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# Circular RNA hsa_circ_0000848 Regulates Cardiomyocyte Proliferation and Apoptosis Under Hypoxia via Recruiting ELAVL1 and Stabilizing SMAD7 mRNA

ORIGINAL INVESTIGATION

## ABSTRACT

**Background:** Myocardial infarction has been recognized globally as a serious problem featured with high mortality and morbidity. In addition, hypoxia represents the central feature of myocardial infarction. Recently, it has been reported that circular RNAs can exert critical functions in the biological processes of diseases. However, the functions of most circular RNAs remain unclear in cells cultured under hypoxic conditions. In this study, we focused on exploring the role of circ_SMAD7 (namely hsa_circ_0000848 in this study) in cardiomyocyte cells cultured under hypoxic conditions to provide a novel insight for future myocardial infarction studies.

**Methods:** Firstly, a real-time quantitative polymerase chain reaction assay was adopted to analyze hsa_circ_0000848 expression. Functional assays were performed to detect the functions of hsa_circ_0000848 in cardiomyocyte cells cultured under hypoxic conditions. Furthermore, mechanism assays were implemented to explore the regulatory mechanism of hsa_circ_0000848.

**Results:** Hsa_circ_0000848 was notably downregulated in hypoxia-induced cardiomyocytes. The silencing of hsa_circ_0000848 hindered the proliferation while accelerating the apoptosis of hypoxia-induced cardiomyocytes cells. Moreover, hsa_circ_0000848 interacted with ELAV-like RNA-binding protein 1 protein to stabilize SMAD family member 7 mRNA. Moreover, SMAD family member 7 overexpression could reverse the suppressive effect of hsa_circ_0000848 knockdown on myocardial infarction progression.

**Conclusions:** Our research was the first in the field to confirm that the hsa_circ_0000848/ELAV-like RNA-binding protein 1/SMAD family member 7 axis could affect the development of cardiomyocyte cells cultured under hypoxia, indicating that hsa_circ_0000848 might function as a novel biomarker in cells under hypoxia thus laying the groundwork for future study on myocardial infarction.

**Keywords:** Hypoxia, hsa_circ_0000848, ELAVL1, SMAD7

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## INTRODUCTION

Cardiovascular disease has been recognized as one of the primary diseases threatening human health.[1] What cannot be ignored is that myocardial infarction (MI) is the leading cause of cardiovascular death.[2] Briefly, MI is the myocardial necrosis secondary to prolonged ischemia.[3] Myocardial infarction may lead to many complications, such as arrhythmias, ventricular free-wall rupture, thromboembolic strokes, and so on. All of those diseases have posed a great threat to the survival of MI patients.[4] Therefore, for the prevention of MI, accurate diagnosis and effective treatment are of great concern.[5] Also, hypoxia represents the major feature of MI. Hence, identifying the biomarkers for the diagnosis and treatment of hypoxia is crucial for future studies on MI.

Circular RNAs (circRNAs) are a class of covalently closed, single-stranded noncoding RNAs.[6] Due to their covalently closed-loop structure, they are resistant to RNA exonuclease.[7] Emerging evidence has demonstrated that circRNAs are important participators in the progression of diverse diseases.[8] For example,

Zhou et al[9] have proposed that circRNA.33186 contributes to osteoarthritis by targeting the miR-127-5p/MMP-13 axis. Liu et al[10] have manifested that circRNA-5692 functions as a ceRNA to suppress the development of hepatocellular carcinoma. Wu et al[11] have disclosed that circRNA_0054633 plays a promoting role in gestational diabetes mellitus. Moreover, circRNAs are also implicated in the pathological progress of MI through certain regulatory mechanisms.[12] For instance, Cai et al[13] have proved that circ-Ttc3 influences MI through serving as a sponge of miR-15b. Si et al[14] have validated the function of circHipk3/miR-133a/Notch1 axis in MI. Zhao et al[15] have illustrated that circMACF1 could impair the process of MI by upregulating EMP1 expression. According to the circBase databse, hsa_circ_0000848 is located in the intron of the SMAD family member 7 (SMAD7) gene which has been verified to play the suppressive role in MI.[16] Hypoxia is one of the central features of MI, and the functions of hsa_circ_0000848 in cardiomyocyte cells cultured under hypoxic condition would be unveiled in this paper. ELAV-like RNA-binding protein 1 (ELAVL1) has been known as HUR, and it has been widely reported to regulate the stability of mRNAs by binding to the 3′-untranslated region (3′-UTR) region of mRNAs. In this paper, we aimed at studying the role of hsa_circ_0000848 in cardiomyocyte cells cultured under hypoxic conditions and deeply delving into the hsa_circ_0000848/ELAVL1/SMAD7 axis in cardiomyocytes under hypoxia.

## METHODS

### Cell Culture

H9c2 cardiomyocyte was obtained from American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (BC-M-002, Biochannel (Nanjing, Jiangsu, China)) added with 10% fetal bovine serum (10270-106, Gibco (Rockville, MD, USA)). The cells were cultured in 60-mm plates at a density of $1\times10^6$ cells/well at 37°C for 30 minutes. An anaerobic incubator (95% $N_2$ and 5% $CO_2$) was used.

### Plasmid Transfection

Genechem (Shanghai, China) was utilized to synthesize the short hairpin RNA (shRNA) targeting hsa_circ_0000848 (sh-hsa_circ_0000848#1/2/3) or ELAVL1 (sh-ELAVL1#1/2), as well as control shRNA. Besides, pcDNA3.1 vectors were subcloned with SMAD7 for overexpression, and an empty pcDNA3.1 vector was used as the negative control. Besides, Lipofectamine 3000 (L3000015, Invitrogen, Carlsbad, CA, USA) was used for cell transfection.

