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# Inhibition of Dectin-1 in mice ameliorates cardiac remodeling by suppressing NF-κB/NLRP3 signaling after myocardial infarction

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ABSTRACT

The myocardial inflammatory response is a consequence of myocardial infarction (MI), which may deteriorate cardiac remodeling and lead to dysfunction in the heart post-MI. Dectin-1 is a c-type lectin, which has been shown to regulate innate immune responses to pathogens. However, the role of Dectin-1 in the heart diseases remains largely unknown. In this study, we aimed to investigate the effects of Dectin-1 on cardiac remodeling post-MI. We found that cardiac Dectin-1 mRNA and protein expressions were significantly elevated in C57BL/6 mice after MI. *In vitro*, hypoxia induced cardiomyocyte injury in parallel with increased Dectin-1 protein expression. Knockdown of Dectin-1 remarkably attenuated cardiomyocyte death under hypoxia and lipopolysaccharide (LPS) stimulation. *In vivo* administration of adeno-associated virus serotype 9 mediated silencing of Dectin-1, which significantly decreased cardiac fibrosis, dilatation, and improved cardiac function in the mice post-MI. At the molecular level, downregulation of Dectin-1 dramatically suppressed up-regulation of nuclear factor-κB (NF-κB), nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3), and the inflammatory genes involved in fibrogenesis and cardiac remodeling after MI. Furthermore, treatment with BAY11-7082, an inhibitor of NF-κB, repressed the activation of NF-κB, and attenuated LPS induced elevation of NLRP3 and cell death in cardiomyocytes. Collectively, upregulation of Dectin-1 in cardiomyocytes post-MI contributes to cardiac remodeling and cardiac dysfunction at least partially by activating NF-κB and NLRP3. This study identified Dectin-1 as a promising therapeutic target for ischemic heart disease.

## 1. Introduction

Myocardial infarction (MI) is the leading cause of mortality worldwide [1,2]. The excessive pro-inflammatory response caused by MI has been demonstrated to exacerbate myocardial injury and dysfunction; anti-inflammatory treatment may be a novel approach that reduces myocardial infarct size and prevents further cardiac remodeling and progression to heart failure [3,4]. However, many anti-inflammatory interventions have shown inadequate efficacy in clinical trials [5]. Understanding the new mechanisms governing cardiac inflammation are therefore crucial to identifying a promising target for treating ischemic heart disease.

Initiation of a sterile inflammatory response after MI is modulated by damage-associated molecular patterns, which are recognized by toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs) [3]. These receptors induce a pro-inflammatory response through activation of nuclear factor-κB (NF-κB) [6,7]. Dectin-1 belongs to the c-type lectin-like superfamily that acts as a transmembrane pattern recognition receptor, containing an extracellular c-type-like domain and immune receptor tyrosine-based activation (ITAM)-like motif in the cytoplasmic region [8]. Dectin-1 is expressed on the surface of myeloid dendritic cells, monocytes and macrophages, which have been shown to play critical roles in the innate immune system, including antifungal immunity to fungal cell walls, and immune responses to other pathogens [9–12]. Upon binding to its ligand, β-glucans, activation of Dectin-1 resulted in recruitment and

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phosphorylation of spleen tyrosine kinase, thereby activating NF-κB, leading to the production and secretion of inflammatory cytokines and chemokines [13–15]. Recent studies have revealed that Dectin-1 also exerts regulatory roles in non-pathogen-mediated sterile inflammation. Under chronic inflammatory stimulation, Dectin-1 was upregulated in liver tissue, and overexpression of Dectin-1 attenuated liver fibrosis by suppressing TLR4 signaling [16]. Furthermore, Dectin-1 was shown to serve as an oncogenic factor by binding a novel ligand other than β-glucans in Pancreatic Carcinoma [17]. This suggests that Dectin-1 is worthy of further investigation in other sterile inflammatory diseases, in particular ischemic heart disease.

In the present study, we examined the dynamic expression of Dectin-1 after MI to elucidate the regulatory roles it has in cardiac remodeling. We demonstrated that upregulation of Dectin-1 promoted myocardial damage, inflammation, fibrosis, and further impaired cardiac dysfunction. Furthermore, we demonstrated that Dectin-1 induced activation of the NF-κB/NLRP3 axis in the infarcted myocardium. Our study reveals a novel link between Dectin-1 in cardiomyocytes and cardiac remodeling, and suggests that targeting Dectin-1/NF-κB/NLRP3 signaling may serve as a promising therapeutic approach against MI.

## 2. Materials and methods

### 2.1. Animals

Approximately ten-week-old male C57BL/6 mice, weighing between 20 and 22 g used for animal studies and pregnant C57BL/6 mice for neonatal myocyte isolation were obtained from Changsheng Biotechnology (Liaoning Province, China) and the animal center at the Second Affiliated Hospital of Harbin Medical University, respectively. Mice were housed on a 12 h light/12 h dark cycle with *ad libitum* access to chow and water. All experiments in this study were approved by the Ethic Committees of Harbin Medical University (the approval number: IRB3009619) and conformed to NIH guide for the Care and Use of Laboratory Animals.

### 2.2. In vivo gene delivery

Before MI surgery, C57BL/6 mice were randomized to receive either the adeno associated virus serotype 9 (AAV9) virus carrying a specific short fragment for silencing Dectin-1 (AAV9-si-Dectin-1) or a scrambled negative control RNA (AAV9-NC) ($1 \times 10^{11}$ plaque-forming units at a volume of 100 μL/animal) via tail vein injection. For data collection following gene delivery, experimental measurements were performed 3 weeks following *in vivo* administration of AAV9-si-Dectin-1 to knock down Dectin-1 expression. 3 weeks later, all the mice either had MI induced, or underwent sham operation.

