosis and autophagy, which were reversed by inhibition of miR-133a.

### XIST Directly Targeted on miR-133a

Then we confirmed the direct binding between XIST and miR-133a by dual-luciferase reporter assay. The predicted binding mode was shown in Figure 3A. Results showed that the luciferase activity was significantly increased when cells were transfected with miR-133a inhibitor and was significantly decreased when cells were transfected with miR-133a mimics in WT-XIST (Figure 3B). However, no significant difference was found in MUT-XIST. Further experiments also showed that the overexpression of XIST significantly downregulated the miR-133a level, and inhibition of XIST remarkably upregulated the miR-133a level (Figure 3C and 3D), suggesting that XIST directly targeted and negatively regulated miR-133a.

### Overexpression of miR-133a Promoted Cell Viability and Inhibited Apoptosis and Autophagy through Regulation of SOCS2

The effects of miR-133a and SOCS2 were then further investigated by overexpression of miR-133a and SOCS2 in H9C2 cells. As shown in Figures 4A and 4B, the expression of SOCS2 was significantly increased when cells were transfected with pcDNA3.1-SOCS2, and the expression of miR-131a was remarkably upregulated when transfected with miR-131a mimics, suggesting the successful transfection. When the miR-131a was overexpressed, the cell viability was significantly upregulated and cell apoptosis was remarkably reduced compared with the NC control (Figures 4C and 4D). However, overexpression of SOCS2 dramatically reversed these effects. Moreover, the ratio of LC3 II/I and the expression of Beclin1 were both significantly downregulated when miR-133a was overexpressed (Figures 4E and 4F), which was remarkably reversed by co-transfection with pcDNA3.1-SOCS2. All of these results suggested that overexpression of miR-133a could reduce the H/R-induced cell injury and autophagy, and the process might be through regulation of SOCS2.

### miR-133a Directly Targeted on SOCS2

By using dual-luciferase reporter assay, we evaluated whether miR-133a could target on SOCS2. The predicted binding mode was shown in Figure 5A. The luciferase activity was significantly increased when miR-133a was suppressed by miR-133a inhibitor and was remarkably decreased when miR-133a was overexpressed by miR-133a mimics in WT-SOCS2 (Figure 5B). However, no