## HIGHLIGHTS

- Circ_0000848 is downregulated in hypoxia-induced cardiomyocytes.
- Inhibition of circ_0000848 hindered cardiomyocyte proliferation and stimulated cardiomyocyte apoptosis after MI.
- Circ_0000848 positively regulates SMAD family member 7 (SMAD7) expression.
- Circ_0000848 recruits ELAV-like RNA-binding protein 1to stabilize SMAD7 mRN.
- Circ_0000848 restrains MI development via enhancing SMAD7 expression.

### Real-Time Quantitative Polymerase Chain Reaction Analysis

Firstly, total RNA was obtained from H9c2 cardiomyocyte with TRIzol Reagent (Invitrogen). RNAs were then reversely transcribed into cDNA by using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, IL, USA). SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was used to quantify RNA levels followed by the $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 as loading controls. The experiment was independently conducted in triplicate.

### Cell Counting Kit-8 Assay

Cells were seeded into 96-well plates ($5\times10^3$ cells/well) and cultured at 37°C. After incubation, Cell Counting Kit-8 (CCK-8) solution was added. Finally, cell proliferation was observed with a spectrophotometer (Thermo Fisher Scientific (Rockford, MD, USA)) after an additional incubation for 2 hours. The experiment was independently conducted in triplicate.

### Flow Cytometry Analysis

PE Annexin V Apoptosis Detection Kit I (BD biosciences (Franklin Lakes, NJ, USA)) was applied during the flow cytometry analysis. After resuspension in 100 mL of binding buffer, $1\times10^6$ cells were harvested and scoured by phosphate-buffered saline (10010049, Gibco, USA). Next, cells were dyed by Annexin-V-fluorescein isothiocyanate isomer (FITC) and propidium iodide in succession. Finally, a flow cytometer was employed to analyze cell apoptosis followed by incubation at 37°C for 15 minutes. The experiment was independently conducted in triplicate.

### Subcellular Fractionation

By using a PARIS™ Kit (Ambion, Austin, TX, USA), cytoplasmic and nucleic elements were separated. Cell cytoplasm was isolated by adding the cell fractionation buffer, and cell disruption buffer was used to collect the cell nucleus. After collecting the nuclear and cytoplasmic fraction, RT-qPCR assay was performed for quantifying hsa_circ_0000848, with GAPDH or U6 as cytoplasmic and nuclear controls. The experiment was independently conducted in triplicate.

### Fluorescent In Situ Hybridization and Immunofluorescence Assay

After fixation with 4% paraformaldehyde for 15 minutes at 37°C and then permeabilized with 0.5% Triton X-100, cardiomyocytes were hybridized with hsa_circ_0000848 probe in buffer, followed by dying with 4′,6-diamidino-2-phenylindole solution. ELAV-like RNA-binding protein 1 primary antibody was used to blot overnight at 4°C and then the blots were incubated with FITC-conjugated secondary antibody. With a confocal laser microscope (Olympus (Tokyo, Japan)), images were obtained. The experiment was independently conducted in triplicate.

### RNA Pull-Down Assay

RNA pull-down was carried out after Bio-miR-513c-5p-Wt, Bio-miR-513c-5p-Mut, and negative control (Bio-NC) were constructed respectively. Streptavidin beads were added to

biotin-labeled miR-513c-5p and spun at 4°C for 2 hours. After that, Cell lysates of $1 \times 10^6$ cells were mixed with streptavidin-coated magnetic beads and then RNAs were purified with TRIzol reagent. The enrichment of hsa_circ_0000848 and SMAD7 was analyzed by RT-qPCR. The experiment was independently conducted in triplicate.

### RNA Immunoprecipitation Assay

RNA Immunoprecipitation assay (RIP) was conducted with the application of Z-Magna RIPTM RNA-binding Protein Immunoprecipitation kit (Millipore Corporation (Bedford, MA, USA)). Anti-ELAVL1 (Abcam) antibody and anti-IgG (Abcam (Cambridge, MA, USA)) antibody were used to immunoprecipitate cell lysates. Finally, the RNA complexes were extracted for RT-qPCR analysis. The experimental procedure was independently carried out in triplicate.

### Western Blot Analysis

Total protein extracted from hypoxia-induced cardiomyocytes (HPC-CMs) was isolated by RIPA buffer, and after being separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA) and cultured in 5% skim milk. Subsequently, the membranes went through incubation with the following primary antibodies against Bcl2-associated agonist of cell death (BCL2), Bax, cleaved caspase-3, total caspase-3, SMAD7, ELAVL1, GAPDH, and β-actin. Afterward, the blots were incubated with a secondary antibody. At last, chemiluminescence system (GE Healthcare, Chicago, IL, USA) was applied to quantify proteins. The experiment was independently conducted at least 3 times.

### Statistical Analysis

Experimental data were analyzed by Statistical Package for the Social Sciences 22.0 statistical software package. All data were expressed as mean ± standard deviation. The suitability of continuous variables to normal distribution was tested by the Shapiro–Wilk normality test and the results demonstrated that the data of our study were normally distributed. The differences between 2 or more groups were analyzed with Student's *t*-test or one-way ANOVA as appropriate. All the experiments were independently performed in triplicate. Differences were considered statistically significant when $P < .05$.

## RESULTS

### Hsa_circ_0000848 Is Downregulated in Hypoxia-Induced Cardiomyocytes

Hypoxia is an important factor leading to MI; therefore, we conducted several assays in HPC-CMs to explore their features. Through CCK-8 assay, we observed that under the condition of hypoxia, cell viability was gradually impaired (Supplementary Figure 1A). Meanwhile, according to the results of flow cytometry assay and western blot analysis, it was revealed that the apoptotic capacity of cardiomyocytes under hypoxia was enhanced (Supplementary Figure 1B-C), suggesting the successful construction of MI model. According to the results of the RT-qPCR assay, it was shown that hsa_circ_0000848 was significantly downregulated in HPC-CMs (Figure 1A). The schematic diagram of the genomic location of hsa_circ_0000848 was presented in Figure 1B. In addition, by conducting the RT-qPCR assay, it was confirmed that linear-SMAD7 mRNA rather than hsa_circ_0000848 was significantly degraded in CMs and HPC-CMs treated by RNase R (Figure 1C). The finding that hsa_circ_0000848 could only be amplified by divergent primers from cDNA instead of genomic DNA further verified the existence of hsa_circ_0000848 (Figure 1D). At the same time, after adding Actinomycin D, which serves as an inhibitor of transcription, we confirmed that hsa_circ_0000848 was more stable than linear-SMAD7 (Figure 1E). Overall, hsa_circ_0000848 was downregulated in hypoxia-induced cardiomyocytes.