### 2.3. Myocardial infarction models

MI modlels were induced as previously described [18]. Briefly, mice were anesthetized by intraperitoneal injection with avertin (200 mg/kg, Sigma-Aldrich Corporation, USA), and intubated with mechanical ventilation during the experiment. The left anterior descending coronary artery (LAD) was permanently ligated using a 7–0 nylon suture. In the Sham + AAV9-NC group, the mice underwent sham-operation, which comprised the same experimental procedures as the MI group, but without ligation of the LAD. Successful occlusion was confirmed by elevation of the S-T segment in lead II. The mice (n = 26) were randomly divided into three groups according to body weight: the Sham + AAV9-NC group (n = 8), the MI + AAV9-NC group (n = 9), and the MI + AAV9-si-Dectin-1 group (n = 9). Overall, only 1 mouse died during the MI surgery (from the MI + AAV9-NC group). The number of animals was based on, and modified in accordance with data from previous experiments [19]. One week later, the animals were sacrificed, and their hearts were quickly excised. The portion of left ventricle (LV) was prepared for Masson staining and other molecular experiments.

### 2.4. Echocardiographic analysis

Echocardiography was used to assess left ventricular (LV) function using a Vevo2100 echocardiographic system (Visualsonics, Toronto, Ontario, Canada) at a probe frequency of 10 MHz. One week after MI, mice were anesthetized with avertin, allowing for non-invasive examination. LV internal dimension at end-diastole (LVIDd) and LV internal dimension at systole (LVIDs) were measured at the maximal and minimal diameters, respectively. Ejection fraction (EF) and fractional shortening (FS) were detected on the M-mode tracings, and based on statistical analysis on an average of three cardiac cycles.

### 2.5. Masson's trichrome staining

Formalin-fixed (10%) paraffin-embedded mid-transverse LV sections from the hearts of MI mice or sham mice were cut in 4 μm thick slices and stained with Masson's trichrome to detect fibrosis. The Masson's Trichrome Staining was performed using a Kit (Solarbio, Beijing, China) following the manufacturer's instructions. Fibrosis fraction as a percentage of the entire section was quantified from a 20× magnification (ScanScope, Aperial Technologies, USA).

### 2.6. Neonatal mouse ventricular cardiomyocytes culture (NMCMs)

Cardiomyocytes were isolated from 1–3 day old neonatal mice using 0.25% trypsin (Solarbio, Beijing, China) as previously described [20]. Briefly, cardiomyocytes were isolated and cultured in Dulbecco's Modified Eagle Medium (DMEM) (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Beyotime, Shanghai, China), and then cultured at 37 °C in 5% $CO_2$ in a humidified incubator. After 90 min for fibroblast adherence, the cell suspension was plated into 6-well plates at $1 \times 10^6$ cells per well with DMEM. 5-bromo-2-deoxyuridine (10 μM) was added into the medium to remove fibroblasts.

### 2.7. Cell transfection and treatment

Small interfering Ribonucleic Acid (siRNA) specific for Dectin-1 (si-Dectin-1) and a scrambled negative control RNA (NC) were synthesized by RiboBio Co. Ltd (Guangzhou, China). These siRNAs were transfected at a final concentration of 50 nM into NMCMs using X-treme GENE Transfection Reagent (Roche, Basel, Switzerland) according to the manufacturer's protocol. After 24 h of transfection, the cells were exposed to hypoxia for 24 h in an anoxic chamber composed of 5% $CO_2$, and 94% $N_2$. Lipopolysaccharide (LPS) was administrated to the cells at a concentration of 1 μg/ml 24 h post-transfection. Treatment with BAY11-7082 at a concentration of 5 μM occurred 1 h before treatment with LPS. The sequence of si-Dectin-1 was 5′-GAAGATGGATATACTC AAT-3′. The negative control sequence was 5′-TTCTCCGAACGTGTCA CGTTT-3′.

### 2.8. Hoechst 33342 and propidium iodide (PI) fluorescent staining

Cardiomyocyte death was evaluated by Hoechst/PI assay. NMCMs were cultured in 24-well plates at a density of $2 \times 10^5$ cells per well. After the designated treatment, NMCMs were incubated with both Hoechst 33342 and PI (Solarbio, Beijing, China) in the dark at 4 °C for 20 min. The fluorescent signal was detected by confocal laser scanning microscopy (FV300, Olympus, Japan).

### 2.9. Western blotting

Western blot analysis was performed as previously described [21].

Proteins were extracted with a lysis buffer (Roche, Switzerland) containing 1% protease inhibitor, and 10% phosphatase inhibitor. The protein concentration was measured by incubation with bicinchoninic acid (BCA) protein kit (Beyotime Institute of Biotechnology, Shanghai, China) at 37 °C for 20 min. Equal amounts of protein were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to nitrocellulose membranes. Next, the membranes were incubated with anti-NLRP3 (#ab97051, Abcam, Cambridge, UK, 1:1000), anti-β-actin (#bs-0061R, BIOSS, Beijing, China, 1:1000), anti-Dectin-1 (#ab140039, Abcam, Cambridge, UK, 1:300), anti-Phospho-NF-κB p65 (#3033, Cell Signaling, Technology, 1:1000) overnight at 4 °C. After washing, the membrane was incubated with a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:10000, LI-COR Bioscience, Lincoln, USA) for 1 h. The intensities of the bands were quantified using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE, USA) to measure the gray-value.

