Dual specificity phosphatase 1 attenuates inflammation-induced cardiomyopathy by improving mitophagy and mitochondrial metabolism

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ABSTRACT

Objectives: Dual specificity phosphatase 1 (DUSP1) is regarded as an anti-inflammatory factor in cardiovascular disorders. Mitophagy removes damaged mitochondria and thus promotes mitochondrial regeneration. We investigated whether DUSP1 could attenuate inflammation-induced cardiomyopathy by improving mitophagy.

Methods: Lipopolysaccharide was used to induce septic cardiomyopathy in wild-type (WT) and DUSP1 transgenic (DUSP1^TG) mice.

Results: Echocardiography revealed that lipopolysaccharide impaired heart function by reducing the cardiac systolic and diastolic capacities of WT mice. Freshly isolated single cardiomyocytes from lipopolysaccharide-treated WT mice also exhibited reduced contractile/relaxation parameters. However, DUSP1 overexpression not only maintained the mechanical properties of cardiomyocytes, but also improved heart performance. Lipopolysaccharide upregulated myocardial inflammatory gene transcription and adhesive factor expression, which increased myocardial neutrophil accumulation and cardiomyocyte apoptosis in WT mice. DUSP1 overexpression inhibited the inflammatory response and therefore promoted cardiomyocyte survival. Lipopolysaccharide disrupted mitochondrial respiration and metabolism in WT cardiomyocytes, but DUSP1 overexpression restored mitochondrial metabolism, maintained the mitochondrial membrane potential and inhibited mitochondrial reactive oxygen species production, possibly by increasing FUN14 domain-containing 1 (FUNDC1)-dependent mitophagy. Silencing of *FUNDC1* abolished the protective effects of DUSP1 overexpression on cardiomyocytes and their mitochondria following lipopolysaccharide treatment.

Conclusion: These results demonstrated that DUSP1 is a novel anti-inflammatory factor that protects against septic cardiomyopathy by improving FUNDC1-induced mitophagy.

Keywords DUSP1; Septic cardiomyopathy; Mitochondria; Mitophagy; FUNDC1

1. INTRODUCTION

Septic cardiomyopathy (SCM) is characterized by acute myocardial depression with reduced left ventricular systolic function. Although abnormal immune responses (e.g., cytokine release, complement system induction and inflammatory cell activation) have been regarded as initial signals of sepsis-induced myocardial injury, numerous clinical observations and animal studies have demonstrated that mitochondrial damage also contributes to the pathogenesis of SCM [1,2]. Striking functional and morphological alterations of mitochondria have been detected during SCM, including reduced mitochondrial adenosine triphosphate (ATP) output [3], disturbed mitochondrial metabolism [4], increased mitochondrial oxidative stress [5], augmented mitochondrial

fission [6], defective mitophagy [7], delayed mitochondrial biogenesis [8] and depolarization of the mitochondrial membrane [9]. Severe mitochondrial damage due to inner and/or outer membrane permeabilization can unleash the enzymatic apoptotic machinery of caspases and induce chromatin degradation, culminating in cardiomyocyte death. Although the pathological changes in mitochondria during SCM have been well investigated, the molecular pathways that induce mitochondrial dysfunction and consequently sensitize cardiomyocytes to apoptosis have not been characterized.

Our previous studies [10–16] have demonstrated that mitophagy attenuates various cardiovascular disorders. Mitophagy was suppressed in the setting of cardiac ischemia/reperfusion injury, whereas the activation of mitophagy reduced the infarct size and normalized heart

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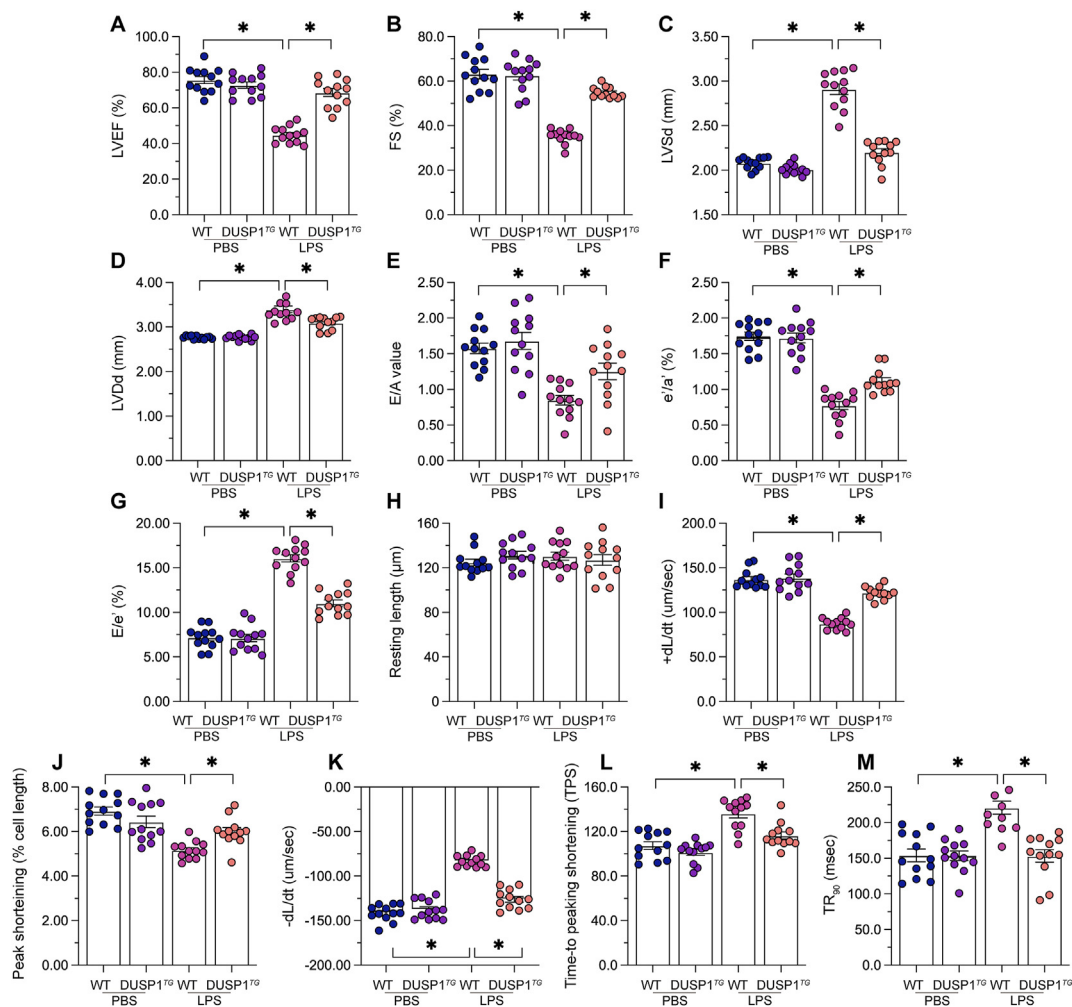**Corresponding author. E-mail: chenwenting326@126.com (W. Chen).