### Inhibition of hsa_circ_0000848 Hinders Cardiomyocyte Proliferation and Stimulates Cardiomyocyte Apoptosis Under Hypoxia

Before the implementation of functional assays to assess the role of hsa_circ_0000848 in hypoxia-induced cardiomyocytes, the interference efficiency of hsa_circ_0000848 was detected (Figure 2A). As the interference efficiency of sh-hsa_circ_0000848#1/2 was better than that of sh-hsa_circ_0000848#3, they were kept for the follow-up assays. After that, the results of the CCK-8 assay demonstrated that when hsa_circ_0000848 was depleted, cell proliferation was greatly impeded (Figure 2B). On the contrary, according to flow cytometry analysis, cell apoptosis was enhanced upon hsa_circ_0000848 silencing (Figure 2C). Moreover, by conducting a western blot assay, the protein levels of apoptosis-associated proteins were detected and the result further proved our previous finding (Figure 2D). Taken together, hsa_circ_0000848 silencing inhibited the progression of hypoxia-induced cardiomyocytes.

### Hsa_circ_0000848 Positively Regulates SMAD7 Expression

Based on RT-qPCR and western blot assays, we observed that SMAD7 expression at mRNA level and protein level in HPC-CMs was dramatically decreased due to hsa_circ_0000848 knockdown (Figure 3A-B). After that, the location of hsa_circ_0000848 in CMs and HPC-CMs was explored by subcellular fractionation and fluorescent in situ hybridization (FISH) assays (Figure 3C-D). The results suggested that hsa_circ_0000848 was mainly distributed in the cytoplasm of cardiomyocytes and hypoxia-induced cardiomyocytes which indicated the possibility of competing for endogenous RNA (ceRNA) mechanism. According to the prediction results from starBase (http://starbase.sysu.edu.cn/index.php), a Venn diagram was generated which determined miR-513c-5p as the target miRNA of SMAD7 (Figure 3E). After that, the binding sites between SMAD7/hsa_circ_0000848 and miR-513c-5p were predicted. However, according to the result of the RNA pull-down assay, it was disclosed that miR-513c-5p could not interact with hsa_circ_0000848 or SMAD7, which meant that hsa_circ_0000848 might not function as a ceRNA in hypoxia-induced cardiomyocytes (Figure 3F). More intriguingly, according to the result of the RT-qPCR assay, it was manifested that hsa_circ_0000848 deficiency could reduce the stability of SMAD7 mRNA in HPC-CMs (Figure 3G).