### 2.10. RNA extraction and quantitative RT-PCR (qRT-PCR)

According to the manufacturer's protocol, RNA samples were extracted from the NMCMs and C57BL/6 mouse heart tissues using TRIZOL (Invitrogen, Carlsbad, CA, USA). The concentration and quality of the RNA samples were detected via NanoDrop ND-8000 (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1 μg) was reverse transcribed to cDNA using a Reverse Transcription kit (Toyobo, Japan). SYBR Green (Toyobo, Japan) was used in real-time PCR assays to quantify Dectin-1, IL-1β, IL-18, ColI, ColIII, α-SMA, CTGF mRNA levels on a 7500 FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Normalized RNA expression was calculated by comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$). Gene expressions were normalized to 18S rRNA in each sample.

### 2.11. Statistical analysis

All values are presented as means ± standard error of the mean (SEM). Student's paired two-tailed *t*-test was used for two-group comparisons, while one-way analysis of variance (ANOVA) followed by Tukey's post-hoc correction was used for multigroup comparisons. GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. A p-value $< 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Upregulation of Dectin-1 in ischemic myocardium and hypoxic NMCMs

To understand the cardiac biology of Dectin-1 under pathological alterations, we conducted a permanent MI mouse model with different time points, and measured Dectin-1 mRNA and protein expression levels. qRT-PCR results showed that Dectin-1 mRNA expression significantly increased within 7 days, and declined at 28 days post-MI (Fig. 1A). This result was further verified by Western blot analysis showing that Dectin-1 protein levels also increased and peaked after 3 days (Fig. 1B). Hypoxia is a vital event in the context of MI, which can contribute to cardiomyocytes injury [22]. In order to examine whether the increased cardiac production of Dectin-1 is derived from cardiomyocytes in the ischemic myocardium, we cultured NMCMs and subjected these cells to hypoxia. We demonstrated that Dectin-1 was remarkably up-regulated in hypoxic cardiomyocytes (Fig. 1C). These data suggest the possible involvement of Dectin-1 in MI-induced cardiomyocytes injury.

### 3.2. Knockdown of Dectin-1 alleviates cardiomyocytes injury in response to hypoxia

To investigate the role of Dectin-1 in hypoxic NMCMs, we performed PI staining to determine cardiomyocytes death. We used specific siRNA to silence Dectin-1, which elicited approximately 50% inhibition of Dectin-1 expression (Fig. 2A). As shown in Fig. 2B, hypoxia induced cardiomyocytes death, as evidenced by increased numbers in PI-positive cells, were prevented by knockdown of Dectin-1. LPS was recognized as a potent stimulator for the induction of an inflammatory response and cardiomyocytes death [23]. To further corroborate our results, the NMCMs were stimulated with 1 μg/ml LPS for 24 h. Deletion of Dectin-1 significantly reduced cardiomyocytes death induced by LPS treatment (Fig. 2C). These data suggest that Dectin-1 contributes to cardiomyocytes injury, and its inhibition yields a protective effect on cardiomyocytes injury in response to pathological stimuli.

### 3.3. Blockade of Dectin-1 inhibits NF-κB/NLRP3 signaling in cardiomyocytes

Next, we sought to elucidate the mechanism by which the inhibition of Dectin-1 attenuated cardiomyocytes death. We therefore decide to examine the expression of NF-κB and NLRP3, which were shown to aggravate cardiomyocytes injury *in vitro* [24]. Western blot analysis showed that LPS induced activation of NF-κB and NLRP3 in NMCMs, which were markedly reduced by silencing Dectin-1 (Fig. 3A–B). Similarly, Dectin-1 inhibition also suppressed the upregulation of NLRP3 protein expression in hypoxic cardiomyocytes (Fig. 3C). These results suggest that a blockade of Dectin-1 protects against cardiomyocytes injury through suppressing the activation of NF-κB and NLRP3. Some studies suggested that NF-κB could regulate the formation of the NLRP3 inflammasome [25,26]. To further uncover the relationship between NF-κB and NLRP3 in cardiomyocytes injury, we treated the cells with the NF-κB inhibitor, BAY11-7082. BAY11-7082 significantly suppressed the activation of NF-κB upon LPS stimulation (Fig. 3D). Additionally, BAY11-7082 significantly reduced LPS-induced upregulation of NLRP3, similarly resulting in decreased cardiomyocytes death (Fig. 3E–F). These data indicate that inhibition of Dectin-1 reduced cardiomyocytes injury, at least by decreasing the NF-κB-mediated transcription of NLRP3.

### 3.4. Dectin-1 silencing protects against cardiac dysfunction after MI in vivo

Our *in vitro* findings led us to hypothesize that cardiomyocytes derived Dectin-1 may play a regulatory role in MI-induced cardiac dysfunction in mice. To this end, we performed a loss-of-function study to knockdown Dectin-1 in the mouse myocardium, by administrating an AAV9 vector carrying a sequence fragment that specifically silences Dectin-1, via tail vein injection. AAV9-si-Dectin-1 was successfully delivered into the mouse myocardium, indicated by the presence of a green fluorescent signal (Fig. 4A). qRT-PCR and Western blot results further verified that AAV9-si-Dectin-1 markedly decreased cardiac Dectin-1 expression induced by MI (Fig. 4B − C). To figure out whether inhibition of Dectin-1 can improve cardiac function in MI-treated mouse hearts, we carried out echocardiographic analysis. The results showed that MI led to significantly decreased EF% and FS%, whereas these effects were blunted by deletion of Dectin-1 (Fig. 4D–F). Moreover, Dectin-1 inhibition also attenuated cardiac dilatation, as indicated by decreased left ventricular diameters in diastole and systole as compared to the MI group (Fig. 4G–H). To corroborate our *in vitro* data, we also measured the expressions of NLRP3 and pro-inflammatory genes involved in myocardial injury and remodeling. Our results also demonstrated that blockade of Dectin-1 markedly reduced MI-induced upregulation of NLRP3 protein expression, and interleukin (IL)-1β and IL-18 mRNA levels (Fig. 4I–K).