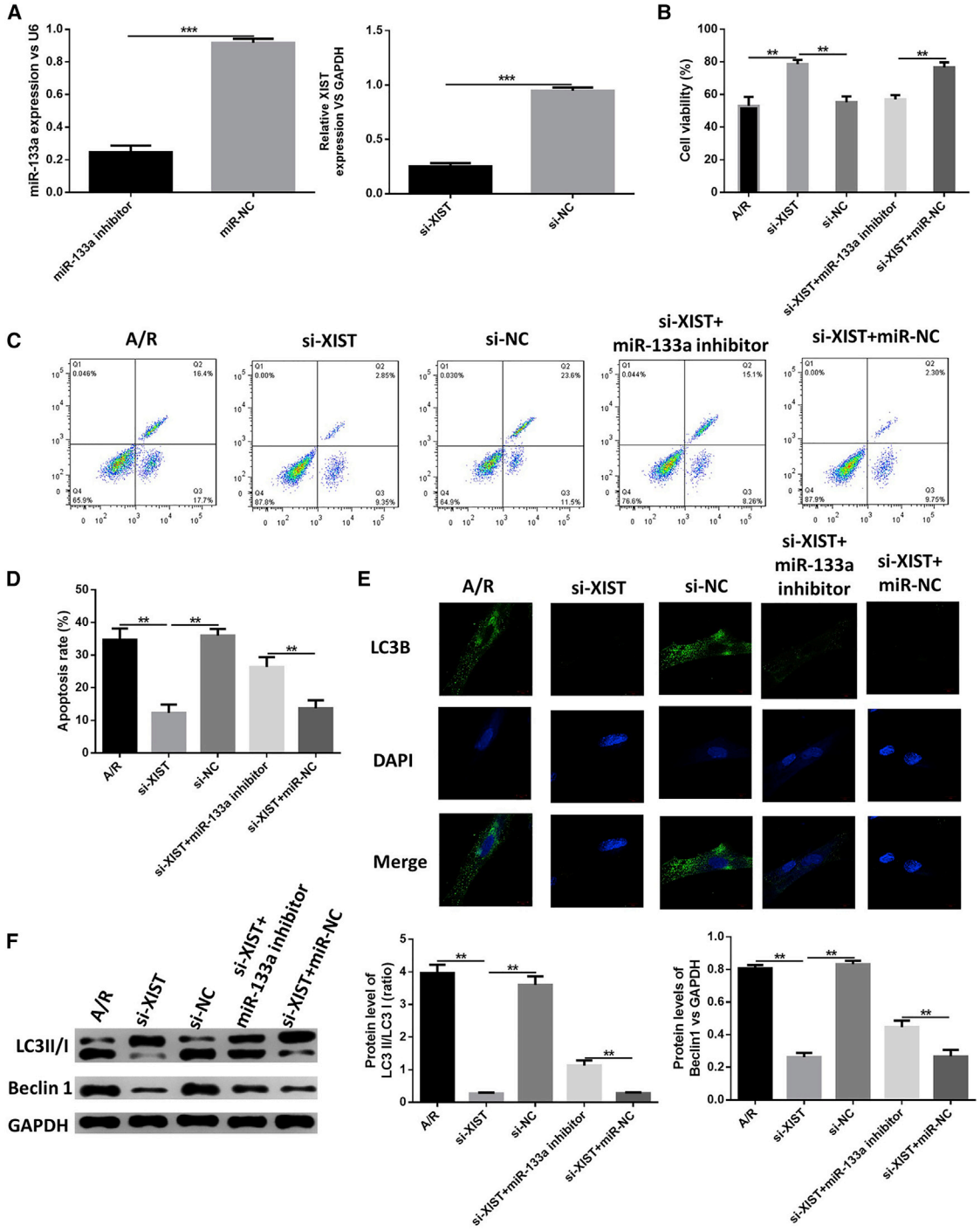


**Figure 2. Inhibition of XIST Promoted Cell Viability and Inhibited Apoptosis and Autophagy through Regulation of miR-133a**

(A) Expression of miR-133a and XIST in different groups of cells by qRT-PCR. (B) Cell viability of different groups of cells by MTT. (C) Cell viability by MTT assay. (D) Cell apoptosis by flow cytometry. (E) Immunofluorescence of LC3 B in different groups of cells. (F) Protein levels of LC3 II/I and beclin1 by western blotting. ***$p < 0.001$, **$p < 0.01$.

significant difference was found in MUT-SOCS2. It was also observed that the expression of SOCS2 was significantly upregulated by inhibition of miR-133a and was remarkably downregulated by overexpression of miR-133a (Figures 5C and 5D), indicating that miR-133a directly targeted and negatively regulated SOCS2.

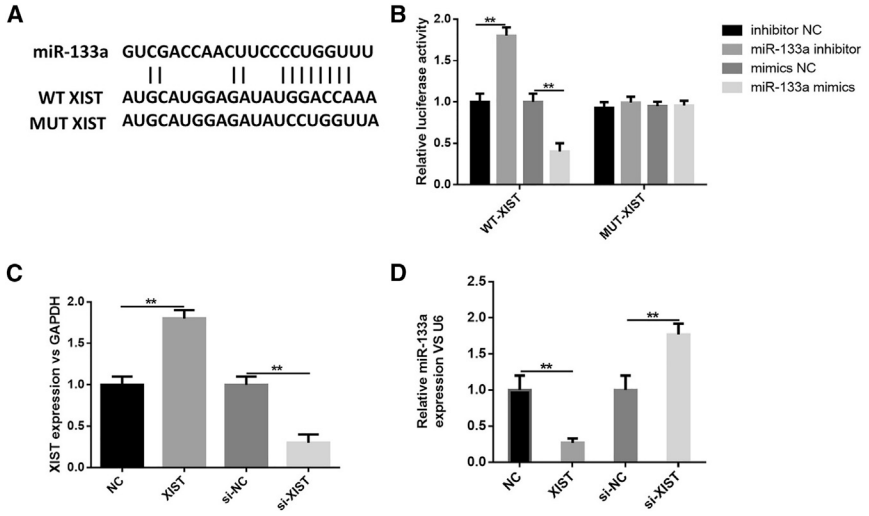


**Figure 3. XIST Directly Targeted on miR-133a**

(A) Predicted binding region between miR-133a and XIST. (B) Relative luciferase activity in WT-XIST and MUT-XIST. (C) Expression of XIST in different groups of cells. (D) Relative miR-133a expression in different groups of cells. $^{**}p < 0.01$.

### Inhibition of XIST Suppressed Cell Apoptosis and Autophagy in Myocardial Tissues of I/R Injury Mice through Regulation of miR-133a

Then, we used an *in vivo* mouse I/R model to further investigate the role of XIST and miR-133a in I/R injury by tail injection of lenti-si-XIST and lenti-miR-133a-inhibitor. Results showed that XIST and SOCS2 were significantly upregulated, whereas miR-133a was remarkably downregulated in myocardial tissues of I/R mice (Figures 6A and 6B). The apoptosis of myocardial cells was then evaluated by TUNEL assay, and it was observed that inhibition of XIST significantly reduced cell apoptosis rate, whereas inhibition of miR-133a remarkably reversed this effect (Figure 6C). Similarly, both LC3 II/I ratio and Beclin1 level were dramatically downregulated by inhibition of XIST, which was reversed by inhibition of miR-133a (Figure 6D). All of these results indicated that inhibition of XIST could improve I/R injury, and these effects might be through regulation of miR-133a.