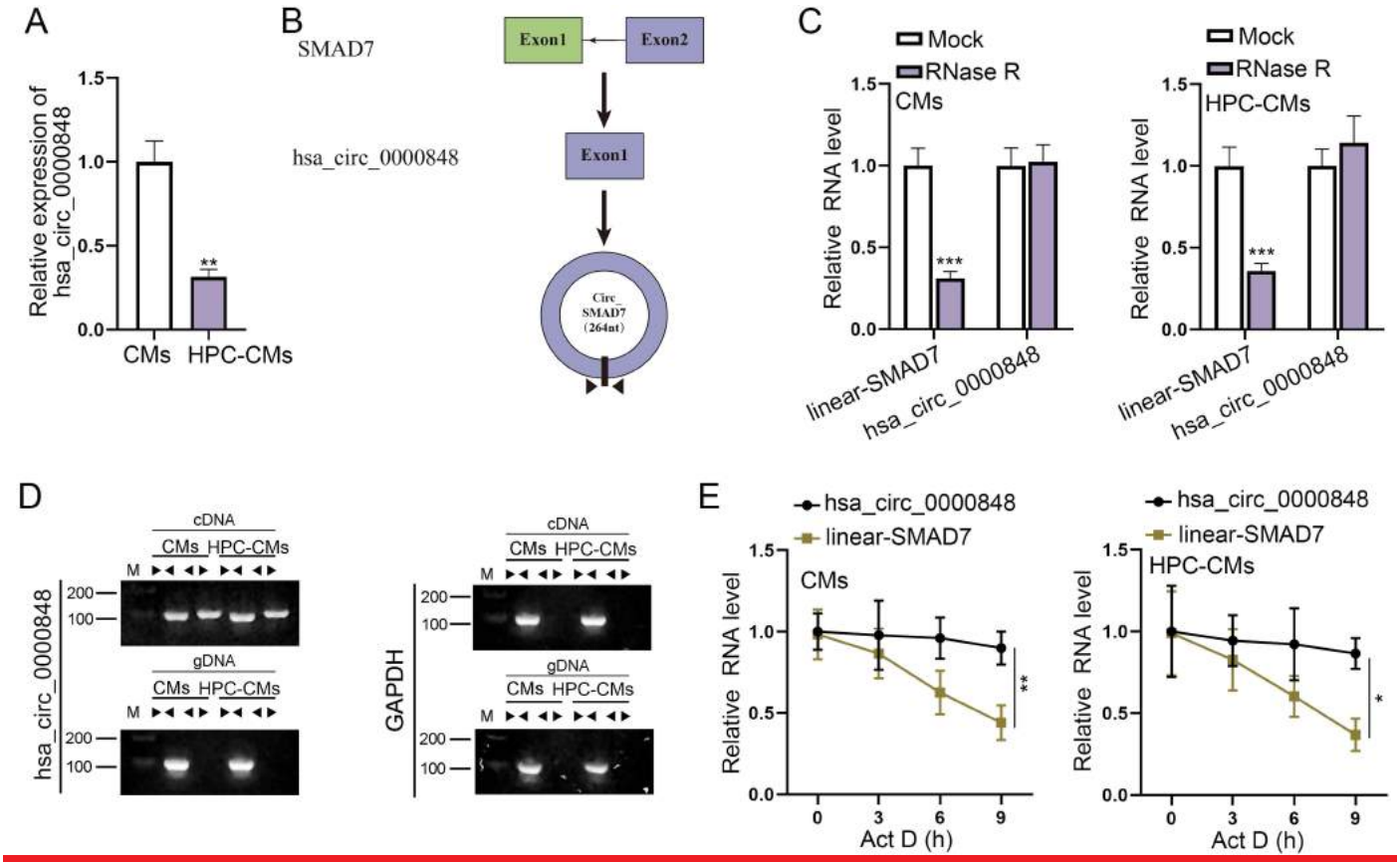


**Figure 1. Hsa_circ_0000848 is downregulated in hypoxia-induced cardiomyocytes. (A) The RT-qPCR assay was conducted to detect hsa_circ_0000848 expression in cardiomyocytes and hypoxia-induced cardiomyocytes. (B) A sequence diagram of cyclization site analysis of hsa_circ_0000848 is presented. (C) The RT-qPCR assay was carried out to detect the expression of hsa_circ_0000848 and linear-SMAD7 in cardiomyocytes and hypoxia-induced cardiomyocytes treated with or without RNase R. (D) Agarose gel electrophoresis was conducted to confirm the existence of hsa_circ_0000848. (E) The stability of hsa_circ_0000848 and linear-SMAD7 was detected after adding Act D. *$P < .05$, **$P < .01$, ***$P < .001$. RT-qPCR, real-time quantitative polymerase chain reaction; SMAD7, SMAD family member 7; Act D, actinomycin D.**

Collectively, hsa_circ_0000848 modulated SMAD7 expression in hypoxia-induced cardiomyocytes.

## Hsa_circ_0000848 Recruits ELAVL1 Protein to Stabilize SMAD7 mRNA

According to the previous findings, we speculated that there might exist an RNA-binding protein (RBP) network in the regulatory mechanism of hsa_circ_0000848 in HPC-CMs. According to the data from starBase and RBPDB (http://rbpdb.ccbr.utoronto.ca/), ELAVL1 was chosen as the potential RBP of hsa_circ_0000848 and SMAD7 (Figure 4A). RNA pull-down assay was then conducted, which displayed the strong affinity of hsa_circ_0000848 to ELAVL1 (Figure 4B). Similarly, according to the result of the RIP assay, it was demonstrated that ELAVL1 could bind to hsa_circ_0000848 and SMAD7 in HPC-CMs (Figure 4C). After that, the co-localization of hsa_circ_0000848 and ELAVL1 was exhibited by FISH and immunofluorescence analysis (Figure 4D). For further verification, the interference efficiency of ELAVL1 was detected by RT-qPCR and western blot assays (Figure 4E-F). According to the results of RT-qPCR and western blot assays, we found that SMAD7 expression at mRNA level and protein level was reduced when ELAVL1 was inhibited (Figure 4G-H). Moreover, the stability of SMAD7 mRNA was also decreased upon ELAVL1 silencing (Figure 4I). To conclude, hsa_circ_0000848 interacted with ELAVL1 protein to stabilize SMAD7 mRNA.

## Hsa_circ_0000848 Regulates Hypoxia-Induced Cardiomyocytes Development Via Elevating SMAD7 Expression

Firstly, the overexpression efficiency of SMAD7 was detected in HPC-CMs (Figure 5A). The cell viability was then detected by CCK-8 assay and it was uncovered that the suppressed cell proliferation caused by hsa_circ_0000848 silencing could be greatly restored by the co-transfection of pcDNA3.1/SMAD7 (Figure 5B). In addition, as demonstrated by flow cytometry and western blot assays, the promoted cell apoptosis induced by hsa_circ_0000848 downregulation was also rescued by the upregulation of SMAD7 (Figure 5C-D). All in all, SMAD7 overexpression could reverse the effect of hsa_circ_0000848 silencing on HPC-CMs.

