Cathepsin B (CTSB), a member of lysosomal cathepsin, is involved in cell autophagy and apoptosis. We previously reported that CTSB increased cardiomyocyte apoptosis in mice heart during pressure overload, while the role of CTSB on diabetic cardiomyopathy has not been fully elucidated. The aim of this study is to explore the role and the underlying mechanism of CTSB on diabetic cardiomyopathy. Mice were subjected to streptozotocin injection to induce a diabetes model. Neonatal rat cardiomyocytes were isolated and cultured with high glucose (33.3 mM) to establish an in vitro model. CTSB protein level was increased in diabetic cardiomyopathy (DCM) mice heart as well as in cardiomyocytes stimulated with high glucose. CTSB knockout mice showed ameliorated cardiac function, cardiac fibrosis, cardiac inflammation, and pyroptosis level. Oppositely, DCM mice with CTSB transgene showed exacerbated cardiac dysfunction, fibrosis, inflammation, and pyroptosis. We found that CTSB could bind to NLR family pyrin domain containing 3 (NLRP3), thus increasing the activation of the NLRP3/caspase-1 inflammasome pathway. When we used a NLRP3 knockout mice, the deteriorating effect of CTSB overexpression via adeno-associated virus (AAV) delivery was abolished. Taken together, CTSB aggravates diabetic cardiomyopathy via promoting NLRP3-mediated pyroptosis.

INTRODUCTION

Diabetes is one of the largest public health problems in the world and accounts for the ten leading causes of death in the world. The prevalence of diabetes worldwide has increased year by year in recent decades. Metabolic disorders caused by diabetes can lead to long-term microvascular and large vascular injuries. Diabetic cardiomyopathy (DCM) is one of its common complications. DCM is a specific form of cardiomyopathy, which is independent of vascular injury. DCM is characterized by myocardial fibrosis, cardiomyocyte hypertrophy, inflammation, and apoptosis. Studies have confirmed that the risk of heart failure in diabetics is 2 to 5 times that of non-diabetic patients, and most patients with diabetes got a worse prognosis. So far, the pathogenesis of DCM has not yet been fully elucidated. Moreover, there is no effective treatment for DCM in clinical.

Pyroptosis is a novel form of programmed necrosis, which is triggered by inflammasome such as nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family. These inflammasomes are stimulated by several types of danger-associated molecular patterns (DAMPs) during cell injury and then activate caspase-1, which consequently activates Gasdermin-D (GSDMD), forming a membrane pore and leading to cell death. Studies have confirmed the essential role of pyroptosis on the progress of DCM. In human diabetic hearts, caspase-1, NLRP3, and GSDMD were found to be up-regulated. Sumit Kar et al. found that mitigating pyroptosis by exercise training could prevent high-fat-diet-induced DCM. Activating caspase-1 by a circular RNA increased pyroptosis in DCM and caused cardiomyocytes death. Thus, inhibiting cardiomyocyte pyroptosis may delay the progress of DCM.

Cathepsin B (CTSB) is a member of lysosomal cathepsin involved in cell autophagy and apoptosis, two kinds of cell death. Recent studies found that CTSB also participates in cell pyroptosis. Previously, we found that CTSB knockout could attenuate cardiomyocyte apoptosis and relieve cardiac remodeling induced by pressure overload in mice. Serum CTSB level in patients with stable coronary heart disease was found to be positively associated with the incidence of cardiovascular events. Moreover, high CTSB levels in heart tissue in patients with coronary heart disease were also reported to be a biomarker for sudden cardiac death. However, the role of CTSB on DCM is unclear. In this study, we used streptozotocin (STZ) injection to induce type 1 diabetes model. Four months after STZ injection, we established a DCM model successfully. To elucidate whether
changing the expression of CTSB could relieve or accelerate the progress of DCM, we used CTSB knockout mice to silence CTSB, and we also used an adeno-associated virus (AAV) gene-delivery system to overexpress CTSB.