### Overexpression of miR-133a Suppressed Cell Apoptosis and Autophagy in Myocardial Tissues of I/R Injury Mice through Regulation of SOCS2

At last, we determined effects of overexpression of miR-133a in the I/R injury mouse model. Similar to above, the overexpression of miR-133a significantly decreased the cell apoptosis and autophagy in myocardial tissues of I/R mice, whereas overexpression of SOCS2 remarkably reversed the effects (Figures 7A and 7B). All of these results suggested that overexpression of miR-133a could reduce I/R-induced myocardial injury, and the effects might be through inhibition of autophagy and regulation of SOCS2.

## DISCUSSION

Although there are advantages in both basic and clinical research for cardiac ischemic-reperfusion injury, the underlying molecular mechanisms for myocardial I/R injury are still unclear. In recent years, both lncRNAs and miRNAs, as well as their interactions, have been proven to play important roles in many diseases, including I/R injury. However, up to now, no studies reported the involvement of XIST in the development of myocardial I/R injury and its relationship between miR-133a/SOCS2 axis. In the present study, we demonstrated for the first time that XIST was upregulated in myocardial I/R injury and the inhibition of XIST could improve I/R-induced myocardial injury through suppressing autophagy and regulation of the miR-133a/SOCS2 axis in both *in vitro* and *in vivo* I/R models.

lncRNA XIST is considered to play important roles in several diseases. Zhu et al.[13] demonstrated that XIST could promote cervical cancer development by upregulating Fus through sponging miR-200a. Chen et al.[14] showed that XIST was upregulated in gastric cancer and accelerated cancer development by spinal miR-101. In a recent study, Zhou et al.[10] found that XIST was upregulated in H/R cells, and the increased XIST could promote cell apoptosis of H/R cells through targeting miR-130a. However, up to now, no study reported a relationship between XIST and miR-133a, and its effect on autophagy in I/R injury. In this research, we found that XIST was upregulated in myocardial I/R injury, and inhibition of XIST could significantly improve I/R injury.

The relationship between miR-133a and improved myocardial I/R injury has been reported in literature researches. Eitel et al.[15] demonstrated that circulating miR-133a was correlated with prognosis of myocardial infarction patients. Another study found that miR-133a was downregulated in myocardial infarction compared with healthy adult and fetal hearts.[16] Chen et al.[17] reported in a recent study that miR-133a could promote the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction. The relationship between XIST and miR-133a was also reported in other diseases. It was reported XIST promoted pancreatic cancer proliferation by sponging miR-133a.[18] In our study, we also found that miR-133a could improve I/R-induced myocardial injury, and moreover, this effect might be through regulation of SOCS2 and autophagy.

Few studies focused on the role of SOCS2 in I/R injury. In a recent study, SOCS2 was reported to facilitate the myocardial I/R injury in diabetic mice and H9c2 cells, and the effects were through regulation of JAK/STAT/IGF-1 signaling.[19] Besides, it was also observed that knockdown of SOCS2 reduced expression of inflammatory factors