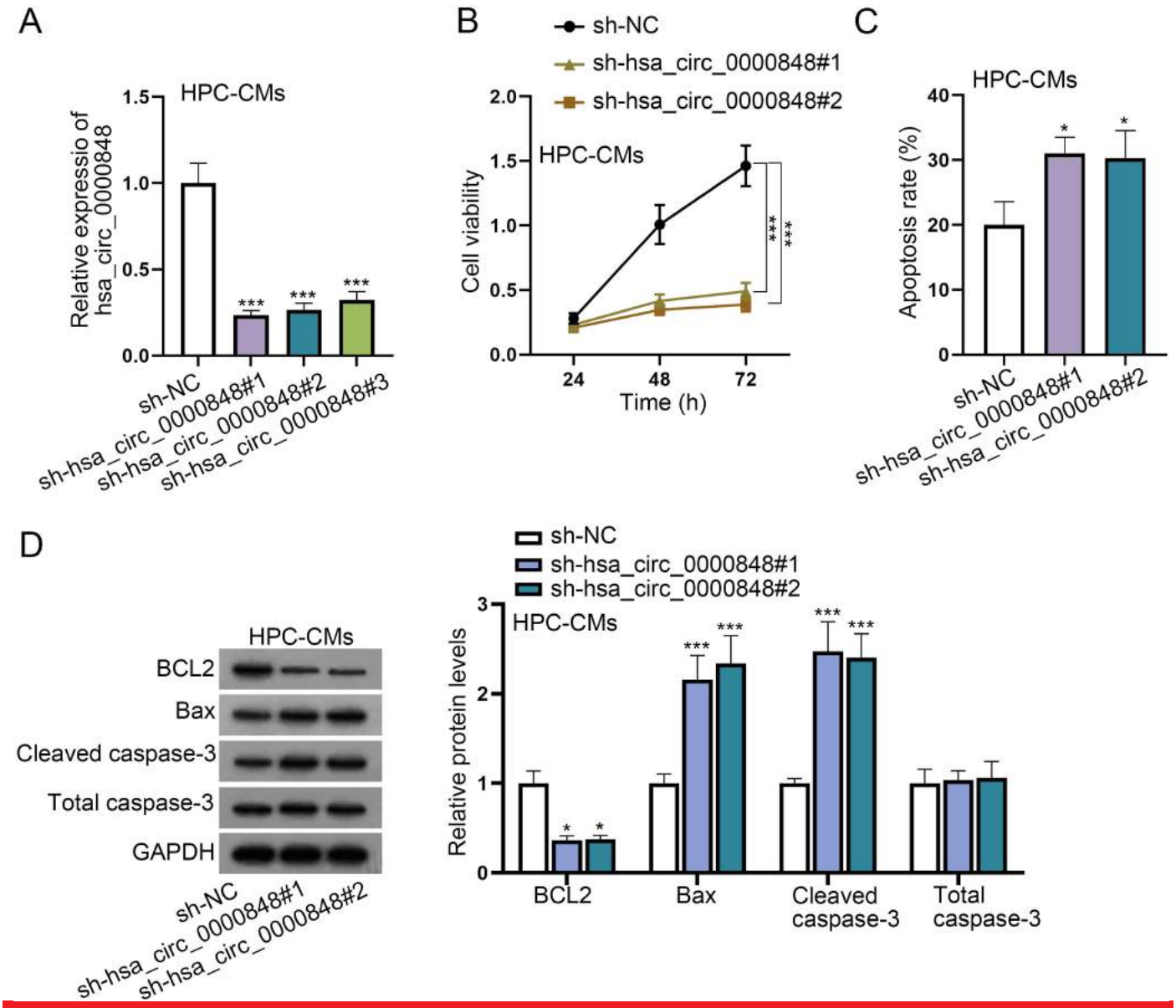


**Figure 2. Inhibition of hsa_circ_0000848 hinders cardiomyocyte proliferation and stimulates cardiomyocyte apoptosis under hypoxia. (A) The interference efficiency of hsa_circ_0000848 was detected. (B) CCK-8 assay was conducted to detect the proliferation of HPC-CMs when hsa_circ_0000848 was downregulated. (C-D) Flow cytometry and western blot assays were implemented to observe cell apoptosis. $^{*}P<.05$, $^{***}P<.001$. CCK-8, Cell Counting Kit-8; HPC-CMs, hypoxia-induced cardiomyocytes.**

## DISCUSSION

It has been commonly acknowledged that circRNAs are the key regulators in MI.[12,17] Hsa_circ_0000848 has been reported to be involved in trophoblast cell proliferation, migration, and apoptosis through interacting with miR-6768-5p.[18] Moreover, it has been reported that hsa_circ_0000848 can promote the cell proliferation of glioma by upregulating PCNA.[19] However, the role of hsa_circ_0000848 has never been studied in hypoxia-induced cardiomyocytes, let alone in MI. Through our investigation, we found that hsa_circ_0000848 expression was rather low in the hypoxia-induced cardiomyocytes. Functional assays revealed that the downregulation of hsa_circ_0000848 hindered cell proliferation while facilitating cell apoptosis in hypoxia-induced cardiomyocytes.

The interaction with RBPs has been determined to be an important part of the functions of circRNAs.[20] Besides, RBPs have been reported to play important roles in human diseases.[21] ELAVL1, which is also called HUR, has been widely reported to regulate the stability of mRNAs by binding to the 3′-untranslated region (3′-UTR) region of mRNAs.[22] For example, Chen et al[23] have elucidated that ELAVL1 has the capacity to stabilize FUT4 mRNA in multiple melanoma. Wang et al[24] have attested that lncRNA EGFR-AS1-recruited HUR enhances the stability of EGFR in renal cancer. Likewise, we observed that hsa_circ_0000848 stabilized