Figure 1: DUSP1 preserves heart function *in vivo* and *in vitro*. DUSP1^{TG} and WT mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg) to induce SCM, and were evaluated after 48 h. **A-G.** Transthoracic two-dimensional B-mode echocardiography was performed to analyze alterations in heart function. **H-M.** Freshly isolated single cardiomyocytes from WT or DUSP1^{TG} mice were used to analyze changes in cardiomyocyte contractile parameters. *$p < 0.05$.

function [17]. Chronic hyperglycemia was associated with mitophagy inactivation and a reduced cardiac relaxation capacity [12]. On the other hand, the upregulation of mitophagy preserved heart function during obesity-associated cardiomyopathy [18]. In accordance with our findings, other studies have also indicated that mitophagy exerts cardioprotective effects by alleviating mitochondrial damage and improving heart performance. Mitophagy activation retarded heart failure through a mechanism involving phosphatase and tensin homolog-induced kinase 1 phosphorylation [19]. Improved mitophagy was found to reduce cardiomyocyte death in doxorubicin-induced cardiotoxicity [20]. However, the involvement of mitophagy and its regulatory network in SCM remains unclear.

Dual specificity phosphatase 1 (DUSP1) is an enzyme that can remove phosphate groups from both tyrosine and threonine residues. We previously reported that DUSP1 overexpression inhibited mitophagy by dephosphorylating/deactivating BCL2 interacting protein 3 in the setting of cardiac ischemia/reperfusion injury [21]. In a subsequent study, DUSP1 overexpression was found to activate mitophagy during diabetic nephropathy [22]. Interestingly, there is ample evidence that DUSP1 is involved in sepsis: for instance, increased DUSP1 expression was shown to suppress inflammation in septic lung injury [23] and to protect

against sepsis-induced myocardial injury by repressing the nuclear factor κB pathway [24]. Based on these findings, we explored whether DUSP1 overexpression could reduce SCM by restoring mitophagy.

2. RESULTS

2.1. DUSP1 preserves heart function *in vivo* and *in vitro*

In the present study, we used lipopolysaccharide to induce septic cardiomyopathy in wild-type (WT) and DUSP1 transgenic (DUSP1^{TG}) mice. We then applied echocardiography to observe changes in heart function. In WT mice, lipopolysaccharide treatment significantly impaired systolic indexes such as the left ventricular ejection fraction (LVEF), fractional shortening (FS) and left ventricular systolic dimension (LVSd) (Figure 1A—G). Lipopolysaccharide also reduced diastolic indexes such as the ratio of early to late transmitral flow velocities (E/A), ratio of diastolic mitral annular velocities (e'/a') and ratio of mitral peak velocity of early filling to early diastolic mitral annular velocity (E/e') and left ventricular diastolic dimension (LVDd) in WT mice (Figure 1A—G). Interestingly, the cardiac systolic and diastolic capacities were restored to near-normal levels in DUSP1^{TG} mice during lipopolysaccharide-induced SCM (Figure 1A—G).
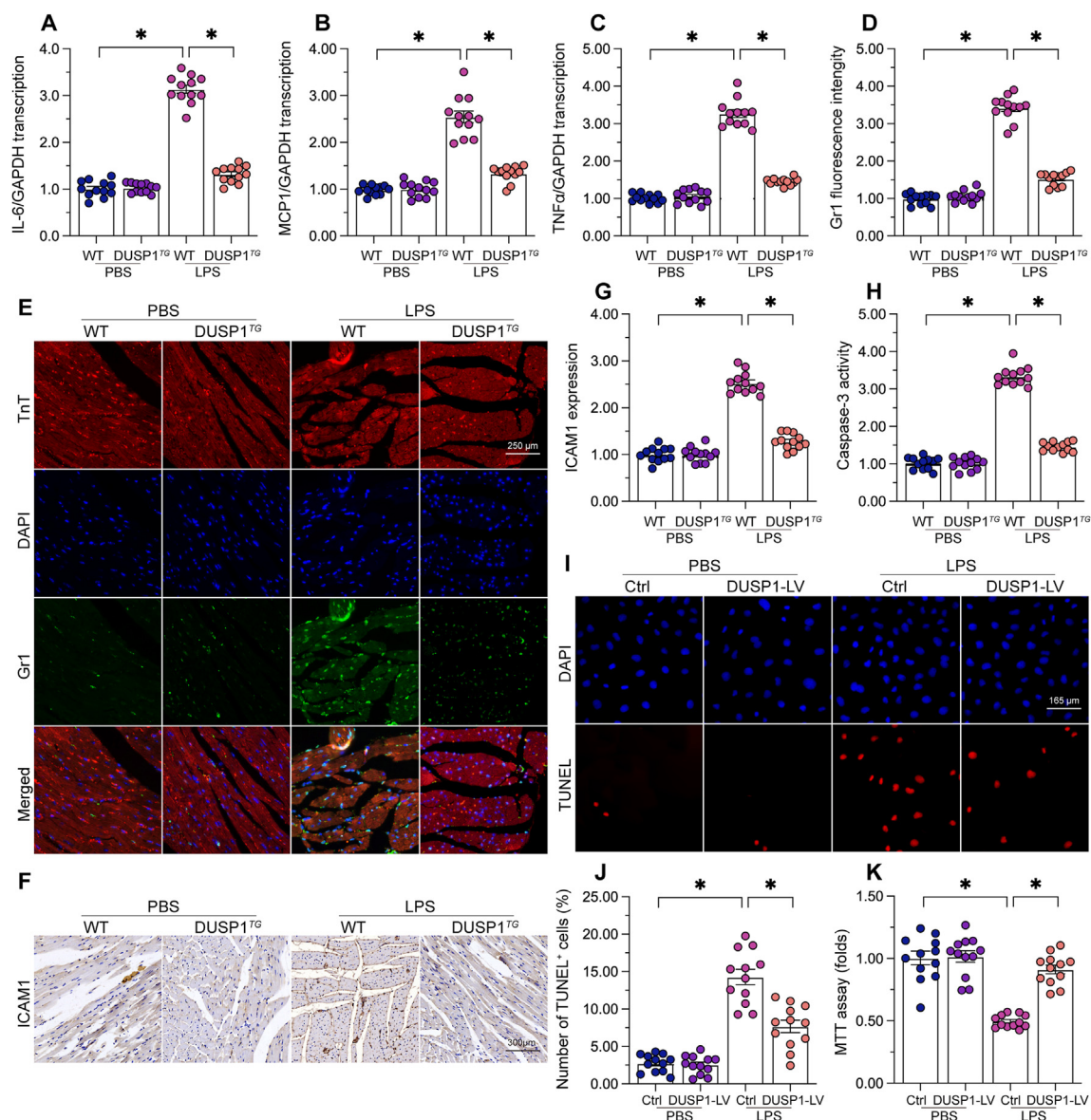
Figure 2: DUSP1 attenuates the inflammatory response and cardiomyocyte death. DUSP1^{TG} and WT mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg) to induce SCM, and were evaluated after 48 h. HL-1 cells were treated with 10 µg/mL lipopolysaccharide for 24 h to induce inflammatory cardiomyocyte damage. HL-1 cells were transfected with the DUSP1-LV to overexpress DUSP1 before lipopolysaccharide treatment. **A-C.** RNA was collected and used for qPCR. The relative levels of *IL-6*, *TNFα* and *MCP1* were normalized to *GAPDH* levels. **D, E.** Immunofluorescence staining was used to assess the expression of Gr1, a neutrophil cell-surface marker. TnT was used to label the myocardium. **F, G.** Immunohistochemistry was applied to observe changes in ICAM1 expression in the myocardium. **H.** An ELISA was used to measure caspase-3 activity in heart tissues. **I, J.** TUNEL staining was applied to observe the number of apoptotic cardiomyocytes. **K.** An MTT assay was used to determine the viability of cardiomyocytes in the presence of lipopolysaccharide. *p < 0.05.