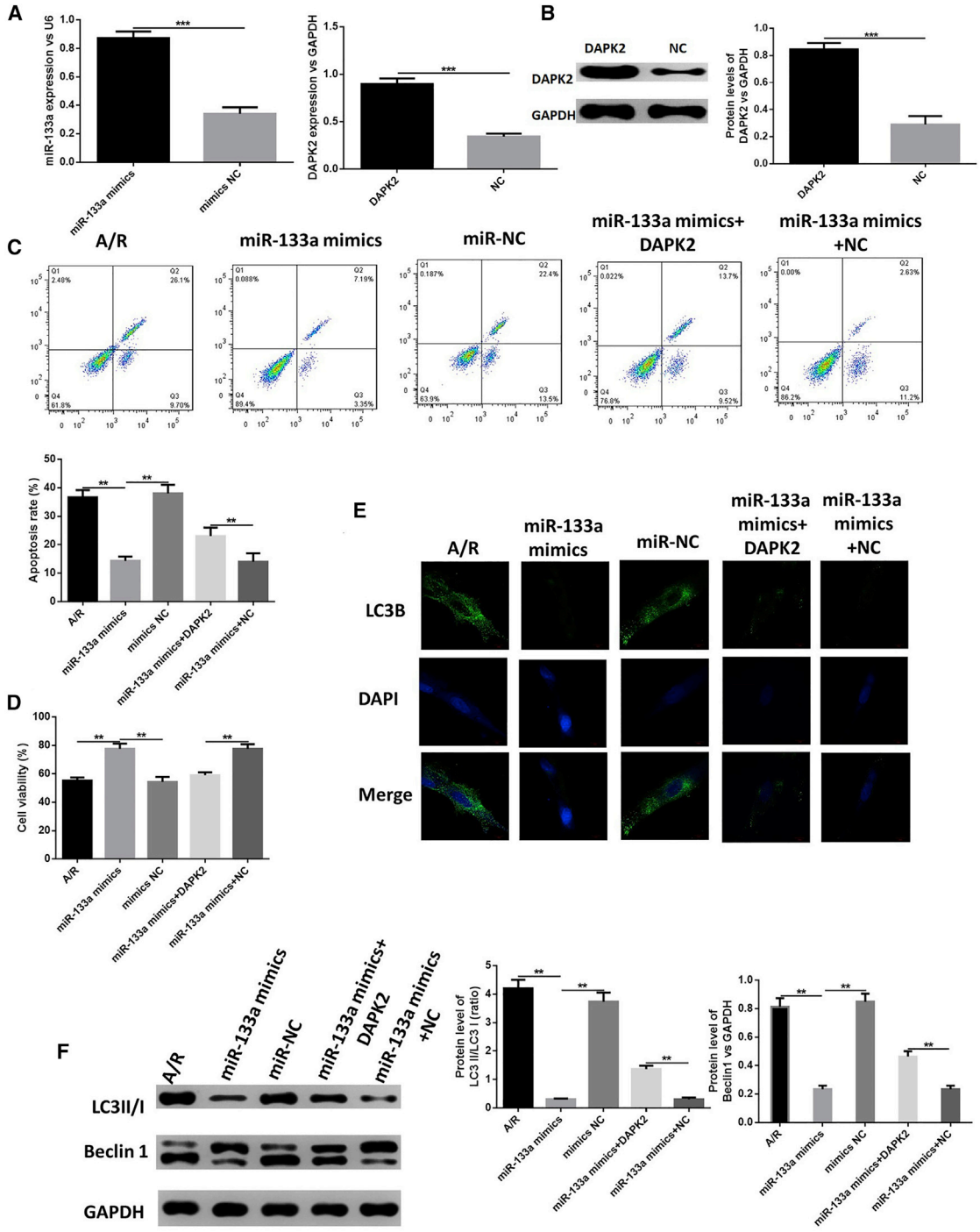


**Figure 4. Overexpression of miR-133a Promoted Cell Viability and Inhibited Apoptosis and Autophagy through Regulation of SOCS2**

(A) miR-133a and DAPK2 expression in different groups of cells by qRT-PCR. (B) Protein level of DAPK2 by western blotting. (C) Cell viability by MTT assay. (D) Cell apoptosis by flow cytometry. (E) Immunofluorescence of LC3 B in different groups of cells. (F) Protein levels of LC3 II/I and beclin1 by western blotting. ***$p < 0.001$, **$p < 0.01$.

of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-10 in a murine model of heart damage.[20] However, no study reported a relationship between miR-133a and SOCS2 in I/R injury. In the present study, we demonstrated that miR-133a could improve I/R injury by directly targeting on SOCS2, and the effect might be associated with regulation of autophagy.