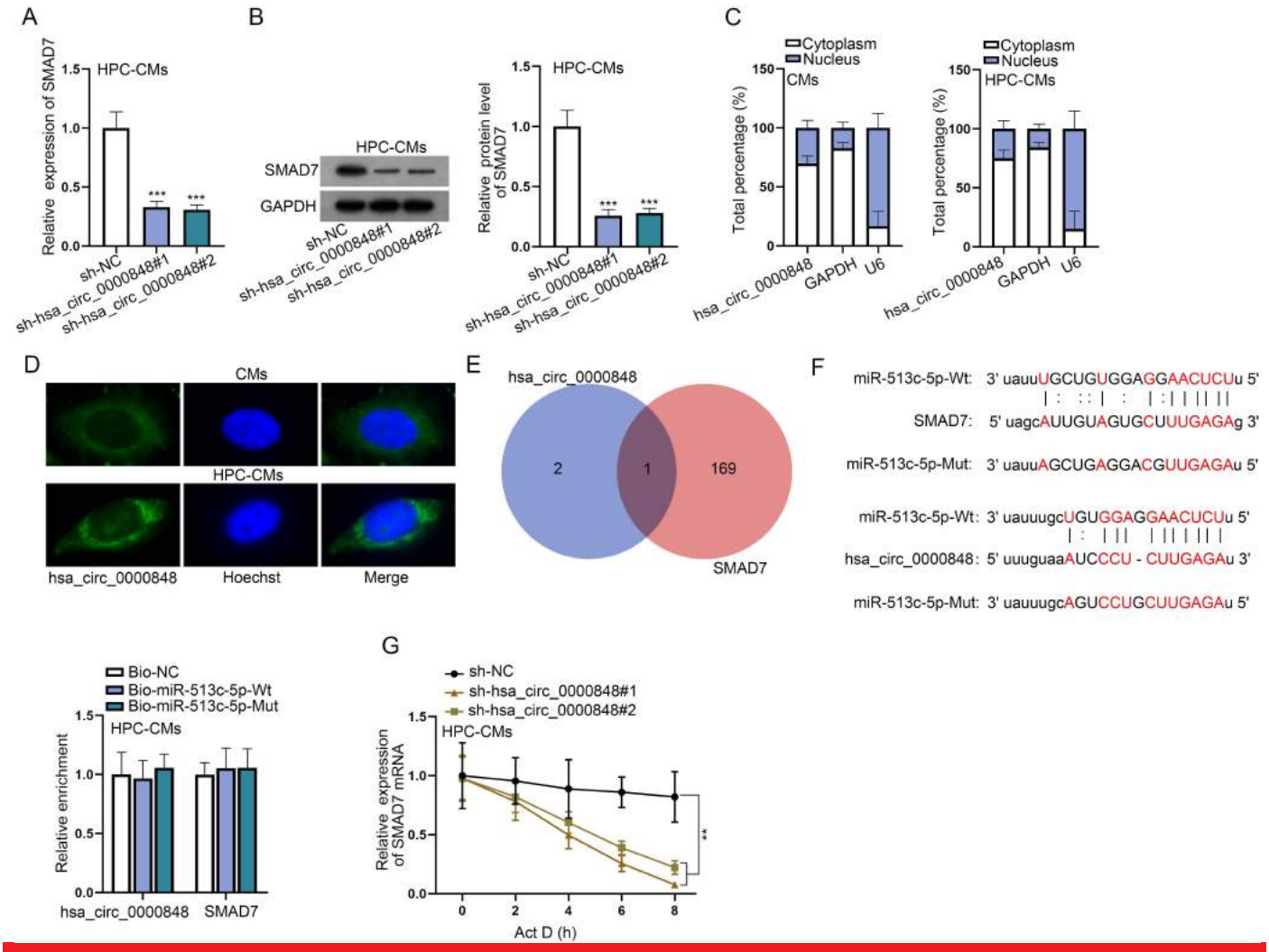


**Figure 3. Hsa_circ_0000848 positively regulates SMAD7 expression. (A-B) SMAD7 expression at mRNA level and protein level was detected when hsa_circ_0000848 was silenced. (C-D) Subcellular fractionation and FISH assays were carried out to explore the distribution of hsa_circ_0000848 in cardiomyocytes and hypoxia-induced cardiomyocytes. (E) A Venn diagram was generated to determine the potential miRNA which could bind to hsa_circ_0000848 and SMAD7. (F) RNA pull-down assay was conducted to examine the binding ability between miR-513c-5p and hsa_circ_0000848 or SMAD7 and the binding sites were predicted. (G) RT-qPCR assay was carried out to analyze the stability of SMAD7 mRNA upon hsa_circ_0000848 silencing after adding Act D. $^{**}P < .01$, $^{***}P < .001$. SMAD7, SMAD family member 7; RT-qPCR, real-time quantitative polymerase chain reaction; Act D, actinomycin D.**

SMAD7 mRNA via recruiting ELAVL1. Moreover, the inhibition of ELAVL1 could down-regulate SMAD7 expression and decrease the stability of SMAD7 mRNA.

SMAD family member 7 has also been widely studied in human diseases. For instance, it has been unveiled that SMAD7 promotes the proliferation of keratinocytes in psoriasis.[25] Also, SMAD7 is involved in the survival of cutaneous melanoma patients.[26] Moreover, SMAD7 has been considered as a therapeutic target for chronic kidney diseases.[27] In addition, SMAD7 has been identified to play a suppressive role in MI.[16,28] What more? It has been proved that hsa_circ_0000848 is located in the intron of the SMAD7 gene. In this study, we discovered that hsa_circ_0000848 modulated SMAD7 expression via binding to ELAVL1 protein. Furthermore, SMAD7 overexpression could rescue the effect of hsa_circ_0000848 silencing on hypoxia-induced cardiomyocytes.

To summarize, hsa_circ_0000848 was demonstrated to be downregulated in hypoxia-induced cardiomyocytes model and it could regulate cardiomyocyte proliferation and apoptosis under hypoxia via recruiting ELAVL1 and stabilizing SMAD7 mRNA. Based on the results of our study, we concluded that the hsa_circ_0000848/ELAVL1/SMAD7 axis might serve as an innovation for exploring novel treatment strategies of hypoxia-induced cardiomyocytes, thus boosting future studies on MI.