We then analyzed contractile parameters in freshly isolated single cardiomyocytes from WT and DUSP1^{TG} mice. Lipopolysaccharide treatment had no influence on the resting cardiomyocyte length (Figure 1H—M). However, parameters related to the cardiomyocyte contractile capacity, such as the peak shortening (PS), maximal velocity of shortening (+dL/dt) and time to peak shortening (TPS), were blunted by lipopolysaccharide in WT cardiomyocytes, but not in DUSP1^{TG} cardiomyocytes (Figure 1H—M). Lipopolysaccharide also repressed relaxation parameters in WT cardiomyocytes, such as the maximal velocity of relengthening (-dL/dt) and time to 90% relengthening (TR90); however, these changes were not observed in DUSP1^{TG}

cardiomyocytes (Figure 1H—M). These results suggested that DUSP1 overexpression preserves heart function.

2.2. DUSP1 attenuates the inflammatory response and cell death in cardiomyocytes

The inflammatory response has been regarded as a key contributor to lipopolysaccharide-induced myocardial injury. Therefore, we used quantitative PCR (qPCR) to analyze myocardial inflammation in the different groups of mice. Lipopolysaccharide exposure significantly elevated the transcription of interleukin 6 (*IL-6*), tumor necrosis factor alpha (*TNFα*) and monocyte chemoattractant protein 1 (*MCP1*) in heart
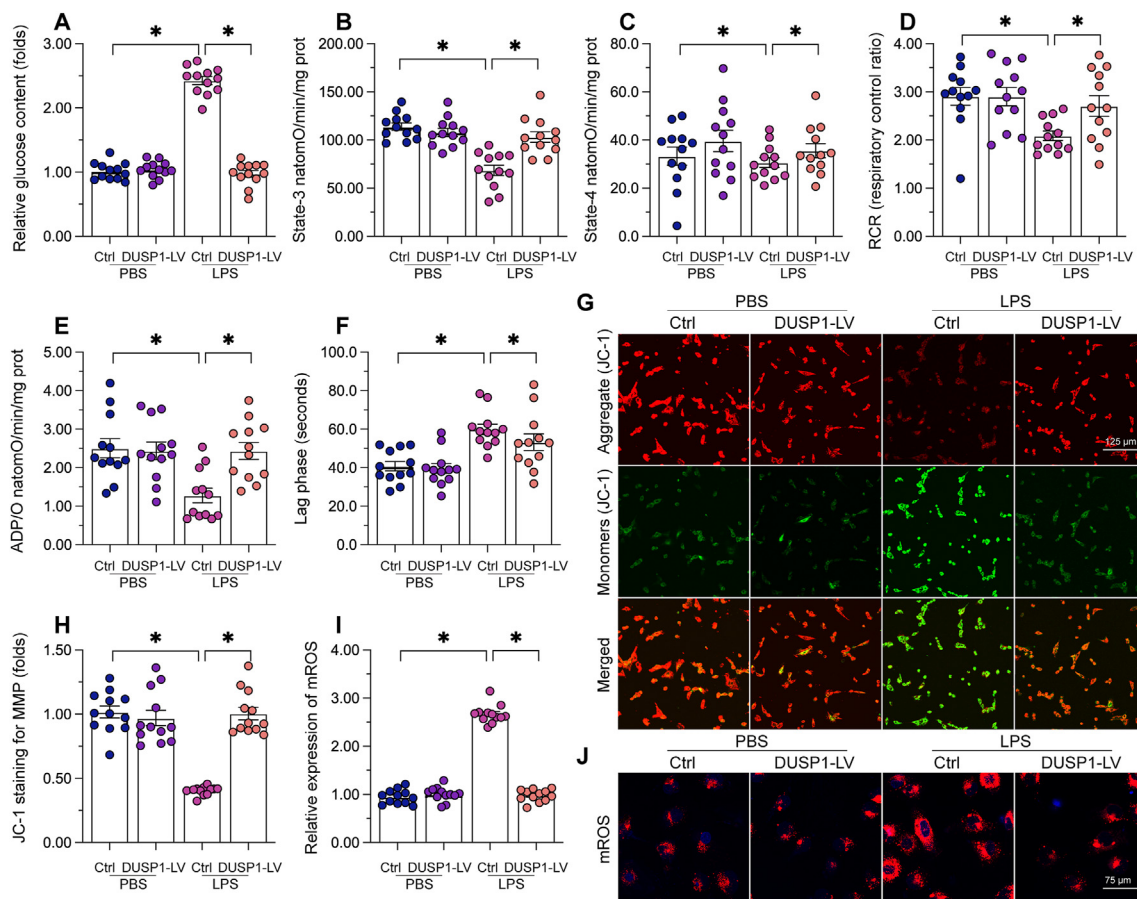
Figure 3: DUSP1 improves lipopolysaccharide-disrupted cardiomyocyte mitochondrial function. DUSP1^{TG} and WT mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg) to induce SCM, and were evaluated after 48 h. HL-1 cells were treated with 10 μg/mL lipopolysaccharide for 24 h to induce inflammatory cardiomyocyte damage. HL-1 cells were transfected with the DUSP1-LV to overexpress DUSP1 before lipopolysaccharide treatment. **A.** The concentration of glucose in the medium was determined through an ELISA. **B-F.** The OCR was used to analyze mitochondrial respiration in the presence of lipopolysaccharide. State-3/4 mitochondrial respiration was recorded, along with the RCR, ADP/O and lag phase. **G, H.** The mitochondrial membrane potential was measured with the JC-1 probe. **I, J.** Mitochondrial ROS production was determined via immunofluorescence. *p < 0.05.