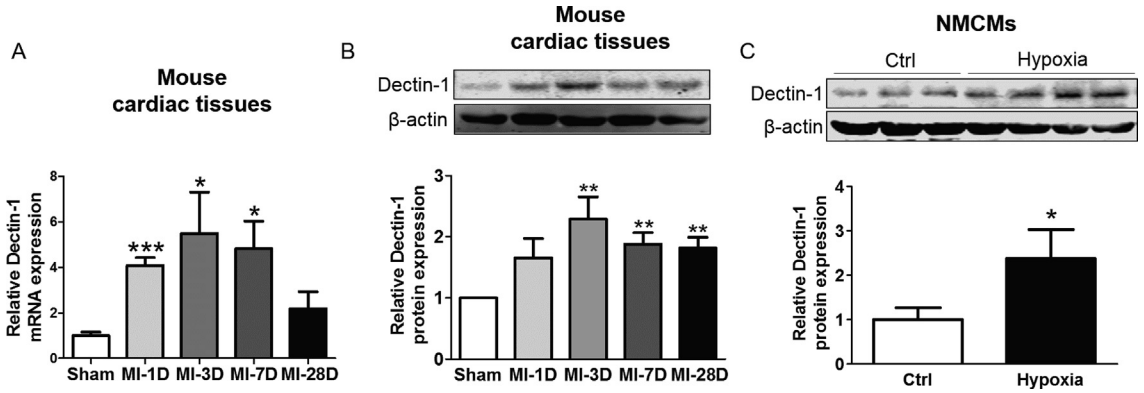


**Fig. 1.** Expression of Dectin-1 in the infarcted myocardium and hypoxic NMCMs. (A) qRT-PCR analysis of Dectin-1 mRNA expression at different time points after MI. 18S rRNA served as an internal control. $n = 5$–6. (B) Western blot analysis of Dectin-1 protein expression at different time points after MI. $n = 5$. Protein expression was normalized to β-actin. (C) Western blot analysis of Dectin-1 protein expression in cardiomyocytes exposed to hypoxia. $n = 3$–4. Protein expression was normalized to β-actin. Data are represented as mean ± SEM; $^{*}P < 0.05$ *vs.* Ctrl, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Sham.

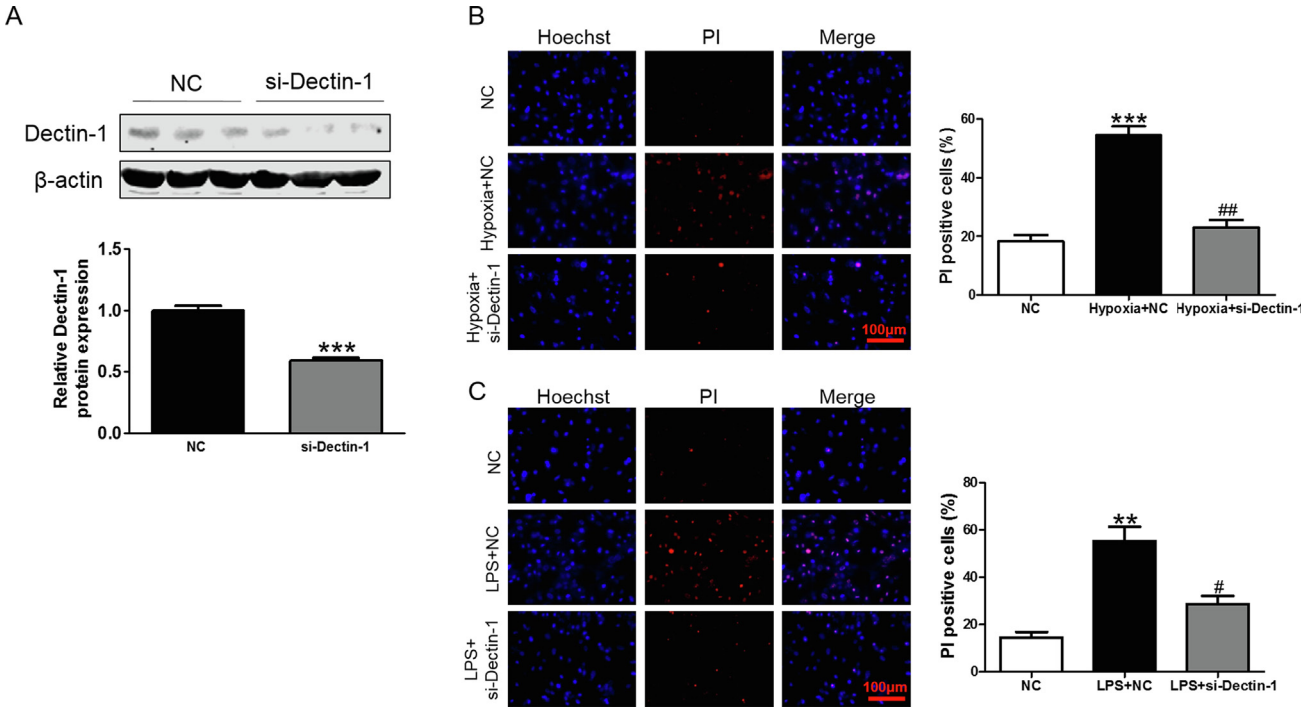


**Fig. 2.** Effects of silencing Dectin-1 on cell death in NMCMs. (A) Dectin-1 protein expression after transfecting with 50 nM si-Dectin-1 in NMCMs. $n = 3$. $^{***}P < 0.001$ *vs.* NC. (B) Representative images and quantitative analysis of Hoechst 33342/PI positive cells in cardiomyocytes. NMCMs were treated with 50 nM si-Dectin-1 for 24 h and then exposed to hypoxic condition for 24 h. The nuclei were stained with Hoechst 33342 (blue), and cells with a ruptured membrane were stained with PI (red). Scale bar = 100 μm. $n = 3$. $^{***}P < 0.001$ *vs.* NC; $^{\#\#}P < 0.01$ *vs.* Hypoxia + NC. (C) Representative images and quantitative analysis of Hoechst 33342/PI positive cells in cardiomyocytes. NMCMs were treated with 50 nM si-Dectin-1 for hours and then administrated with 1 μg/ml LPS for 24 h. The nuclei were stained with Hoechst 33342 (blue), and cells with a ruptured membrane were stained with PI (red). Scale bar = 100 μm. $n = 3$. $^{**}P < 0.01$ *vs.* NC; $^{\#}P < 0.05$ *vs.* LPS + NC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### *3.5. Inhibition of Dectin-1 attenuates cardiac fibrosis in the MI hearts*