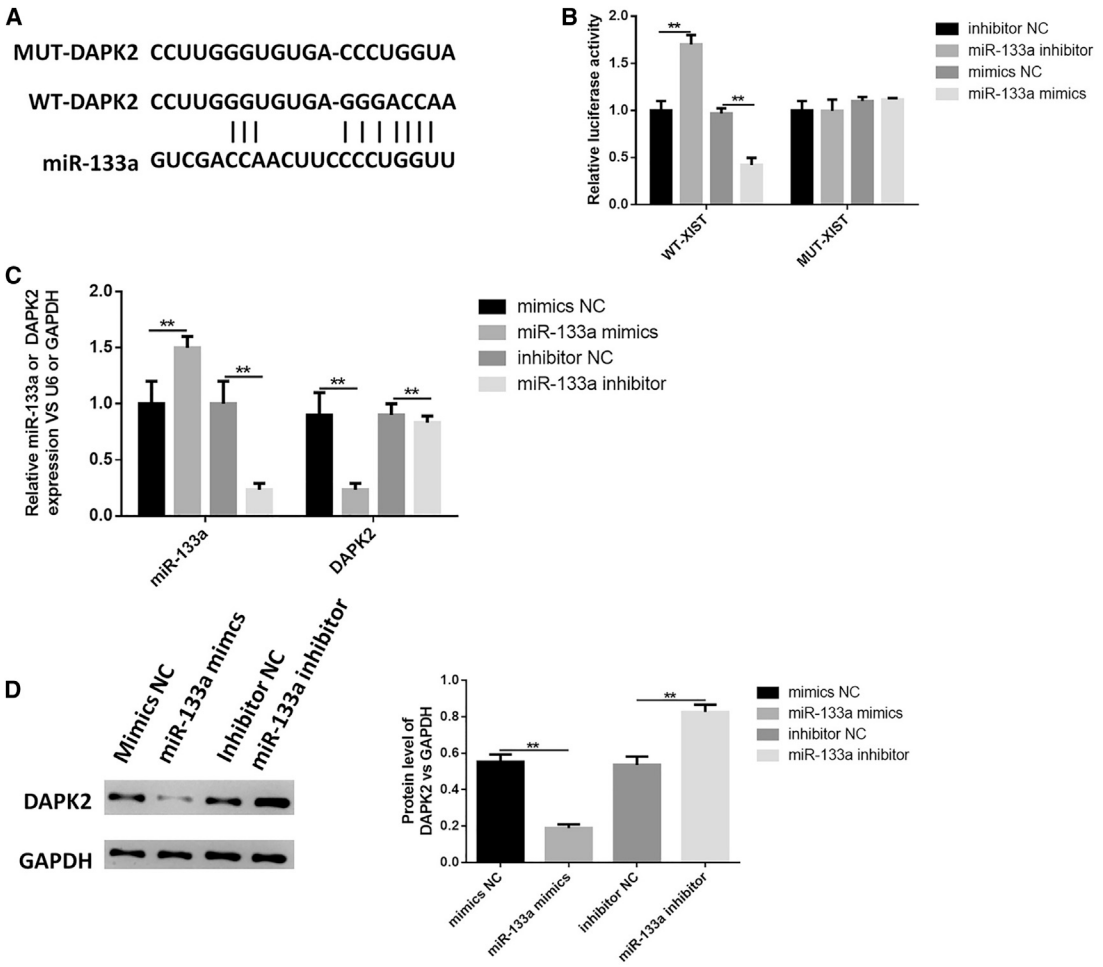


**Figure 5. miR-133a Directly Targeted on SOCS2**

(A) Predicted binding region between miR-133a and SOCS2. (B) Relative luciferase activity in WT-SOCS2 and MUT-SOCS2. (C) Expression of miR-133a and SOCS2 in different groups of cells. (D) Protein expression of DAPK2 in different groups of cells. **$p < 0.01$.

The role of autophagy in I/R injury has been reported in many studies. Generally, it is considered that the moderate activation of autophagy is protective for I/R injury, and the overactivation leads to damage for organelle or protein clearance in the later phase of the I/R process.[21] Wang et al.[22] reported that lncRNA APF improved myocardial infarction by inhibition of autophagy and targeting miR-188-3p. It was also found that hesperidin protected the myocardial I/R injury by inhibition of autophagy and activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway.[23] In this study, we also found that inhibition of XIST could improve I/R injury by inhibition of autophagy, and the effects were through regulation of the miR-133a/SOCS2 axis.

It was reported that inhibition of XIST enhanced the chemosensitivity of non-small-cell lung cancer (NSCLC) cells via suppression of autophagy.[24] miR-133a was reported to inhibit autophagy in gastric cancer.[25] A study also showed that SOCS2 might be associated with the autophagy process. It was found that overexpression of ATG5 could activate LC3 by interacting between LC3 and SOCS2; however, the relationship between SOCS2 was not clear.[26] In the present study, we also observed that inhibition of XIST and overexpression of miR-133a could suppress autophagy, and overexpression of SOCS2 reversed the effects. However, further studies are still needed to illuminate deeper insights for the interaction between the XIST/miR-133a/SOCS2 axis and autophagy.

In conclusion, we conducted both *in vitro* and *in vivo* experiments to investigate the role of lncRNA XIST in myocardial I/R injury and found that the inhibition of XIST could improve myocardial I/R injury by regulation of miR-133a/SOCS2 axis and inhibition of autophagy. This study might give deeper insights for mechanisms of XIST in myocardial I/R injury and provide novel potential therapeutic targets.