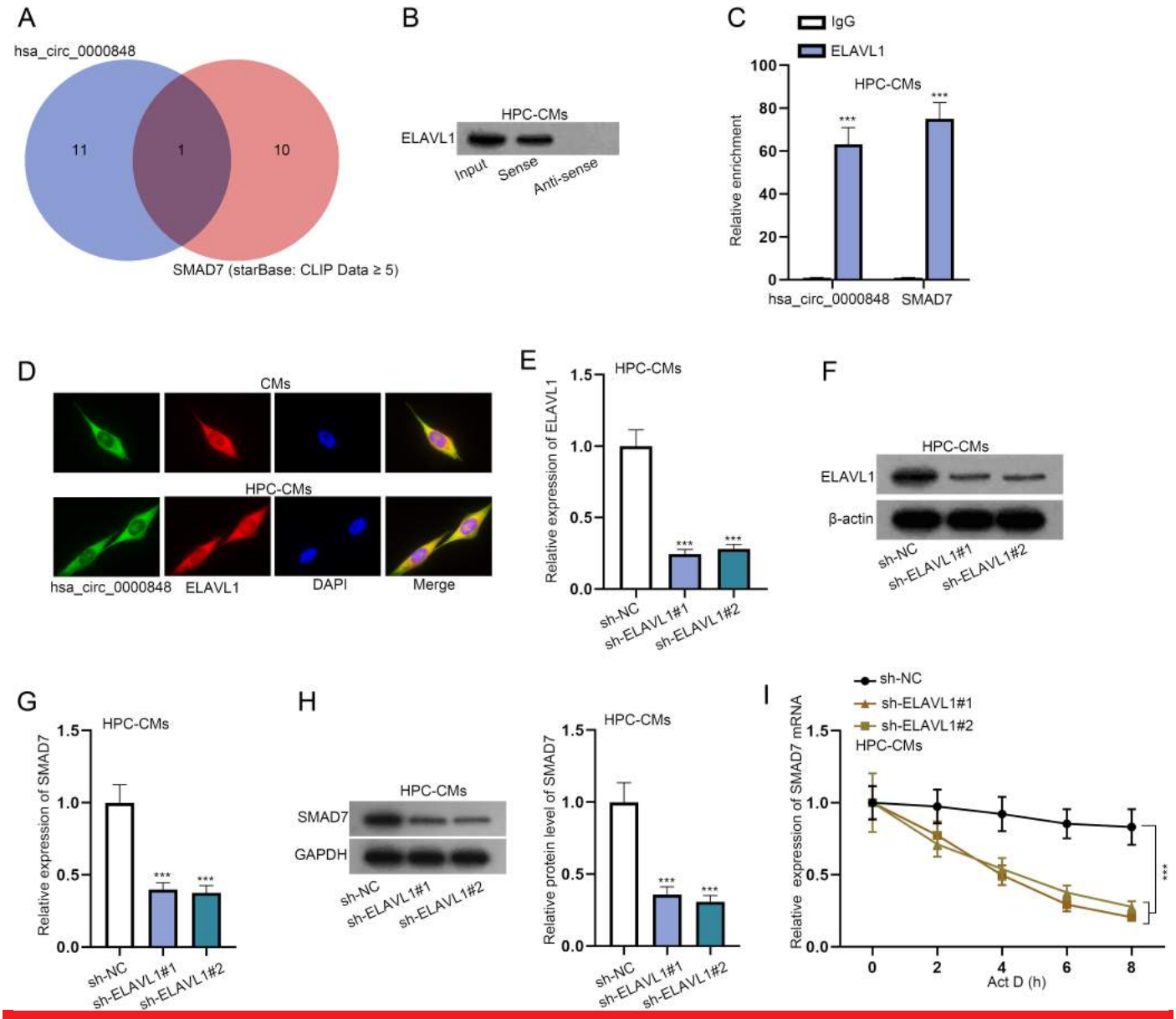


**Figure 4. Hsa_circ_0000848 recruits ELAVL1 protein to stabilize SMAD7 mRNA. (A) A Venn diagram was generated to determine the protein which could bind to both hsa_circ_0000848 and SMAD7. (B) RNA pull-down assay was used to confirm the affinity of hsa_circ_0000848 to ELAVL1. (C) RIP assay was conducted to display the relationship among hsa_circ_0000848, ELAVL1, and SMAD7. (D) FISH and IF assays were utilized to determine the co-localization of hsa_circ_0000848 and ELAVL1. (E and F) The interference efficiency of ELAVL1 was detected. (G-H) The mRNA and protein levels of SMAD7 were tested when ELAVL1 was depleted. (I) The stability of SMAD7 mRNA was assessed when ELAVL1 was inhibited. ***$P < .001$. SMAD7, SMAD family member 7; RIP, RNA immunoprecipitation assay; ELAV1, ELAV-like RNA-binding protein 1; FISH, fluorescent in situ hybridization; IF, immunofluorescence.**

However, though hypoxia is the central feature of MI and we have unveiled the function of hsa_circ_0000848 in hypoxia-induced cardiomyocytes, the specific role of such circRNA in MI still requires detailed analysis. In addition, our work lacks clinical trials to verify the therapeutic effect of hsa_circ_0000848 on MI development which requires more in-depth research in the future. Therefore, in our future studies, we will focus our energy on revealing the relationship between hsa_circ_0000848, or even more circRNAs, and MI.

**Ethics Committee Approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – S.C. C.L. L.L. G.Z. Y.J. J.F.; Design – S.C. C.L. L.L. G.Z. Y.J. J.F.; Supervision – S.C. C.L. L.L. G.Z. Y.J. J.F.; Funding – S.C. C.L. L.L. G.Z. Y.J. J.F.; Materials – S.C. C.L. L.L. G.Z. Y.J.