Next, histological sections were stained with Masson's trichrome to investigate the effect of Dectin-1 on cardiac fibrosis. MI caused a significant increase in cardiac fibrosis as compared to the sham + NC group, which was significantly diminished by AAV9-si-Dectin-1 treatment (Fig. 5A–B). In further verifying this finding, we determined the expression of the genes related to fibrogenesis using qPCR. MI induced a significant increase in Collagen (ColI and ColIII) mRNA expression, but this was reversed by the knockdown of Dectin-1 (Fig. 5C–D). Furthermore, alpha-smooth muscle actin (α-SMA), an indicator of cardiac fibroblast activation and myofibroblast transformation, was induced in the infarcted myocardium but decreased in AAV9-si-Dectin-1 treated hearts (Fig. 5E). Meanwhile, Co-treatment with AAV9-si-Dectin-1 prominently reduced connective tissue growth factor (CTGF) expression as compared to MI mouse; CTGF is involved in extracellular cardiac remodeling (Fig. 5F). These data support an important role of Dectin-1 in the development of cardiac fibrosis post-MI.

## 4. Discussion

Our findings demonstrated that the innate immune response driven by Dectin-1 is causally linked to the myocardial injury associated with MI. We revealed that Dectin-1 expression is increased in cardiomyocytes by MI or hypoxia stimuli. Deletion of Dectin-1 attenuates cardiomyocytes death in response to hypoxia or LPS *in vitro*. Furthermore,