RESULTS

CTSB was up-regulated in DCM mice heart and cardiomyocytes

Firstly, we explore the expression of CTSB on DCM hearts. As shown in Figures 1A and 1B, the protein level and activity of CTSB was up-regulated in mice heart with DCM. Immunostaining also revealed that the expression of CTSB was increased in cardiomyocytes in hearts with DCM (Figure 1C). Neonatal rat cardiomyocytes (NRCMs) were stimulated with high glucose (HG), and hypertonic (HO; with 27.5 mM mannitol) control. As expected, the protein level and activity of CTSB was up-regulated in the HG group. No difference was observed in the negative control group and the HO group (Figures 1D and 1E). Immunofluorescence staining also showed that a high level of CTSB in the HG group was mainly located in the cytoplasm and lysosome (Figure 1F). We also detected other Cathepsin family members, as shown in Figure 1A and 1D. The expression of CTSL was not changed in both diabetic mouse hearts and cardiomyocytes under hyperglycemic stresses, but the protein level of CTSS was up-regulated in heart tissue and cardiomyocytes under hyperglycemic stresses.

CTSB knockout relieved cardiac dysfunction, fibrosis, and inflammation in DCM mice

Then, we used a CTSB full knockout mice to elucidate the role of CTSB on the progress of DCM. One week after the final STZ injection, the blood glucose was elevated in both wild-type (WT)-DCM and knockout (KO)-DCM groups as shown in Table S3. CTSB-KO did not change the blood glucose 4, 8, 12, and 16 weeks after the final STZ injection (Table S3). The body weight was increased gradually, and was decreased at 3, and 4 months after STZ injection. CTSB-KO did not change the body weight compared with the corresponding WT groups (Figure S1A). We also detected the insulin level and triglyceride level 16 weeks post STZ injection; as expected, the insulin level was reduced and the triglyceride level was increased in DCM groups. But, CTSB KO did not affect insulin and triglyceride levels when compared with the corresponding control group (Figure S1A). Sixteen weeks after STZ injection, mice in both DCM groups developed cardiac dysfunction as assessed by increased left ventricular end diastolic diameter (LVEDd), LV end systolic diameter (LVESd) and decreased LV ejection fraction (LVEF), and LV fractional shortening (LVFS).

Figure 1. CTSB was up-regulated in DCM mice heart and cardiomyocytes

(A) Protein levels of CTSB, CTSL, and CTSS on diabetic cardiomyopathy (DCM) mice heart tissue (n = 6). (B) The activity of CTSB on DCM mice heart tissue (n = 6). (C) Immunohistochemistry of CTSB on DCM mice heart (n = 5). (D) Protein levels of CTSB, CTSL, and CTSS on neonatal rat cardiomyocytes (NRCMs) stimulated with high glucose (HG) and hypertonic (HO; 27.5 mM mannitol) control (n = 6). (E) The activity of CTSB on NRCMs (n = 6). (F) Immunofluorescence staining of CTSB and lysosome tracker in NRCMs (n = 6). (*p < 0.05 versus control (CON)/HO group). Student’s unpaired t test was used to compare the data in (A) and (B). One-way ANOVA was used in (D), (E) and (F).
shortening (LVFS) (Figure 2A). The cardiac catheter results also showed that mice with DCM developed reduced maximal/minimal rates of pressure development ($dp/dt_{max}$ and $dp/dt_{min}$, respectively), while CTSB-KO attenuated these changes and improved cardiac function (Figures 2A and 2B). Cardiac fibrosis and inflammation, other characteristics of DCM, were also assessed. As shown in Figure 2C, the increased fibrosis fraction in DCM hearts was hampered in the CTSB-KO group. The increased numbers of CD45- and CD68-labeled inflammatory cells were also reduced in DCM mice hearts in the CTSB-KO group (Figure 2D). The mRNA levels of those inflammation markers (tumor necrosis factor $\alpha$ [TNF-$\alpha$], interleukin-6 [IL-6], and monocyte chemoattractant protein-1 [MCP-1]) and fibrosis markers (collagen I, collagen III, and connective tissue growth factor [CTGF]) were dropped in CTSB-KO-DCM mice hearts (Figures S1C and S1D).

CTSB KO attenuated pyroptosis in DCM mice heart

A recent study found the associated between CTSB and cell pyroptosis.11 We explored the role of CTSB on pyroptosis under DCM pathological state. As shown in Figure 3A, TUNEL- and caspase-1-positive cells in DCM heart were increased, while they were reduced in the CTSB-KO group. We also assessed the protein markers of pyroptosis, GSDMD and GSDMD P30. Both GSDMD and GSDMD P30 were elevated in DCM mice heart but decreased in CTSB-KO-DCM mice hearts (Figures 3B and 3C). We also detected the activity of caspase-1. Consistently, the activity of caspase-1 was increased in the WT-DCM group but dropped in the CTSB-KO-DCM group (Figure 3D). ELISA assay also found that the level of IL-1$\beta$ and IL-18 was decreased in the CTSB-KO-DCM group when compared with the WT-DCM group (Figure 3E).