tissues from WT mice (Figure 2A−C). Due to the inflammatory response, the number of neutrophils also increased in WT heart tissues, as evidenced by the increased fluorescence intensity of the neutrophil cell-surface marker Gr1 (Figure 2D,E). Interestingly, overexpression of DUSP1 prevented the transcriptional upregulation of these inflammatory factors (Figure 2A−C) and the accumulation of neutrophils (Figure 2D,E) in heart tissues following lipopolysaccharide treatment. Moreover, the adhesive factor intercellular adhesion molecule 1 (ICAM1) was upregulated in the myocardium in lipopolysaccharide-treated WT mice, while DUSP1 overexpression reduced ICAM1 expression (Figure 2F,G), which may have contributed to the reduced neutrophil infiltration and cytokine transcription in the DUSP1^{TG} heart.

Abnormal inflammatory responses have been associated with increased cardiomyocyte damage, including apoptosis. We found that caspase-3 activity was significantly elevated in heart tissues from lipopolysaccharide-treated WT mice (Figure 2H). *In vitro,* lipopolysaccharide treatment also augmented the number of apoptotic HL-1 cardiomyocytes (Figure 2I,J). Overexpression of DUSP1 prevented caspase-3 activation *in vivo* (Figure 2H) and therefore reduced the number of apoptotic cardiomyocytes *in vitro* (Figure 2I,J) following lipopolysaccharide treatment. Similarly, a 3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide (MTT) cell viability assay revealed that lipopolysaccharide promoted cardiomyocyte death, while a DUSP1 lentivirus (DUSP1-LV) reversed this effect (Figure 2K). These results indicated that DUSP1 can attenuate the inflammatory response and cardiomyocyte death following lipopolysaccharide treatment.

2.3. DUSP1 improves lipopolysaccharide-disrupted mitochondrial function

Impaired mitochondrial function has been identified as a downstream event in response to lipopolysaccharide-induced SCM [1,25,26]. Thus, we assessed whether DUSP1 could protect cardiomyocyte mitochondria against lipopolysaccharide-induced stress. The primary function of mitochondria is to consume glucose and produce ATP. We used an enzyme-linked immunosorbent assay (ELISA) to measure glucose levels in media from HL-1 cardiomyocytes treated with lipopolysaccharide and/or the DUSP1-LV. As shown in Figure 3A, media from lipopolysaccharide-treated HL-1 cardiomyocytes contained significantly higher glucose levels than media from control cells, while this effect was abolished in cells transfected with the DUSP1-LV. Due to impaired glucose metabolism, ATP production in cardiomyocytes was reduced after exposure to lipopolysaccharide, possibly due to blunted state-3 and state-
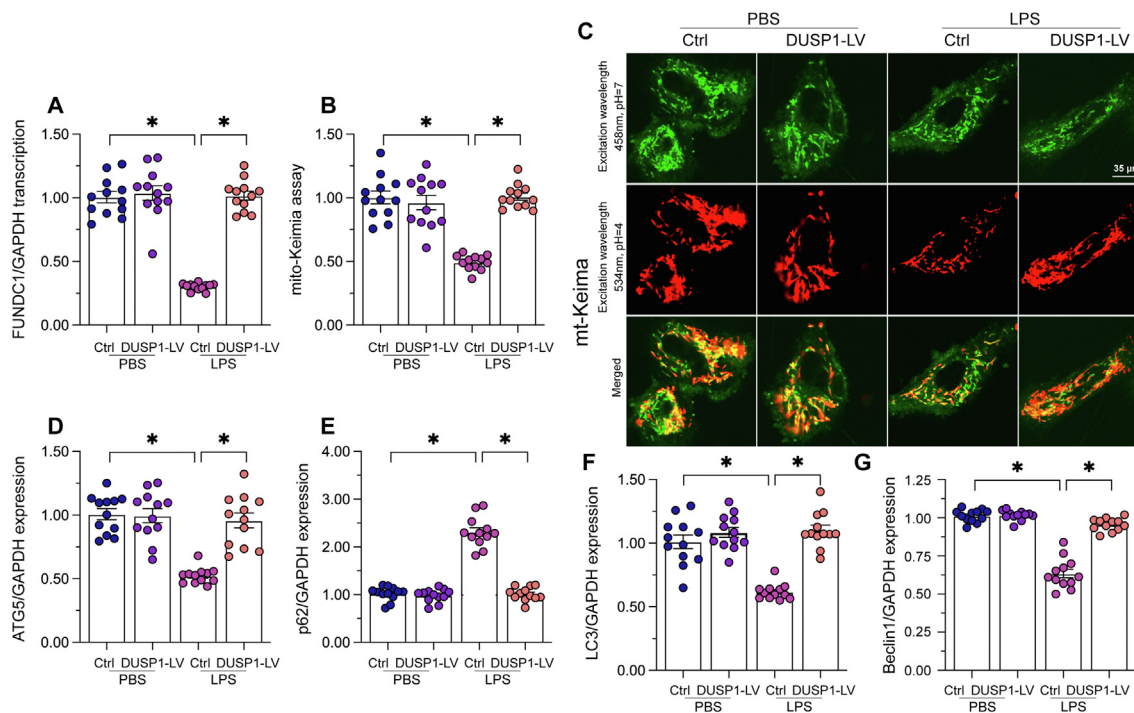
Figure 4: DUSP1 restores FUNDC1-dependent mitophagy in SCM. DUSP1^{TG} and WT mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg) to induce SCM, and were evaluated after 48 h. HL-1 cells were treated with 10 μg/mL lipopolysaccharide for 24 h to induce inflammatory cardiomyocyte damage. HL-1 cells were transfected with the DUSP1-LV to overexpress DUSP1 before lipopolysaccharide treatment. **A.** RNA was collected and used for qPCR. The relative expression of *FUNDC1* was normalized to *GAPDH* expression. **B, C**. Mitophagy activity was observed through an immunofluorescence assay with the mito-Keima probe. **D-G.** The protein levels of Atg5, Beclin1, p62 and LC3II were measured through Western blotting. *p < 0.05.