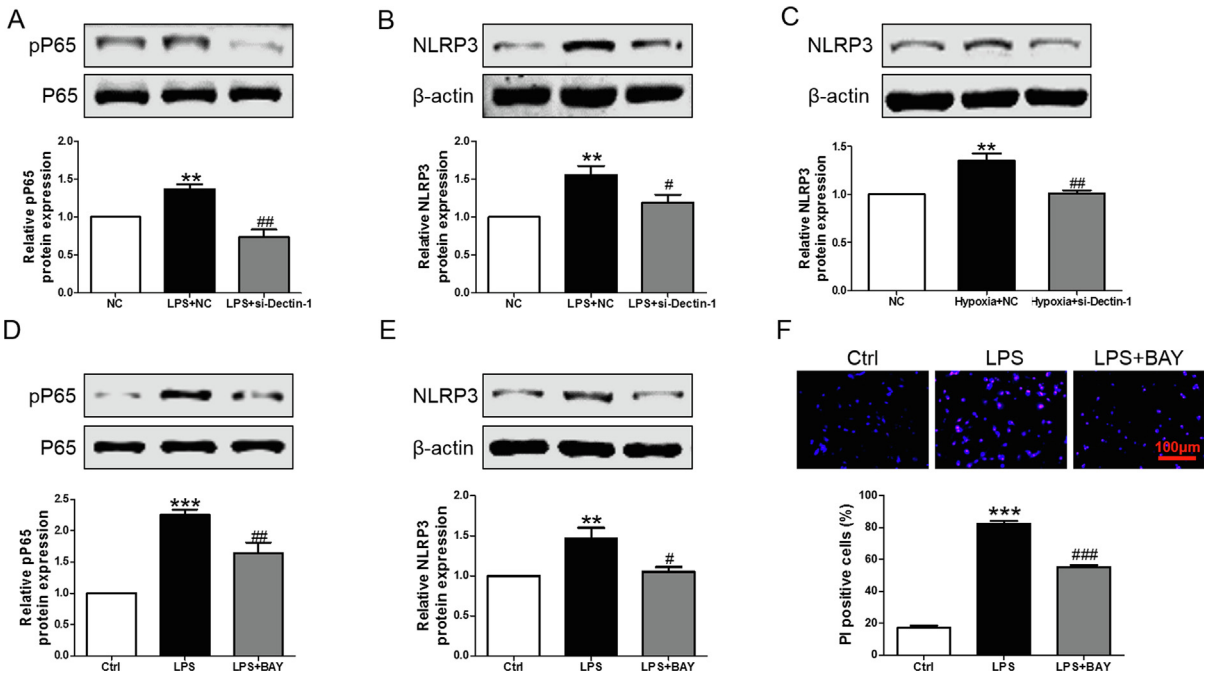


**Fig. 3.** Knockdown of Dectin-1 attenuates activation of NF-κB/NLRP3 signaling in NMCMs. (A–B) Western blot images and analysis of phosphorylated NF-κB (pP65) and NLRP3 in LPS treated NMCMs. Protein expression was normalized to β-actin. $n = 4$–6. $^{**}P < 0.01$ *vs.* NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* LPS + NC. (C) Western blot images and analysis of protein level of NLRP3 in NMCMs subjected to hypoxia for 24 h. Protein expression was normalized to β-actin. $n = 5$. $^{**}P < 0.01$ *vs.* NC; $^{\#\#}P < 0.01$ *vs.* Hypoxia + NC. (D-E) Western blot images and analysis of phosphorylated NF-κB (pP65) and NLRP3 in NMCMs were pretreated with 5 μM BAY11-7082 for 1 h prior to 1 μg/ml LPS treatment for 24 h. Protein expression was normalized to β-actin. $n = 5$–6. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Ctrl; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* LPS. (F) Representative images of Hoechst 33342/PI staining and quantitative analysis of PI-positive cardiomyocytes from each group. The nuclei were stained with Hoechst 33342 (blue), and cells with a ruptured membrane were stained with PI (red). Scale bar = 100 μm. $n = 4$. $^{***}P < 0.001$ *vs.* Ctrl; $^{\#\#\#}P <$ 0. 001 *vs.* LPS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inhibition of Dectin-1 in the mouse heart reduces cardiac inflammation, fibrosis and improves cardiac function upon permanent MI, along with depressed activation of NF-κB and NLRP3 signaling.

Dectin-1, a C-type lectin receptor (CLR) that is highly expressed in macrophages, and other myeloid-derived cells [27], which has been well recognized to play vital roles in innate immunity against fungal infections [28]. Dectin-1 deficiency was shown to be associated with a high risk for developing vulvovaginal candidiasis in humans [29]. During the course of our study, a finding reported that Dectin-1 was elevated in the infarcted myocardium, reaching a peak at 24 h, thereafter returning to baseline level after 3 days in response to cardiac ischemia/reperfusion (I/R) [30]. They showed that increased Dectin-1 expression is mainly from bone marrow-derived macrophages that infiltrate into the heart, and further study indicated by using genetic knockout mouse model, that deficiency of Dectin-1, and Dectin-1 neutralizing antibodies can significantly improve cardiac function and reduce cardiomyocytes apoptosis by suppressing macrophage polarization to the M1 phenotype, increased and neutrophil recruitment. In addition, the same group also demonstrated Dectin-2, another CLR, was mainly expressed in macrophage and upregulated in the early phase permanent MI hearts [31]. In contrast to Dectin-1, Dectin-2 results in cardiac rupture and induces cardiac remodeling by modulating Th1 differentiation instead of controlling macrophage polarization post-MI [31]. Our results showed the similar findings that Dectin-1 protein level was up-regulated in the permanent MI mouse heart. Since cardiomyocytes constitute the most important cells of the heart, and their loss is a crucial determinant of cardiac remodeling after MI [32,33]. This led us to hypothesize whether Dectin-1 is expressed in cardiomyocytes and affected by MI. To test our hypothesis, we cultured NMCMs to measure Dectin-1 expression under pathological conditions. Interestingly, our study demonstrated that Dectin-1 was markedly increased in hypoxia or LPS stimulated NMCMs, suggesting that in addition to macrophages, the cardiomyocytes are also a major source of the elevated Dectin-1 expression observed in the heart post-MI. Another difference compared with I/R study is that we observed Dectin-1 was progressively up-regulated from 1 day to 3 days post-MI. One possible explanation for this observation is that the permanent MI caused much severe damage in cardiomyocytes and sustained stronger macrophages infiltration [3], which collectively contributed to Dectin-1 expression. Our study showing Dectin-1 expression does not further increase 3 days post-MI can be accounted for the decline in the number of macrophage populations and the fact that more cardiomyocytes are lost over time [34]. Furthermore, we proposed the regulatory roles of cardiomyocyte-derived Dectin-1 in the heart using an animal model of MI and a cellular model of hypoxia. We demonstrated the knockdown of Dectin-1 reduced cardiomyocytes injury as compared to LPS or hypoxia-treated cells. Since AAV9 preferentially transduced into cardiac myocyte we undertook the loss of function study to block Dectin-1 expression in cardiomyocytes post-MI. Our *in vivo* study demonstrated that Dectin-1 inhibition in cardiomyocytes led to a significant improvement in cardiac function in the MI-treated mouse heart. Our *in vivo* and *in vitro* studies provided a novel insight into the function and role of Dectin-1 in cardiomyocytes in regulating myocardial injury and heart function.

Dectin-1 regulates the inflammatory response through its ITAM-like motifs, thereby recruiting and activating spleen tyrosine kinase, along with the subsequent activation of the CARD9–Bcl10–Malt1 (CBM) scaffold [35]. Activation of this pathway by Dectin-1 can lead to the activation of NF-κB, that promotes transcription of pro-inflammatory cytokines [36]. It was reported that Dectin-1 is able to regulate numerous cellular responses involves phagocytosis, autophagy, the respiratory burst by inducing the production of some cytokines including IL-6 and IL-1β [37]. NF-κB is a key transcription factor that promotes pro-inflammatory responses by inducing cytokine transcription upon MI, and the blockade of NF-κB has gained the beneficial effects on