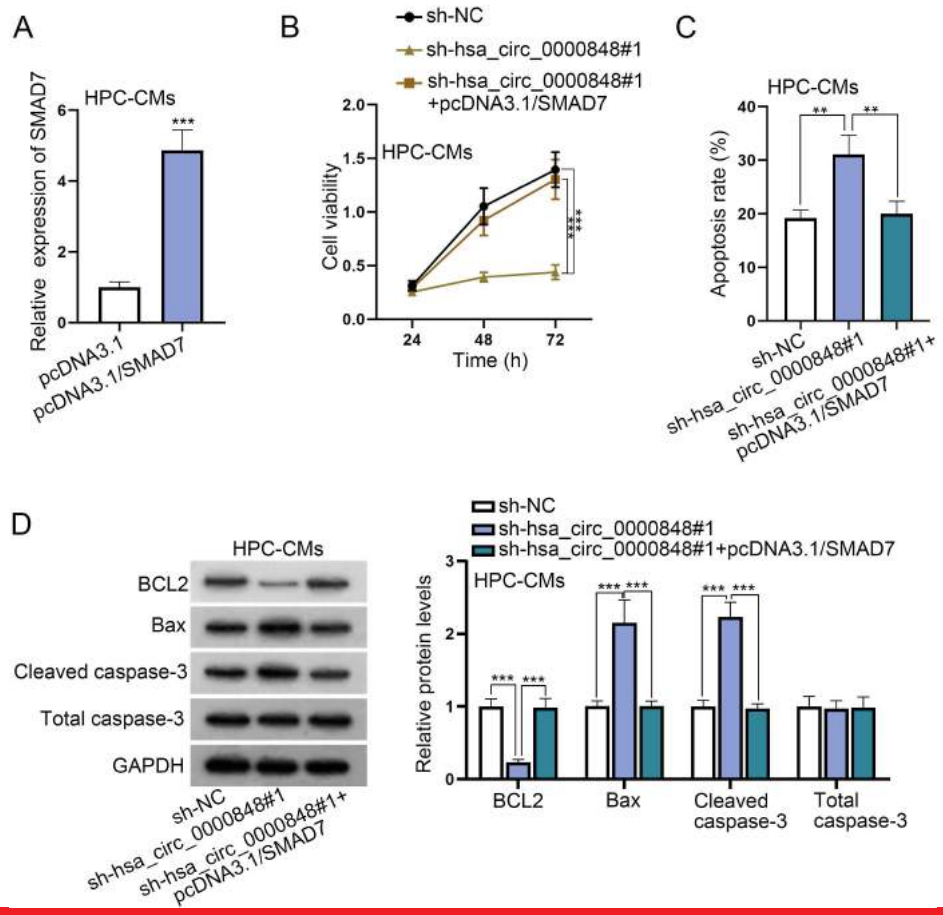


**Figure 5. Hsa_circ_0000848 regulates hypoxia-induced cardiomyocytes development via elevating SMAD7 expression. (A) The overexpression efficiency of SMAD7 was detected in hypoxia-induced cardiomyocytes. (B) CCK-8 assay was implemented to test the proliferation of HPC-CMs transfected with sh-NC, sh-hsa_circ_0000848#1, and sh-hsa_circ_0000848#1+pcDNA3.1/SMAD7 plasmids, respectively. (C and D) Cell apoptosis was observed in sh-NC, sh-hsa_circ_0000848#1, and sh-hsa_circ_0000848#1+pcDNA3.1/SMAD7 groups. $^{**}P < .01$, $^{***}P < .001$. SMAD7, SMAD family member 7; HPC-CMs, hypoxia-induced cardiomyocytes; CCK-8, Cell Counting Kit-8.**

J.F.; Data Collection and/or Processing – S.C. C.L. L.L. G.Z. Y.J. J.F.; Analysis and/or Interpretation – S.C. C.L. L.L. G.Z. Y.J. J.F.; Literature Review – S.C. C.L. L.L. G.Z. Y.J. J.F.; Writing – S.C. C.L. L.L. G.Z. Y.J. J.F.; Critical Review – S.C. C.L. L.L. G.Z. Y.J. J.F.

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**Declaration of Interests:** No conflicts of interest exist.

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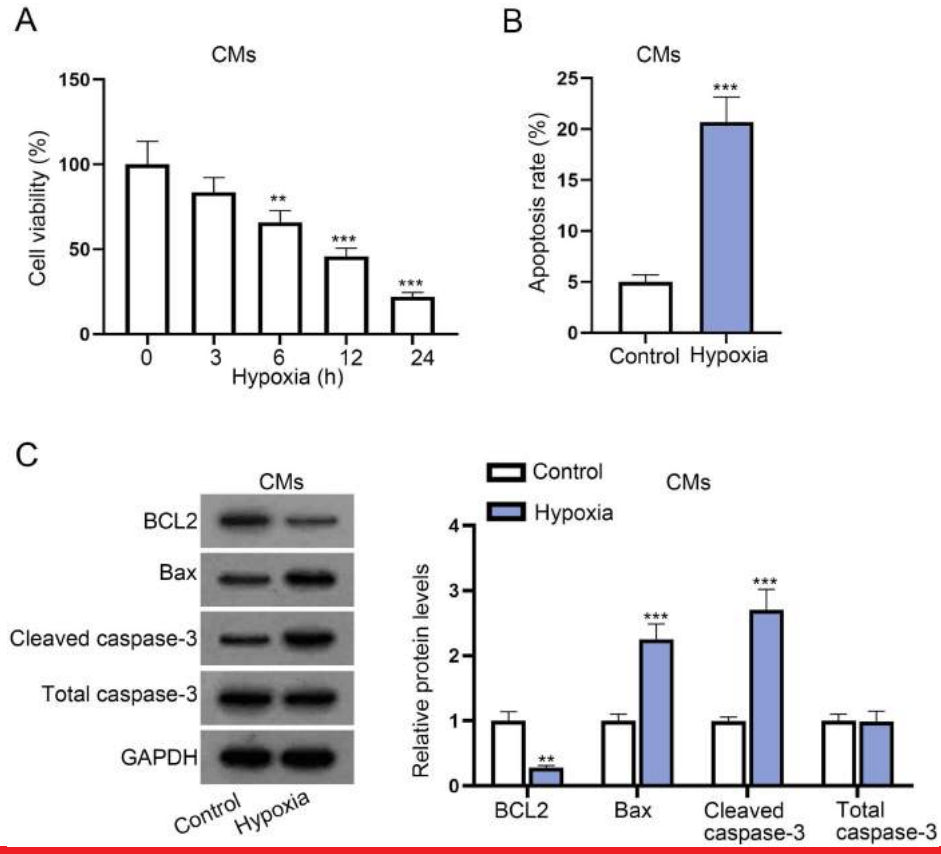


**Supplementary Figure 1. The establishment of the hypoxia model. (A) CCK-8 assay was conducted to detect cell viability during the induction of hypoxia. (B and C) Flow cytometry and western blot assays were utilized to detect cell apoptosis during the induction of hypoxia.** $^{**}P < .01$, $^{***}P < .001$.