CTSB knockdown ameliorated cardiomyocyte pyroptosis in HG-stimulated NRCM model

To confirm the role of CTSB on cardiomyocytes, NRCMs were transfected with CTSB small interfering RNA (siRNA) to knockdown CTSB (Figure 4A). HG induced cardiomyocyte death as evidenced by higher TUNEL-positive and Propidium Iodide (PI)-positive cells (Figures 4B and 4A), while CTSB silence decreased those TUNEL-positive and PI-positive cells (Figures 4B and S2A). CTSB silence also reduced caspase-1 activity (Figure S2B) as well as lactate dehydrogenase (LDH) release (Figure 4C). Decreased GSDMD and GSDMD P30 levels (Figure 4D) and reduced release of IL-1$\beta$ and
IL-18 (Figure S2C) were also observed in cells with CTSB silence after HG stimulation. We also found that CTSB KO reduced cleavage caspase-3 activity and Annexin V-labeled cell apoptosis under HG stimulation (Figures S2D and S2E).

**Figure 3. CTSB knockout attenuated pyroptosis in DCM mice heart**

(A) TUNEL and caspase-1 staining and quantification of TUNEL-positive cell numbers in DCM mouse hearts (n = 6). (B) and (C) Protein level of GasderminD and GasderminD P30 in DCM mouse hearts (n = 6). (D) Caspase-1 activity in DCM mouse hearts (n = 6). (E) ELISA detection of IL-1β and IL-18 levels in DCM mouse hearts (n = 6). *p < 0.05 versus corresponding CON; #p < 0.05 versus WT-DCM. Two-way ANOVA was used in (A)–(E).

CTSB transgene deteriorated cardiac dysfunction, fibrosis, and inflammation in DCM mice

To assess the role of human CTSB protein, mice were injected with AAV9-CTSB to overexpress human CTSB protein (Figure S3A).

**Figure 4. CTSB knockdown ameliorated cardiomyocyte pyroptosis in HG-stimulated NRCMs model**

(A) Protein level of CTSB in NRCMs transfected with CTSB siRNA (n = 6). (B) TUNEL staining and quantification of TUNEL-positive cell numbers in NRCMs stimulated with HG (n = 6). (C) Lactate dehydrogenase (LDH) release from NRCMs (n = 6). (D) Protein level of GasderminD and GasderminD P30 in NRCMs (n = 6). *p < 0.05 versus corresponding HO; #p < 0.05 versus ScRNA-HG. Student’s unpaired t test was used to compare the data in (A). Two-way ANOVA was used in (B)–(D).
Two, four, and six weeks after AAV9 injection, hearts revealed high levels of CTSB protein. Blood glucose was increased in DCM mice (Table S4), while body weight was dropped gradually after 2 months of STZ injection (Figure S3B). Overexpressing human CTSB did not affect blood glucose and body weight in both physical conditions and DCM conditions (Table S4; Figure S3B). Echocardiography results showed that CTSB transgene aggravated cardiac dysfunction as evidenced by increased LVEDd and reduced LVEF as well as decreased maximal rate of pressure development and decay. (C) PSR staining and left ventricular collagen volume in DCM mouse hearts (n = 6). (D) CD45 and CD68 staining and quantification of CD45- and CD68-positive cell numbers in DCM mouse hearts (n = 6). *p < 0.05 versus corresponding CON, †p < 0.05 versus AAV9-NC-DCM. Two-way ANOVA was used in (A–D).

CTSB transgene deteriorated cardiac dysfunction, fibrosis, and inflammation in DCM mice

(A) Echocardiography in mice injected with AAV9-CTSB after 16 weeks of STZ injection (n = 8). (B) Hemodynamic parameters (n = 8). LVEDd, left ventricular end diastolic dimension; LVEDd, left ventricular end systolic dimension; LVEF, LV ejection fraction; LVFS, LV fractional shortening; HR, heart rate; dp/dt max/min, decreased maximal rate of pressure development and decay. (C) PSR staining and left ventricular collagen volume in DCM mouse hearts (n = 6). (D) CD45 and CD68 staining and quantification of CD45- and CD68-positive cell numbers in DCM mouse hearts (n = 6). *p < 0.05 versus corresponding CON, †p < 0.05 versus AAV9-NC-DCM. Two-way ANOVA was used in (A–D).