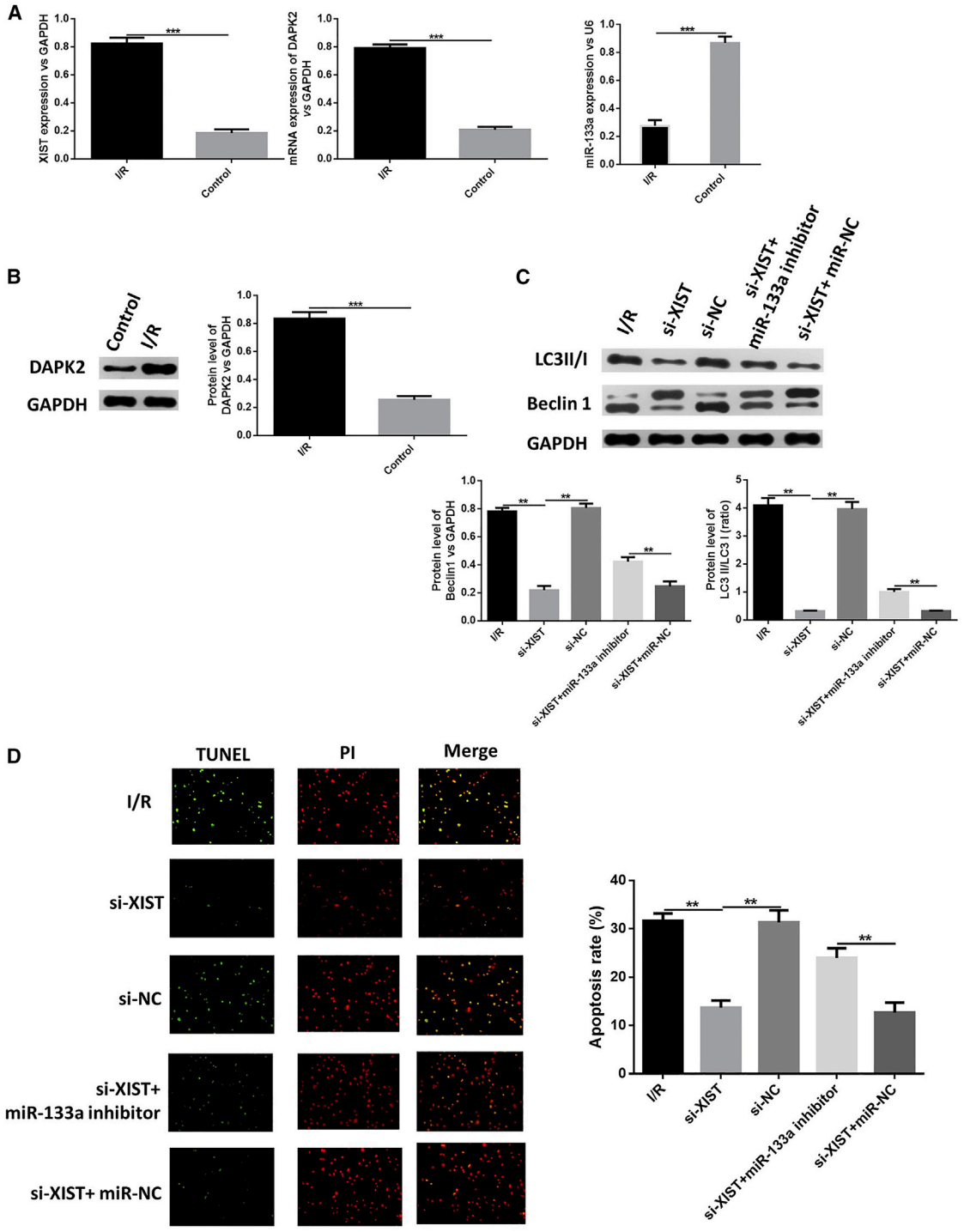


**Figure 6. Inhibition of XIST Suppressed Cell Apoptosis and Autophagy in Myocardial Tissues of I/R Injury Mice through Regulation of miR-133a**

(A) XIST, miR-133a, and DAPK2 mRNA expression by qRT-PCR. (B) Protein expression of DAPK2 in I/R and control by western blotting. (C) Protein levels of LC3 II/I and beclin1 by western blotting. (D) Cell apoptosis of different groups of cells by TUNEL staining. ***$p < 0.001$, **$p < 0.01$.