4 mitochondrial respiration (Figure 3B—F). Moreover, lipopolysaccharide disturbed the respiratory control ratio (RCR), ADP/O and lag phase in cardiomyocytes (Figure 3B—F). DUSP1 overexpression significantly restored mitochondrial respiratory function in lipopolysaccharide-treated cardiomyocytes by normalizing state-3/4 mitochondrial respiration, the RCR, ADP/O and lag phase and improving ATP synthesis (Figure 3B—F). A JC-1 probe revealed that the mitochondrial membrane potential in cardiomyocytes was destroyed by lipopolysaccharide but restored to near-normal levels following DUSP1-LV transfection (Figure 3G,H). On the other hand, lipopolysaccharide promoted the production of mitochondrial reactive oxygen species (ROS), a byproduct of glucose metabolism, while this effect was not observed in cardiomyocytes transfected with the DUSP1-LV (Figure 3I,J). These results indicated that DUSP1 over-expression protected mitochondrial function against lipopolysaccharide stress.

2.4. DUSP1 restores FUN14 domain-containing 1 (FUNDC1)-dependent mitophagy in SCM

Mitophagy is a protective mechanism that removes damaged mitochondria and thus maintains normal mitochondrial behavior. Our previous studies have highlighted the cardioprotective effects of FUNDC1-induced mitophagy in various cardiovascular disorders [17,27]. Therefore, we investigated whether DUSP1 protected cardiomyocyte mitochondria during SCM by promoting FUNDC1-dependent mitophagy. A qPCR assay revealed that lipopolysaccharide treatment significantly downregulated *FUNDC1* in cardiomyocytes, while DUSP1-LV transfection stabilized *FUNDC1* transcription (Figure 4A). Due to reduced *FUNDC1* transcription, mitophagy activity was rapidly downregulated in lipopolysaccharide-treated

cardiomyocytes, as evidenced by a mito-Keima assay; however, overexpression of DUSP1 restored mitophagy activity (Figure 4B,C).

At the molecular level, mitophagy is regulated by several proteins, including autophagy-related 5 (Atg5), Beclin1, p62 and microtubule-associated protein 1 light chain 3-II (LC3II). Western blotting indicated that lipopolysaccharide treatment significantly reduced Atg5, Beclin1 and LC3II levels in HL-1 cardiomyocytes, while it increased p62 accumulation (Figure 4D—G). DUSP1 overexpression normalized the expression of Atg5, Beclin1, LC3II and p62 in lipopolysaccharide-treated cells (Figure 4D—G). These results suggested that DUSP1 promotes FUNDC1-induced mitophagy in SCM.

2.5. *FUNDC1* deficiency abolishes the beneficial effects of DUSP1 on cardiomyocyte mitochondria

To determine whether the mitochondrial protective effects of DUSP1 in lipopolysaccharide-treated cardiomyocytes depended on FUNDC1-induced mitophagy, we transfected cardiomyocytes with short hairpin RNA (shRNA) against *FUNDC1* before transfecting them with the DUSP1-LV, and then assessed their mitochondrial function and cell viability after lipopolysaccharide treatment. *FUNDC1* shRNA completely disrupted mitochondrial respiratory function in DUSP1-overexpressing cells, as evidenced by reduced state-3 and state-4 mitochondrial respiration and an abnormal RCR, ADP/O and lag phase following lipopolysaccharide treatment (Figure 5A—E). Moreover, DUSP1 overexpression inhibited mitochondrial ROS production in lipopolysaccharide-treated cardiomyocytes, while *FUNDC1* shRNA abolished this effect (Figure 5F,G).

An MTT assay demonstrated that DUSP1 sustained cardiomyocyte viability in the presence of lipopolysaccharide, while silencing of *FUNDC1* promoted cardiomyocyte death despite DUSP1-LV

transfection (Figure 5H). Moreover, an ELISA indicated that DUSP1 inhibited caspase-3 activity in lipopolysaccharide-treated cardiomyocytes, while this action was undetectable in cells transfected with *FUNDC1* shRNA (Figure 5I). These data illustrated that *FUNDC1* deficiency reverses the mitochondrial protective effects of DUSP1 in cardiomyocytes.

3. DISCUSSION

The results of this study demonstrated that DUSP1 overexpression could attenuate the progression of SCM. DUSP1 overexpression induced three major protective mechanisms in cardiomyocytes. Firstly, DUSP1 overexpression elevated FUNDC1-dependent mitophagy, although this mechanism remains to be fully delineated. Secondly, increased mitophagy maintained mitochondrial function, especially mitochondrial respiration and metabolism. Thirdly, DUSP1 overexpression alleviated the inflammatory response and cardiomyocyte death. Thus, FUNDC1-dependent mitophagy seems to be a key protective program to reduce sepsis-induced mitochondrial damage and cardiac dysfunction. Moreover, DUSP1 overexpression could be

considered as a novel strategy to restore heart function and mitochondrial homeostasis during sepsis.