Two, four, and six weeks after AAV9 injection, hearts revealed high levels of CTSB protein. Blood glucose was increased in DCM mice (Table S4), while body weight was dropped gradually after 2 months of STZ injection (Figure S3B). Overexpressing human CTSB did not affect blood glucose and body weight in both physical conditions and DCM conditions (Table S4; Figure S3B). Echocardiography results showed that CTSB transgene aggravated cardiac dysfunction as evidenced by increased LVEDd and reduced LVEF as well as dropped dp/dt max and dp/dt min, as assessed by cardiac catheter (Figures 5A and 5B). Cardiac fibrosis and inflammation were also exacerbated in CTSB transgene mice with DCM as evidenced by increased fibrosis fraction (Figure 5C), increased CD45- and CD68-labeled cells (Figure 5D), and elevated mRNA levels of fibrosis markers and inflammation markers (Figures S3C and S3D).

CTSB transgene increased pyroptosis in DCM mice heart

Cell pyroptosis was assessed in CTSB transgene mice hearts. As expected, CTSB overexpression enhanced TUNEL- and caspase-positive cells in DCM heart (Figure 6A), increased protein levels of GSDMD and GSDMD P30 (Figure 6B), and increased levels of caspase-1 activity and release of IL-1β and IL-18 (Figures 6C and 6D).

CTSB overexpresses aggravated cardiomyocyte pyroptosis in HG-stimulated NRCM model

We transfected NRCMs with Ad-CTSB to overexpress human CTSB (Figure S4A). CTSB overexpression increased TUNEL- and PI-positive cells (Figures S4B and S4C), enhanced caspase-1 activity (Figures S4D and S4E), and increased the levels of GSDMD and GSDMD P30 (Figure S4F) as well as the release of LDH, IL-1β, and IL-18 (Figures S4G and S4H). CTSB overexpression increased c-caspase-3 activity and annexin V-labeled cell apoptosis (Figure S4I).

NLRP3 overexpresses counteracted protective effects of CTSB silence in vitro

To confirm that NLRP3 is the target of CTSB, we transfected cardiomyocytes with Ad-NLRP3 to overexpress human NLRP3 (Figure S5A). CTSB silence reduced HG exposure-induced TUNEL- and PI-positive cells, while NLRP3 overexpression increased these trends (Figures S5B and S5C). Cells with both NLRP3 overexpression and CTSB knockdown showed increased TUNEL- and PI-positive cells (Figures S5B and S5C). We also detected caspase-1 activity and IL-1β and IL-18 release. As expected, cells with both NLRP3 overexpression and CTSB knockdown showed increased caspase-1 activity and IL-1β and IL-18 release compared with cells with merely CTSB knockdown.
Taken together, NLRP3 may act as a target of CTSB in DCM progress.

CTSB activated NLRP3 inflammasome
NLRP3 is one of the DAMPs that accounts for the activation of pyroptosis signaling. Whether CTSB affects NLRP3 in cardiomyocytes is unknown. We found that the levels of NLRP3, ACS, and caspase-1 were elevated in DCM mouse hearts (Figure 7A), while CTSB-KO reduced protein levels of NLRP3, ACS, and caspase-1 (Figure 7A). The elevated levels of NLRP3, ACS, and caspase-1 were also observed in mice with CTSB transgene when compared with negative control DCM mice (Figure 7B). In NRCMs with CTSB silence, the levels of NLRP3, ACS, and caspase-1 were dropped, while in NRCMs with human CTSB overexpressed, the levels of those NLRP3 inflammasome proteins were increased (Figures 7C and 7D). We detected whether CTSB could bind to NLRP3 protein. As shown in Figures 7E and 7F, co-immunoprecipitation (coIP) assay showed that CTSB could bind to NLRP3 and that HG increased the bind of these two proteins.