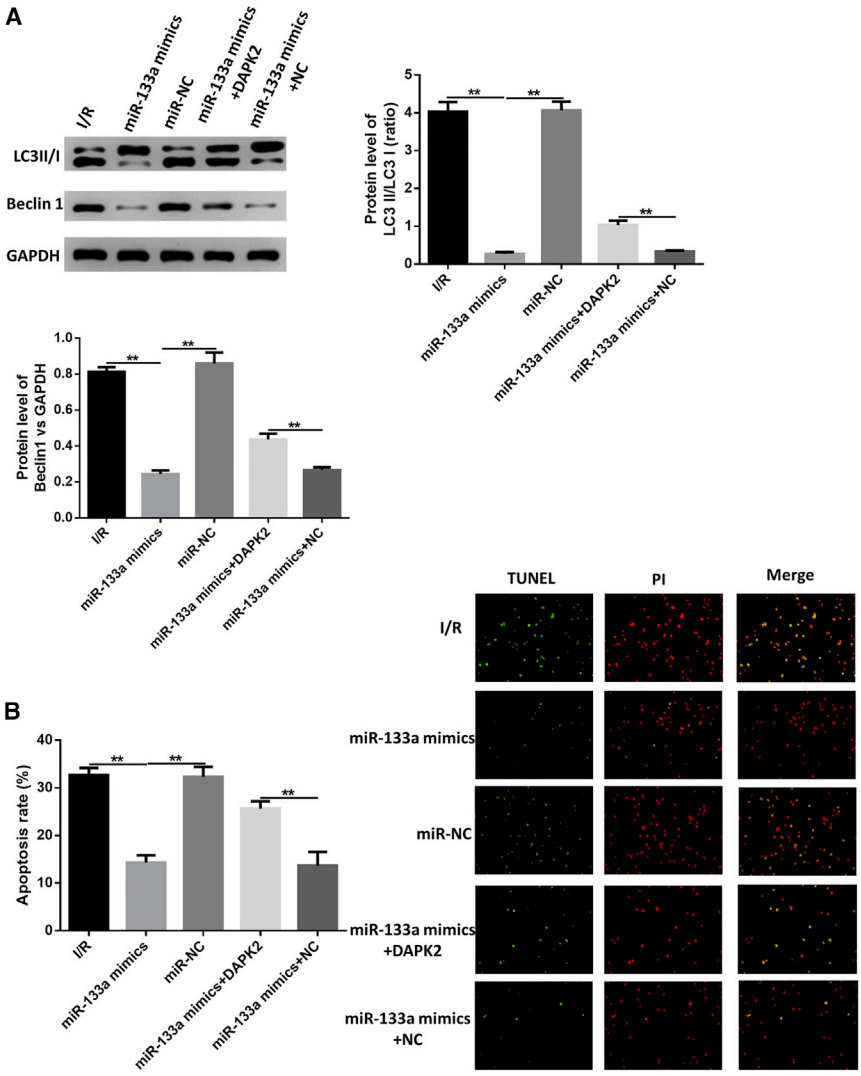


**Figure 7. Overexpression of miR-133a Suppressed Cell Apoptosis and Autophagy in Myocardial Tissues of I/R Injury Mice through Regulation of SOCS2**

(A) Protein levels of LC3 II/I and beclin1 by western blotting. (B) Cell apoptosis of different groups of cells by TUNEL staining. **p < 0.01.

## MATERIALS AND METHODS

### Cell Culture and Treatment

In brief, the myocardial cell line H9C2 cells, which were obtained from ATCC (Manassas, VA, USA), were cultured in DMEM (GIBCO, Gaithersburg, MD, USA) with 10% FBS (GIBCO) and 1% PS (100 μg/mL penicillin, 100 μg/mL streptomycin) at 37°C and 5% $CO_2$. The hypoxia/reoxygenation (HR) model was established by the following method: the cells were cultured in the hypoxia cabin under 2% $O_2$, 93% $N_2$, and 5% $CO_2$ for 2 h and then were moved to a normoxic condition of 37°C and 5% $CO_2$ for 4 h. The control cells were cultured under the normoxic condition of 37°C and 5% $CO_2$ for 6 h.

### Transfection

For cell transfection, the XIST small interfering RNA (siRNA) (si-XIST) and si-negative control (NC), miR-133 mimics, inhibitor, and corresponding negative controls, as well as overexpressing SOCS2 and XIST recombinant plasmids and NC, were all designed and synthesized by GeneChem (Shanghai, China). Cells were transfected with si-XIST, miR-133 mimics, inhibitor, pcDNA3.1-SOCS2, pcDNA3.1-XIST, or corresponding NCs (5 nM for all) using Lipofectamine 2000 (Invitrogen) in serum-free Opti-MEM medium (GIBCO) according to the manufacturer's instruction. The transfection efficiency was determined by quantitative real-time PCR after 48 h.

### Measurement of Cell Apoptosis

Cell apoptosis was measured by flow cytometry analysis. In brief, cells with a density of $2 \times 10^5$/well were seeded into six-well plates and stained with Annexin V/PI double-staining kit (BD Biosciences, MA, USA). The cell apoptosis was then determined using a FSCAN flow cytometer (BD Biosciences).

### Measurement of Cell Viability

Cell viabilities were evaluated using MTT assay. In brief, after 48 h of transfection, cells were seeded at a density of $2 \times 10^5$ in 96-well plates, and 10 μL MTT solution (5 mg/mL) was added. Then cells were cultured for 4 h at 37°C and 5% $CO_2$, and MTT was replaced with 180 μL DMSO. The optical density (OD) value was evaluated under 490 nm.

### Immunofluorescence

The expression of LC3 was evaluated using an immunofluorescence method. In brief, the cells were collected, fixed, and permeabilized. Then cells were incubated with anti-LC3B antibody (Abcam, Cambridge, MA, USA) at 4°C overnight and were incubated with a corresponding secondary antibody at room temperature for 1 h. DAPI was used for counterstaining the nucleus. Pictures were taken by using a Leica TCS-SP laser scanning confocal microscope.

### Dual-Luciferase Reporter Assay

The predicted binding sites between XIST and miR-133a, as well as miR-133a and SOCS2, were obtained from bioinformatic software TargetScan 7.2 and starbase. The XIST (or SOCS2) 3′ UTR with the predicted wild-type (WT) miR-133a binding site region or mutant

(MUT) was amplified and sub-cloned into a pGL4.10 luciferase reporter vector. The cells were co-transfected with either the vectors, miR-133a mimics, or NC using Lipofectamine 2000. After 48 h of transfection, the luciferase activity was determined by luciferase assay using a Bright-Glo Luciferase Assay System (Promega, USA).