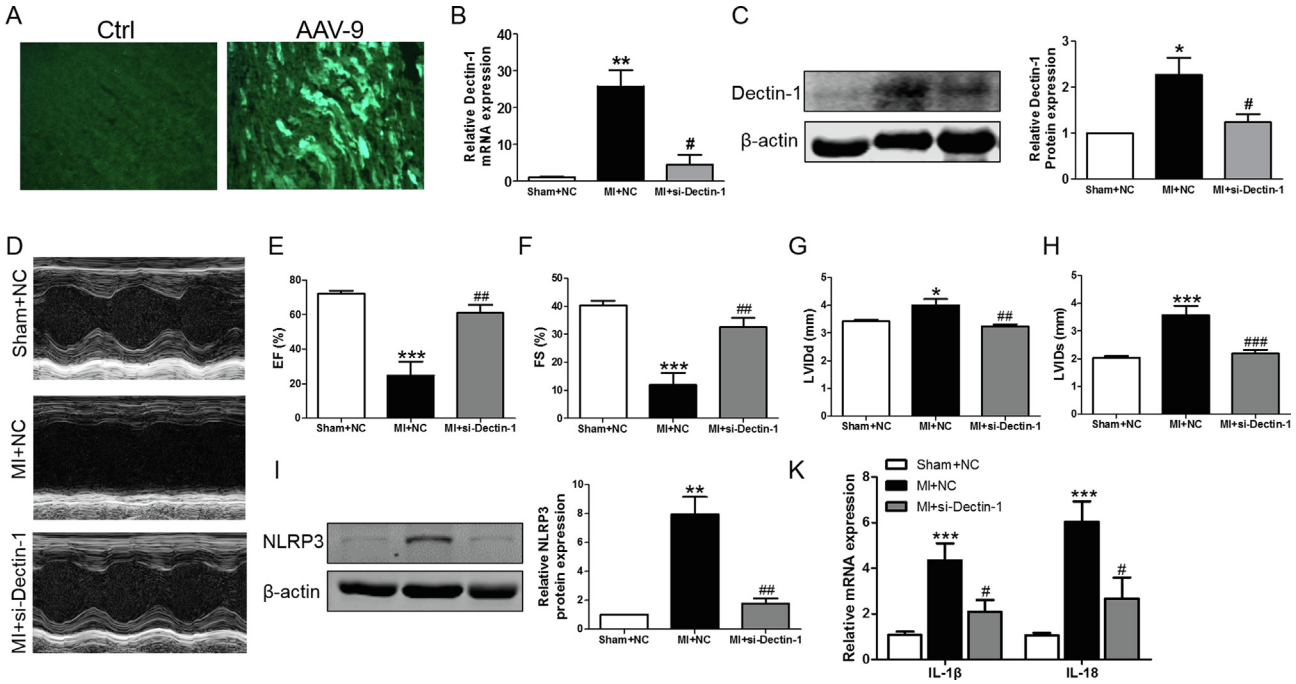


**Fig. 4.** Deletion of Dectin-1 improves cardiac function *in vivo*. (A) Representative cardiac sections showing the successful delivery of si-Dectin-1 into mouse myocardium after 3 weeks *in vivo*, which was indicated the significant presence of fluorescence elicited by GFP attached to the AAV9-NC. (B) qRT-PCR analysis of Dectin-1 mRNA expression measured in LV at 1 week post-MI in AAV9-si-Dectin-1 or AAV9-NC treated mice hearts. 18S rRNA served as an internal control. $n = 3$–4. (C) Western blot analysis of Dectin-1 protein level at 1 week post-MI in AAV9-si-Dectin-1 or AAV9-NC treated hearts. Protein expression was normalized to β-actin. $n = 4$. (D–H) Cardiac functional parameters was measured using echocardiography 1 week after MI in AAV9-si-Dectin-1 or AAV9-NC treated hearts, including ejection fraction (EF%), fractional shortening (FS%), LV internal dimension at end-diastole (LVIDd), and LV internal dimension at systole (LVIDs). $n = 8$–9. (I) Western blot analysis of NLRP3 protein level at 1 week after MI in AAV9-si-Dectin-1 or AAV9-NC treated hearts. $n = 4$. Protein expression was normalized to β-actin. (K) qRT-PCR analysis of IL-1β, IL-18 mRNA expressions in mouse hearts. 18S rRNA served as an internal control. $n = 6$–8. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Sham + NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* MI + NC.

attenuation of cardiac remodeling and improvement on cardiac function [38]. LPS was shown to induce inflammation and injury of cardiomyocytes by activating NF-κB both *in vivo* and *in vitro* [23,24,39]. Our *in vitro* study indicated that LPS induced the upregulation of NF-κB, which was significantly reversed by silencing Dectin-1. The same was true for our *in vivo* data, where AAV9-si-Dectin-1 mediated inhibition of Dectin-1 in the myocardium reduced both IL-1β and IL-18 expressions. These data can explain why we observed that inhibition of Dectin-1 reduces cardiomyocytes apoptosis, inflammation and confers protective effects on cardiac function.

It has been reported that Dectin-1 can also activate NLRP3/caspase1 in a Syk-dependent manner, thereby inducing the generation of IL-1β