The pathology of SCM has been linked with mitochondrial dysfunction. An increased inflammatory response is associated with pathological mitochondrial fission and an abnormal mitochondrial morphology [6]. Fragmented mitochondria are characterized by a reduced mitochondrial membrane potential and blunted mitochondrial respiration [6]. Inhibiting mitochondrial fission by disrupting the binding between dynamin-related protein 1 and mitochondrial fission 1 protein was found to sustain cardiac function and thus reduce mortality [6]. Another study revealed the functional importance of the mitochondrial anti-oxidative system in the pathogenesis of SCM using an H9C2 cellular model [7]. A reduced anti-oxidative capacity was associated with oxidative stress-induced mitochondrial apoptosis in lipopolysaccharide-stimulated cardiomyocytes [7]. Our recent study demonstrated that a defective mitochondrial unfolded protein response can also contribute to the development of SCM [28]. Although the mitochondrial unfolded protein response was slightly activated by lipopolysaccharide stress, the endogenous mitochondrial unfolded protein response failed to preserve heart function and
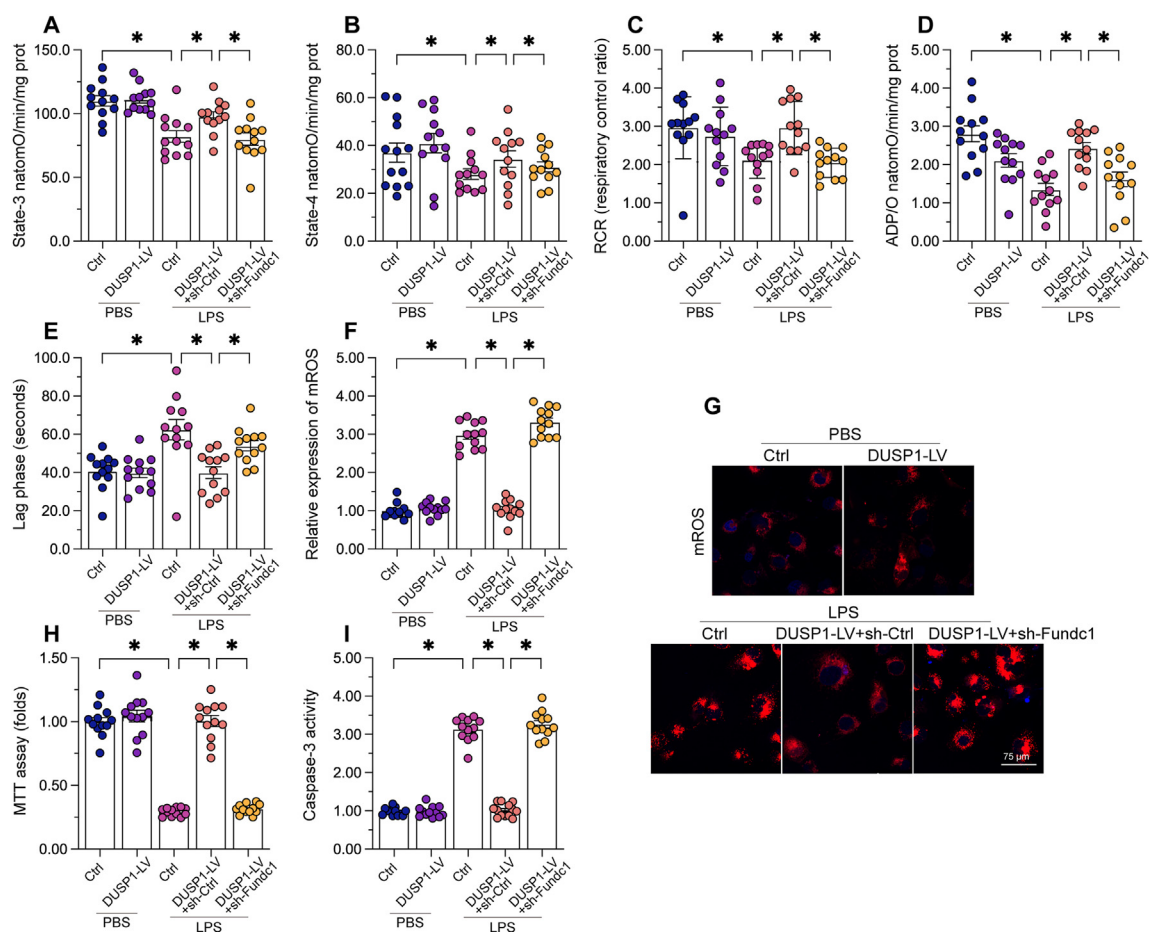

Figure 5: *FUNDC1* **deficiency abolishes the beneficial effects of DUSP1 on cardiomyocyte mitochondria.** DUSP1^{TG} and WT mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg) to induce SCM, and were evaluated after 48 h. HL-1 cells were treated with 10 μg/mL lipopolysaccharide for 24 h to induce inflammatory cardiomyocyte damage. HL-1 cells were transfected with the DUSP1-LV to overexpress DUSP1 before lipopolysaccharide treatment. HL-1 cells were transfected with shRNA against *FUNDC1* (sh-FUNDC1) before DUSP1-LV transfection. **A-E.** The OCR was used to analyze mitochondrial respiration in the presence of lipopolysaccharide. State-3/4 mitochondrial respiration was recorded, along with the RCR, ADP/O and lag phase. **F, G.** Mitochondrial ROS production was determined via immunofluorescence. **H.** An MTT assay was used to determine cell viability. **I.** An ELISA was used to determine caspase-3 activity. *$p < 0.05$.

mitochondrial integrity [28]. As mitochondria are centers of intracellular metabolism, their biogenesis is vital for cardiomyocyte contraction. Interestingly, a previous study indicated that lipopolysaccharide administration impaired mitochondrial biogenesis by attenuating sirtuin-3 transcription, whereas sirtuin-3 overexpression normalized mitochondrial biogenesis and therefore ameliorated SCM [8].

The present study revealed the changes in mitophagy and its regulatory network during SCM. Defective mitophagy was accompanied by mitochondrial dysfunction and cardiomyocyte death, which may have worked together to induce the inflammatory response and impair cardiac function. Our findings have provided novel insights into the complex mechanisms of mitochondrial behavior in SCM. Therapeutic approaches that prevent cardiomyocyte mitochondrial damage may offer additional benefits for patients with SCM, although clinical data are needed to validate this concept.

DUSP1 is a classical protective factor in cardiovascular illness. Early studies identified DUSP1 as a regulator of angiotensin II signaling, since DUSP1 controls three mitogen-activated protein kinase (MAPK) pathways: extracellular signal-regulated kinase, c-Jun N-terminal kinase and p38 [29]. Therefore, DUSP1 may regulate hypertension by affecting angiotensin II-induced vascular contraction. Subsequent research revealed that DUSP1 maintains cardiac muscle cell morphology by controlling actin degradation [30]. Other experiments indicated that DUSP1 deficiency induced cardiac myocyte hypertrophy [31], and that reduced DUSP1 expression promoted insulin resistance in neonatal rat cardiomyocytes [32].

Of note, the impact of DUSP1 on the inflammatory response was first observed in atherosclerosis. Reduced DUSP1 expression was detected in endothelial cells exposed to shear stress, and this promoted the expression of adhesion factors such as vascular cell adhesion molecule 1 [33]. DUSP1 deficiency was found to induce hypotension by augmenting inducible nitric oxide synthase expression [34]. Numerous recent reports have described the influence of DUSP1 on the inflammatory response. DUSP1 suppressed the MAPK pathway, while MAPK induced inflammation through G-protein coupled receptors in lipopolysaccharide-treated cardiomyocytes [35]. DUSP1 may inhibit the inflammatory response not only by repressing MAPK, but also by suppressing prostaglandin E2 generation via cyclooxygenase-2 [35]. Moreover, *Clostridium difficile* Toxin B was reported to induce the colonic inflammatory response through the DUSP1 and nuclear factor κB pathways [36]. In a clinical study of adult cardiovascular disease patients and controls, circulating DUSP1 levels were linked to high-sensitivity C-reactive protein and oxidized low-density lipoprotein concentrations [37]. Therefore, serum DUSP1 is believed to be a potential marker of chronic subclinical inflammation and residual risk in cardiovascular disease patients [37]. In line with these findings, our molecular and functional experiments confirmed the involvement of DUSP1 in SCM. Overexpression of DUSP1 attenuated inflammation-induced myocardial injury by maintaining the mitochondrial integrity. Considering the classical anti-inflammatory properties of DUSP1 in cardiovascular disease, DUSP1 could be a pharmacological target for new drugs designed to treat abnormal myocardial inflammatory responses.