NLRP3 KO abolished the deteriorating effects of CTSB transgene in DCM mice
To confirm that NLRP3 is the target of CTSB during DCM progress, we used NLRP3 KO mice (Figure 8A) and injected mice with AAV9-CTSB to overexpress CTSB. As shown in Figure 8B, the blood glucose level of DCM mice was increased, while their body weight decreased significantly. Sixteen weeks after STZ injection, the levels of cardiac dysfunction, fibrosis, and inflammation were increased in WT mice with DCM. NLRP3-KO mice revealed relieved cardiac dysfunction, fibrosis, and inflammation, while no significant difference was observed between NLRP3-KO mice and NLRP3-KO + AAV9-CTSB mice (Figures 8C and 8D). NLRP3-KO reduced cell pyroptosis of DCM mice, but no significant difference was observed about the level of pyroptosis between NLRP3-KO mice and NLRP3-KO + AAV9-CTSB mice as assessed by TUNEL-positive cells, caspase-1 activity, and levels of GSDMD and GSDMD P30 (Figures 8E–8G).

These data indicate that CTSB binds to NLRP3, activating the NLRP3-mediated cardiomyocyte pyroptosis.

DISCUSSION
CTSB, a cysteine proteolytic enzyme, expresses in cardiomyocytes and is mainly located in the lysosomes. Its roles in cell death, such as autophagy and apoptosis, have been fully elucidated, while the role of CTSB on cardiomyocyte pyroptosis is unclear. In this study, we used a DCM mouse model and found that CTSB was up-regulated in DCM mice and cardiomyocytes. CTSB deficiency led to relieved cardiac remodeling with ameliorating cardiac dysfunction, fibrosis, and inflammation, as well as cardiomyocyte pyroptosis, while CTSB overexpression led to aggravating cardiac remodeling with deteriorating cardiac dysfunction, fibrosis, inflammation, and cell pyroptosis. Although CTSB affects cardiac remodeling under DCM situation, knocking out or overexpressing the CTSB gene does not affect the basic function of the heart.

Pyroptosis is a new kind of programmed cell death that is different from apoptosis and autophagy. It is characterized by the formation of holes in the cell membrane, leading to the release of pro-inflammatory cytokines, and subsequently, cell lysis. Both pyroptosis and apoptosis are characterized by TUNEL staining. Pyroptosis is an important innate
Oxidative stress activates NLRP3 in macrophage, which, in combination with ACS, promotes the activation of caspase-1. On the one hand, activated caspase-1 cleaves GSDMD to form a polypeptide containing the N-terminal active domain of GSDMD, which leads to perforation of cardiomyocytes, rupture of cell membrane, release of cell contents, and cell death. On the other hand, caspase-1 cleaves pro-IL-1β and pro-IL-18, activating IL-1β and IL-18 and leading to a cascade amplification of inflammation.

A recent study reported that CTSB activated the NLRP3 and promoted the caspase-1-induced pyroptosis in a mice acute pancreatitis model. CTSB was also been found to activate pyroptosis and autophagy in INS-1 cells. Here, in this study, we found that cardiomyocyte pyroptosis was increased in DCM mice heart and in cardiomyocytes stimulated with HG. Moreover, CTSB deficiency decreased the extent of cardiomyocyte pyroptosis in both in vivo and in vitro experiments. Human CTSB overexpression increased cardiomyocyte pyroptosis during the process of DCM. On the other hand, CTSB overexpression also increased IL-1 and IL-18 levels, while CTSB KO reduced these activated inflammasomes.

The activation of NLRP3 is the initial part of cardiomyocyte pyroptosis. As a DAMP, NLRP3 can be activated by both dead...
cardiomyocytes and increased reactive oxygen species (ROS) levels. During the progress of DCM, both hyperglycemia and hyperlipidemia could provoke an increased production of advanced glycation products (AGEs) and ROS. On the other hand, damaged cardiomyocytes also trigger the activation of NLRP3 to form more inflammasomes and provoke pyroptosis. The association of CTSB and NLRP3 has been elucidated in previous studies. Wang H. found that CTSB could interact with NLRP3 in a rheumatoid arthritis model. In a peritoneal and gouty inflammation model, CTSB was found to be released from lysosome and activate NLRP3 inflammasosome. In our study, we also confirm that CTSB could directly bind to NLRP3 in cardiomyocytes. Under HG stimulation, the expression of both CTSB and NLRP3 increased, thus leading to the higher level of CTSB-NLRP3 interaction. The mechanism by which CTSB releases from the lysosome is unclear. Maybe various factors such as ROS and inflammation lead to cell damage and lysosomal membrane leakage. We used a NLRP3 KO mouse to confirm that NLRP3-triggered pyroptosis is the target of CTSB during DCM pathology. As expected, NLRP3 deficiency could ameliorate cardiac remodeling in DCM heart and reduce cell pyroptosis. However, human CTSB overexpression could no longer exert aggravate phenotype. Thus, the increased CTSB during DCM activates NLRP3-triggered pyroptosis, which deteriorates the pathology of DCM.