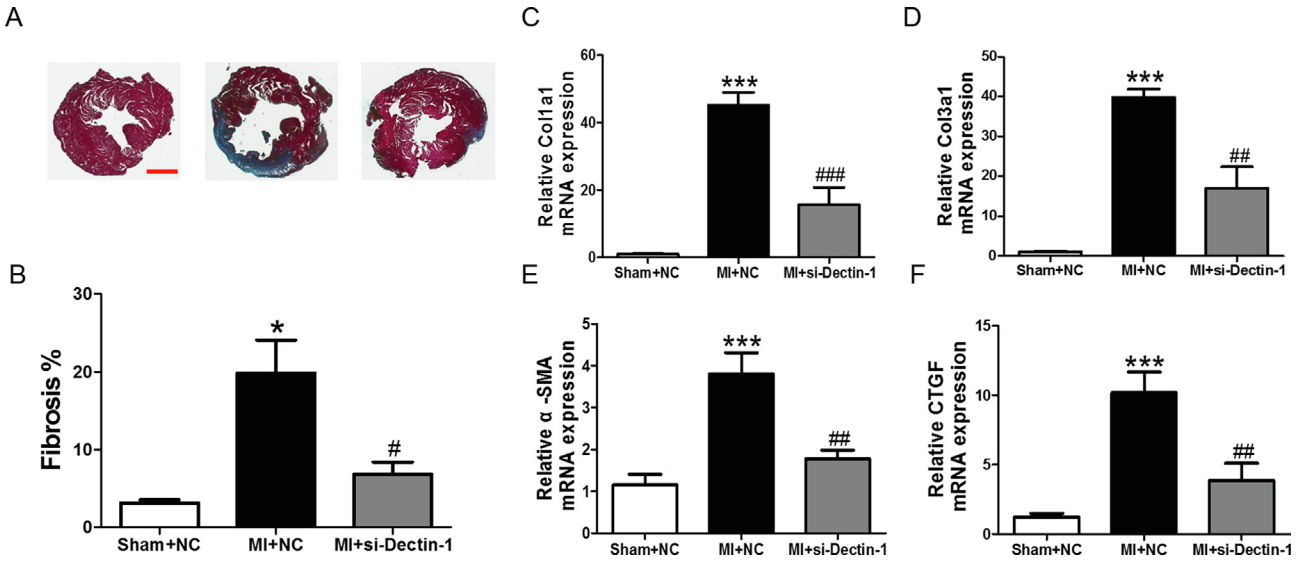


**Fig. 5.** Inhibition of Dectin-1 ameliorates cardiac fibrotic remodeling 1 week after MI. (A–B) Representative images and quantitative analysis of Masson's trichrome stained heart sections in the AAV9-si-Dectin-1 or AAV9-NC treated hearts at 1 week post-MI. Scale bar = 1 mm. $n = 3$–4. (C–F) qRT-PCR analysis of Col1α1, Col3α1, α-SMA and CTGF mRNA expressions at 1 week post-MI in AAV9-si-Dectin-1 or AAV9-NC treated hearts. 18S rRNA served as an internal control. $n = 8$. $^{*}P < 0.05$, $^{***}P < 0.001$ *vs.* Sham + NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* MI + NC.

[37,40]. NLRP3 is one of the most well studied inflammasomes, which was reported to amplify the inflammatory response to aggravate myocardial injury upon MI by inducing the production of IL-1β and IL-18 [41,42]. Inhibition of NLRP3 has been shown to reduce infarct size and protect against cardiac dysfunction in some experimental MI models [43,44]. Our data showed that Dectin-1 silencing in cardiomyocytes significantly decreased the elevation in NLRP3 expression both *in vivo* and *in vitro*, indicating its therapeutic potential for ischemic heart disease. This was supported by our recent publication showing that inhibition of NLRP3 expression in cardiomyocytes ameliorated pyroptotic cell death [24]. Considering the similar expression pattern of NF-κB and NLRP3 after inhibiting Dectin-1 as well as the previous studies reported that NF-κB could regulate NLRP3 inflammasome in epithelia and cancer cells [45,46], we asked ourselves whether there exits such a regulatory mechanism in cardiomyocytes. Therefore, we performed additional studies by using BAY11-7082, an inhibitor of NF-κB to test our hypothesis. Our results indicated that BAY11-7082 significantly reduced cardiomyocytes injury and NLRP3 expression induced by LPS. Our study also supported a novel role of Dectin-1, where it controls myocardial injury and remodeling by regulating NLRP3 expression through NF-κB signaling pathway.

Dectin-1 functions as a regulator of immunity by binding its ligand, β-glucans [8]. In addition to β-glucans, another novel ligand of Dectin-1 has been revealed to promote pancreatic carcinoma and peritumoral immune tolerance once ligation of receptor-ligand [17]. Although a previous study supports our conclusion that Dectin-1 promotes myocardial injury at least by inducing NF-κB activation [30], we both failed to uncover the ligand of Dectin-1 in cardiomyocytes and macrophages post-MI. Further studies are needed to explore this underlying mechanism. Our findings showing that silencing Dectin-1 reduced fibrotic scarring and related genes, which are probably as a result of the resolution of inflammation. Whether Dectin-1 can directly regulate fibroblast activation is worthy of investigating. A study challenged the common consensus of inflammation-driven action of Dectin-1 by ours and other studies. They reported the opposite effects that Dectin-1 activation exerted protective effects on liver fibrosis, and sterile inflammation, and LPS induced sepsis, by suppressing TLR4 expression and consequent activation, thereby reducing liver injury and remodeling [15]. But they failed to uncover the mechanism by which Dectin-1 suppressed TLR4 activation in liver tissue. This study implies that Dectin-1 might function in a tissue, cell or even disease-specific manner. Since TLR4 activation was shown to exacerbate myocardial injury by activating NF-κB signaling post-MI [47], we have to figure out whether Dectin-1 can affect TLR4 expression in the ischemic myocardium and the underlying mechanism.

In conclusion, our study characterized the role of Dectin-1 in cardiac remodeling and function in mice with MI. We found that Dectin-1 was up-regulated in cardiomyocytes in the post-MI mouse heart, aggravating cardiomyocytes death, the cardiac inflammatory response, and fibrosis, further deteriorating cardiac dysfunction. Dectin-1 could result in NF-κB/NLRP3 axis activation, contributing to enhancing myocardial injury. We observed the beneficial effects by knocking-down Dectin-1 *in vivo* and *in vitro*, identifying Dectin-1 as a novel therapeutic target for ischemic cardiac disease.

## Author contributions

WJD, XL and BY designed the present study; XL, BY, STY, PP, XZW, YLG, YLG, QL and KWL performed the experiments; XL, BY and STY analyzed the data; LX, WJD wrote the draft; WJD and YY revised paper.

## CRediT authorship contribution statement

**Xin Li:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Yu Bian:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Shuting Yu:** Investigation. **Ping Pang:** Investigation. **Xiuzhu Wang:** Investigation. **Yuelin Gao:** Investigation. **Kuiwu Liu:** Investigation. **Qian Liu:** Investigation. **Ye Yuan:** Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision. **Weijie Du:** Conceptualization, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition, Supervision.

## Declaration of Competing Interest

The authors have declared that there are no conflicts of interest.

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