FUNDC1-dependent mitophagy is a well-known protective mechanism that removes damaged mitochondria and thus promotes mitochondrial regeneration [38]. An early study identified FUNDC1 as a hypoxia-dependent protein, since hypoxia promoted FUNDC1 dephosphorylation and thus increased its affinity for LC3 [39]. In a subsequent study, ischemic preconditioning was found to promote FUNDC1 phosphorylation, thus increasing mitophagy [40]. Various stressors are known to repress mitophagy, including cardiac ischemia/reperfusion injury [41], obesity [18] and hyperglycemia [42]. Consistent with these findings,

we found that the lipopolysaccharide-induced inflammatory response repressed mitophagy. Interestingly, DUSP1 overexpression restored mitophagy in SCM, suggesting that DUSP1 may promote FUNDC1-dependent mitophagy. However, it remains unclear whether DUSP1 induces FUNDC1 at the transcriptional or post-transcriptional level, so further research is needed.

Overall, our results illustrated that DUSP1 can attenuate sepsis-induced myocardial injury. DUSP1 overexpression improved FUNDC1-induced mitophagy and thus promoted mitochondrial turnover, ultimately stabilizing mitochondrial respiration and metabolism. By sustaining the mitochondrial integrity, DUSP1 increased the cardiomyocyte survival rate and reduced the inflammatory response, thereby increasing heart function.

4. MATERIALS AND METHODS

4.1. Animals
All mice were maintained in a barrier facility on a 12-hour light/dark cycle (ambient temperature of 23 °C) and fed with a standard laboratory diet. All mouse studies were approved by the Animal Care and Use Committee of Southern Medical University and Chinese PLA General Hospital, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. DUSP1^{TG} mice were generated by our group as previously described [21]. WT mice served as controls. For the SCM model, mice were injected intraperitoneally with a single dose of lipopolysaccharide (20 mg/kg, Sigma-Aldrich) and evaluated after 48 h, as we previously described [28].

4.2. Echocardiography
Transthoracic two-dimensional B-mode echocardiography was performed using a Vevo 2100 (VisualSonics, Toronto, Canada) equipped with a 30-MHz transducer. Echocardiographic studies were performed on mice anesthetized with a mixture of 1.5% isoflurane and oxygen (1 L/min). The left ventricular wall thickness, end-systolic volume (LVESV) and end-diastolic volume (LVEDV) were recorded, and the percent fractional shortening (%FS) and ejection fraction (%EF) were calculated as mean values based on nine measurement points.

4.3. Histological analysis
At sacrifice, heart tissues were removed, formalin-fixed, paraffin-embedded and sectioned at 5-micron intervals. Slides were analyzed immunohistochemically using primary antibody ICAM (clone BM8; BMA Biomedicals, Switzerland) at a dilution of 1:200. Standard immunohistochemical protocols were used thereafter, as we previously described [27]. ICAM1 staining was conducted as previously described [43]. Histological images were captured on an Olympus BX51 microscope equipped with a four-megapixel Macrofire digital camera (Optronics, Goleta, CA) using the Picture Frame Application 2.3 (Optronics). All images were cropped and assembled using Photoshop CS2 (Adobe Systems, Inc., Mountain View, CA) [44].

4.4. Isolation, culture and treatment of neonatal mouse cardiomyocytes
Neonatal mouse cardiomyocytes were isolated using a neonatal heart dissociation kit (#130-098-373, Miltenyi Biotechnology). Briefly, 20 hearts were harvested, and the left ventricles were cut into 1-mm pieces. Enzyme mix 1 (2362.5 μL) was added to enzyme mix 2 (137.5 μL), and the tissue pieces were incubated with the enzyme mixture for 15 min at 37 °C. Dissociation was completed via gentle trituration for 2 min. The enzyme incubation and dissociation were repeated two more times. Enzyme activity was blocked through the

addition of 7.5 mL of high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The mixture was passed through a 70-μm strainer and centrifuged at $600 \times g$ for 5 min. The cell pellets were resuspended and plated in culture medium for 1.5 h at 37 °C. The non-attached cells were collected and transferred onto plates that had been pre-coated with 0.5% laminin, and then 0.1 mol/L 5-bromo-2′-deoxyuridine (#B9285, Sigma-Aldrich) was added to inhibit fibroblast proliferation. Primary cardiomyocytes were incubated in culture medium for 24 h, and then the medium was replaced with fresh medium. For the induction of inflammatory cardiomyocyte damage, HL-1 cells were treated with 10 μg/mL lipopolysaccharide for 24 h.

4.5. Lentiviral expression system

The DUSP1-overexpressing lentivirus and *FUNDC1* shRNA lentivirus were constructed and synthesized by Shanghai GeneChem Co., Ltd. (Shanghai, China). The lentiviruses were stored at −80 °C. Cardiomyocytes were plated in six-well plates, and transfection was performed when the cell density reached 60—70%. ENiS (Enhanced Infection Solution, infection enhancement solution), the target and control viruses, and the appropriate amount of Polybrene were added to the wells after the original medium was discarded. The multiplicity of infection was adjusted according to the manufacturer's instructions. The medium was changed after 8—12 h of transfection, and the cells were then cultured for 48—72 h, depending on the cell density. The fluorescence intensity was observed with a microscope 24 h after transfection. The interference or overexpression effects of the lentiviruses were detected through RT-PCR and Western blotting.

4.6. Cardiomyocytes contraction analysis

All confocal imaging described in this manuscript was performed using a Leica SP8 confocal microscope equipped with a 63 × 1.4 numerical aperture oil objective. Cultured mouse ventricular myocytes were loaded with Fluo-3 AM (Invitrogen) at room temperature for 10 min in Ca^{2+}-free Tyrode's solution, and then were washed for 8 min in Tyrode's solution containing 1 mmol/L Ca^{2+}. The myocytes were perfused with Tyrode's solution (1 mmol/L Ca^{2+}) at room temperature during recordings [45]. Myocytes were paced via field stimulation at 2 Hz with platinum electrodes. To test for the propensity of triggered activity, ventricular myocytes were paced for 20 s, and the latency between the last pacing stimulus and the subsequent spontaneous Ca^{2+} wave was calculated. Fluo-3 AM was excited at 488 nm, and fluorescence emission was collected at wavelengths of 500—550 nm in line scan mode at a sampling rate of 200 Hz.