#### Animals and Treatment

The C57BL/J6 mice (~8–10 weeks, 20–30 g) were obtained from Beijing Children's Hospital, Capital Medical University, National Center for Children's Health. All animals were kept in a light-controlled room under a 12 h/12 h light/dark cycle and controlled temperature (23°C–25°C), and had free access to food and water according to the *Guide for the Care and Use of Laboratory Animals*. In particular, any effort was put forth to avoid unnecessary pain of the animals. The whole study was approved by the Institutional Animal Care Committee at Beijing Children's Hospital, Capital Medical University, National Center for Children's Health.

For establishment of the myocardial I/R model, mice were anaesthetized using 4% isoflurane, and an 8-mm skin incision was made at the fourth intercostal space. The myocardial I/R injury was induced by temporary ligation of the left anterior descending coronary artery. Occlusion was confirmed by balancing of the left ventricular (LV) myocardium below the suture after removing the ligature. The transitory ligation was maintained for 45 min followed by 3 h of reperfusion. For the control group, sham operation was conducted, but no placement of the ligature.

The construction of lentivirus vectors to inhibit XIST or miR-133a was conducted by GeneChem. For inhibition of XIST in mice, the lentivirus vector transfected with si-XIST (lenti-si-XIST, 100 μL, virus titer $2 \times 10^7$ transduction unit [TU]/mL) was given by tail vein injection, and the control mice received a tail vein injection of lenti-si-NC. For inhibition or overexpression of miR-133a in mice, mice received tail vein injection of lenti-miR-133a-inhibitor (or lenti-miR-133a-mimics, 100 μL, virus titer $2 \times 10^7$ TU/mL) or the NC. For overexpression of SOCS2, adenovirus vector to overexpress SOCS2 (ad-SOCS2, 0.5 mL of $4 \times 10^8$ plaque-forming units [PFUs]/mL) was used for tail vein injection of the mice, and the control received irrelevant sequence control.

#### TUNEL Assay

The TUNEL assay was used for measuring the apoptosis of mouse myocardial cells. The cells were stained with an Apoptosis *In Situ* Detection Kit (Abcam), and the number of TUNEL-positive cells was calculated as percent of total number of cells. The staining was observed and photographed using a Leica TCS-SP laser scanning confocal microscope (Leica Microsystems).

#### RNA Extraction and qRT-PCR Assay

The expression levels of XIST, miR-133a, and SOCS2 were measured using qRT-PCR. In brief, the extraction of total RNA was conducted using the TRIzol reagent (Tiangen Biotech, Beijing, China), and the mirVana miRNA isolation kit (Ambion, Austin, TX, USA) was used to extract the miRNA. A PrimeScript One Step RT-qPCR kit (Takara Biotechnology, Dalian, China) was used to convert RNA to cDNA for mRNA, and a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems Life Technologies) was used to convert RNA to cDNA for miRNA, respectively. The PCR analysis was performed in an Applied Biosystems 7500 Real Time PCR system (Applied Biosystems, Thermo Fisher Scientific). All primers used in PCR were designed and synthesized by GeneChem. GAPDH and U6 small nuclear RNA (U6 snRNA) were used as internal references for mRNA and miRNA, respectively. The relative expression level was calculated by the $2^{-\Delta\Delta Cq}$ method.[27]

#### Western Blotting

Western blotting was used to test the protein expression levels of LC3 I/II, Beclin1, and SOCS2, with GAPDH as a loading control. In brief, the extracted proteins were subjected to 10% SDS-PAGE, transferred to polyvinylidene fluoride (PVDF) membranes, and blocked by 5% non-fat milk at room temperature for 1 h. After incubation with a primary antibody anti-LC3 I/II (ab128025; Abcam), anti-Beclin1 (ab210498; Abcam), or anti-SOCS2 (ab3692; Abcam) at 4°C overnight, the membranes were incubated with a goat anti-rabbit immunoglobulin G secondary antibody (ab205718; Abcam) at 37°C for 45 min. An EasySee Western Blot Kit (Beijing TransGen Biotech, Beijing, China) was used to scan the films.

#### Statistical Analysis

The measurement data were expressed by mean ± SD. Comparison between two groups was performed using the Student's t test. Comparison among three or more groups was conducted using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered as statistically significant. All calculations were made using SPSS 20.0.

#### Ethics Approval

The ethic approval was obtained from the Ethic Committee of Beijing Children's Hospital, Capital Medical University, National Center for Children's Health.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Z.L.; Performed the experiments: Z.L., Y. Zhang; Analyzed the data: Z.L., Y. Zhang, N.D.; Contributed reagents/materials/analysis tools: Z.L., Y. Zhang, N.D., Y. Zhao, Z.Y., L.S., H.Y., Y. Zhu.

## CONFLICTS OF INTEREST

The authors declare no competing interests.

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