Figure 8. NLRP3 knockout abolished the deteriorating effects of CTSB transgene in DCM mice
NLRP3-KO mice were injected with AAV9-CTSB. (A) The protein levels of NLRP3 and CTSB in the indicated group (n = 6). (B) Body weight and blood glucose in the indicated group (n = 12). (C) Echocardiography parameters in mice in the indicated group (n = 8). (D) PSR and CD45 staining, and quantification of collagen fraction and CD45-positive cell numbers in mice heart (n = 6). (E) TUNEL staining and quantification of TUNEL-positive cell numbers in hearts (n = 6). (F) Caspase-1 activity in hearts (n = 6). (G) Protein levels of GasderminD and GasderminD P30 in hearts (n = 6). *p < 0.05 versus WT-CON; #p < 0.05 versus WT-DCM. Two-way ANOVA was used in (A)–(G).
In summary, CTSB aggravates cardiac remodeling induced by DCM and deteriorates cardiac dysfunction by regulating cardiomyocyte pyroptosis. By interacting with NLRP3, CTSB regulates inflammasome formation and provokes both NLRP3-mediated pyroptosis and inflammation. Thus, CTSB may become a target of new therapeutic methods for treating DCM.

MATERIALS AND METHODS

Animals
All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University (protocol no. 00019275). All treatment and subsequent analyses were performed in a blinded manner. Male and female CTSB−/+ heterozygous mice were purchased from the Jackson Laboratory, as described in our previous study.13 The WT littermates were used as control. The animals were allowed free access to food and water and were maintained on a 12 h light/dark cycle in a controlled temperature (20°C–25°C), humidity (50% ± 5%) and specific pathogen-free environment. The mice were grouped according to a random number table to either a control or DCM group.

Animal model
The diabetic model was established by intraperitoneal STZ injection (dissolved in 0.1 mol/L citrate buffer [pH 4.5]) at a dose of 50 mg/kg for 5 consecutive days. Control mice were injected with an equal volume of citrate buffer. One week after the final STZ injection, fasting blood glucose (FBG) was detected. Diabetes was defined as FBG ≥ 11.1 mmol/L in three independent measurements.27

CTSB overexpression
For CTSB overexpression experiments, C57/BL6j male mice (8–10 weeks), purchased from the Chinese Academy of Medical Sciences (Beijing, China), were used. Mice received tail vein injection of either AAV9-CTSB or AAV9-negative control (NC) 10 weeks after the final STZ injection (each mouse received a total of 60–80 µL AAV9-CTSB or AAV9-NC [5.0–6.5 × 10^13 VG/mL]).

NLRP3 KO experiment
For mechanism experiments, NLRP3-KO mice (purchased from the Jackson Laboratory, which are a gift from Dr Jing Zong, Department of Cardiology, The Affiliated Hospital of Xuzhou Medical University) received tail vein injection of either AAV9-CTSB or AAV9-NC 10 weeks after final STZ injection (each mouse received a total of 60–80 µL AAV9-CTSB or AAV9-NC [5.0–6.5 × 10^13 VG/mL]).

Supplemental methods
Sixteen weeks after final STZ injection, mice were subjected to echocardiography and cardiac catheter, and then mice were sacrificed, and hearts were removed. For other details about the AAV9 vector, echocardiography and cardiac catheter, histological analysis, NRCM isolation and culture, real-time PCR and western blotting, Elisa assay, co-immunoprecipitation assays, and statistical analysis, please see the supplemental information.

Data availability
The datasets used and/or analyzed in the current study are available from the corresponding authors upon reasonable request.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2022.09.019.

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AUTHOR CONTRIBUTIONS
Q.Q.W., conceived and designed the experiments; C.L., Y.Y., Q.Y., T.H., Q.X., and J.N., performed the experiments; Z.C. and J.Z., analyzed the data; Q.Q.W., wrote and revised the manuscript.

DECLARATION OF INTERESTS
The authors declare that they have no competing interests.

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