4.7. qPCR

Total RNA was extracted using an RNeasy Micro Kit (Qiagen). Depending on the total RNA yield, 50—500 ng of total RNA was reverse-transcribed using Superscript II Reverse Transcriptase (Invitrogen) [46]. The reaction mixture for each well was prepared using KAPA SYBR FAST (Kapa Biosystems), forward and reverse primers, nuclease-free water and cDNA, per the manufacturer's recommendations [47]. Each reaction was performed in technical duplicate. Following amplification, the threshold for all genes was set to 0.1. The genes were normalized to the geometric mean of the housekeeping genes ubiquitin (*UBC*) and porphobilinogen deaminase (*PBGD*). The following primer sequences were used: *TNFα* (Forward, 5′-AGATG-GAGCAACCTAAGGTC-3′; Reverse, 5′-GCAGACCTCGCTGTTCTAGC-3′), *IL-6* (Forward, 5′-CAGACTCGCGCCTCTAAGGAGT-3′; Reverse, 5′-GATAGCCGATCCGTCGAA-3′), *MCP1* (Forward, 5′-GGATGGATTGCA-CAGCCATT-3′; Reverse, 5′-GCGCCGACTCAGAGGTGT-3′).

4.8. Western blotting

Cells were washed with phosphate-buffered saline. Radioimmunoprecipitation assay protein isolation buffer supplemented with a protease inhibitor was used to lyse the cells [48]. A bicinchoninic acid protein assay kit with bovine serum albumin (BSA) as the standard was used to determine the protein concentrations in the cell lysates [49]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to separate the proteins, which were then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% dry milk (nonfat) or BSA in Tris-buffered saline and 0.05% Tween 20 (TBS-T) for 1 h, and then were incubated with specific primary antibodies in 5% milk or BSA at 4 °C overnight. The membranes were washed three times with TBS-T and then incubated with secondary immunoglobulin G for 1 h at room temperature. Chemiluminescence was detected using ECL Western blotting substrate (Thermo) and visualized using an Amersham Imager 600. The following primary antibodies from Abcam were used at 1:1000 dilutions: Atg5 (#ab108327), Beclin1 (#ab207612), p62 (#ab96134) and LC3II (#ab192890) [50].

4.9. ELISA

Caspase-3 activity was measured with a Caspase-3 Activity Assay Kit (fluorometric, #ab252897, Abcam). Glucose levels in cell culture media were determined with a Glucose Assay Kit (#ab65333, Abcam) [43].

4.10. Bioenergetics

A Seahorse instrument was used to measure changes in the extracellular oxygen flux in the medium surrounding cells seeded in XF96-well plates. The assay was performed one day after cell plating [51]. The regular cell medium was removed, and the cells were washed twice with DMEM running medium (XF assay modified, supplemented with 11 mM glucose, 2 mM L-glutamine and 1 mM sodium pyruvate, pH 7.4). The cells were incubated at 37 °C without CO_2 for 1 h to allow the cells to pre-equilibrate with the assay medium. Oligomycin, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and antimycin/rotenone diluted in DMEM running medium were loaded into port-B, port-C and port-D, respectively. The final concentrations of these reagents in the XF96 cell culture microplates were 1.5 μM oligomycin, 20 μM FCCP, 2.5 μM antimycin and 1.25 μM rotenone. The following sequence of measurements was used unless otherwise described. The basal oxygen consumption rate (OCR) was measured five times, and then port-A was injected and mixed for 3 min, after which the OCR was measured three times for 3 min. The same protocol was used for port-B and port-C. The OCR was measured after each injection to determine the mitochondrial and non-mitochondrial contributions to the OCR. All measurements were normalized to the average of three measurements of the basal (starting) cellular OCR for each well. Each sample was measured in three to five wells [52]. The experiments were repeated three to five times with different cell preparations. The non-mitochondrial OCR was determined based on the OCR after antimycin/rotenone injection. Maximal respiration was calculated as the maximum OCR after FCCP injection minus the non-mitochondrial OCR. ATP production was calculated as the last OCR measurement before oligomycin injection minus the minimum OCR measurement after oligomycin injection.

4.11. Immunofluorescence microscopy and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis

Cells grown in 2D were rinsed with Dulbecco's PBS with calcium chloride and magnesium chloride (Gibco) before being fixed for 20 min in a cold 4% paraformaldehyde (ChemCruz) solution. The cells were blocked and permeabilized in 10% Donkey Serum (Jackson

ImmunoResearch)/0.1% Triton-X (Sigma) for 30 min at room temperature. The cells were then incubated overnight at 4 °C with the primary antibody (diluted 1:100 in 1% Donkey Serum/0.1% Triton-X) [53]. Subsequently, the cells were incubated with the secondary antibody (diluted 1:1000 in 1% Donkey Serum/0.1% Triton-X) for 1 h at room temperature. For nuclear staining, cells were incubated with 4′,6-diamidino-2-phenylindole (DAPI, 1:5000 in Dulbecco's PBS; Thermo Scientific) for 5 min at room temperature. A primary antibody for Tom 20 (#ab232589, Abcam) was used at a 1:500 dilution. TUNEL staining was performed with a TUNEL Assay Kit (FITC; #ab66108, Abcam) as we previously described [14].

4.12. Mitochondrial membrane potential, mitochondrial ROS and mito-Keima assays

The mitochondrial membrane potential was measured with a JC-1 Mitochondrial Membrane Potential Detection Kit (#30001, Biotium, Inc.) as we previously described [54]. Mitochondrial ROS were detected with a Mitochondrial ROS Detection Assay Kit (#701600, Cayman Chemical) based on our previous report [11]. Mitophagy was assessed with the mito-Keima probe (pMT-mKeima-Red, #AM-V-251; MBL Medical & Biological Laboratories, Co., Ltd., Woburn, MA) as we previously described [17].

4.13. Statistical analysis and image processing

All statistical calculations were performed using GraphPad Prism. An unpaired two-tailed Student's t-test was used for statistical analysis. $P < 0.05$ was considered statistically significant. Quantitative data are shown as the mean ± standard deviation. All figures, labels, arrows, scale bars and outlines were assembled and drawn using Adobe Photoshop software.

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AUTHORS' CONTRIBUTIONS

Ying Tan, Yue Zhang, and Jing He conceived the original experiments. Hao Zhou, Feng Wu, Di Wu, Nengxian Shi, and Weifeng Liu carried out all the *in vivo* experiments and molecular investigation *in vitro*. Ying Tan, Ziying Li, Wenqian Liu, and Hao Zhou wrote the whole manuscript. Wenting Chen revised the final version of manuscript. All the authors read the article and approved the submission.

DATA AVAILABILITY

Data will be made available on request.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in the present work.

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