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# The mechanism of dexmedetomidine regulation of the HIF-1α/FUNDC1 axis in myocardial ischemia/reperfusion injury

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**Summary.** Objective. Myocardial ischemia/reperfusion injury (MIRI) is a life-threatening event that typically follows reperfusion therapy for myocardial infarction. Regarding the effects of dexmedetomidine (Dex) in MIRI, we explored its specific mechanism.

Methods. The MIRI rat model was treated with Dex, Topotecan [a hypoxia-inducible factor-1α (HIF-1α) inhibitor], and lentiviral-overexpressing FUN14 domain-containing protein 1 (Lv-oe-FUNDC1), with rat heart rate analysis. The pathological damage of rat myocardial tissue was evaluated by hematoxylin-eosin (HE) and Masson staining. Positive expression levels of PTEN-induced kinase 1 (PINK1), Parkin, microtubule-associated protein 1 light chain 3 (LC3) II/I, p62 and Beclin1 proteins, HIF-1α and FUNDC1 messenger RNA (mRNA), and HIF-1α and FUNDC1 were assessed by western blot, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and immunohistochemical staining, respectively. HIF-1α-FUNDC1 binding sites and targeted binding relationships were predicted and verified via databases and dual-luciferase assay. HIF-1α enrichment levels in the FUNDC1 promoter region were evaluated using a ChIP assay.

Results. MIRI rats exhibited myocardial injury and severe myocardial dysfunction, with elevated left ventricular diastolic pressure and p62 expression, reduced left ventricular systolic pressure, and maximum rate of change in left ventricular pressure and PINK1, Parkin, LC3 II/I ratio and Beclin-1 protein levels, which were reversed by Dex treatment. MIRI rats had increased HIF-1α and FUNDC1 expression levels, which were further boosted after Dex treatment. Dex promoted mitophagy to ameliorate myocardial injury in MIRI rats via the HIF-1α/FUNDC1 axis.

Conclusion. Dex promoted mitophagy by up-regulating HIF-1α to facilitate the transcriptional expression of FUNDC1, thereby ameliorating myocardial injury in MIRI rats.

**Key words:** Myocardial ischemia/reperfusion injury, Dexmedetomidine, Mitophagy, Hypoxia-inducible factor-1α, FUN14 domain-containing protein 1, Beclin-1

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## Introduction

Myocardial ischemia-reperfusion injury (MIRI) is a fatal killer for patients with cardiovascular diseases, posing high morbidity, disability, and mortality on a global scale (Zheng et al., 2019; Wang et al., 2021; Dong et al., 2023). It is characterized by inadequate oxygen supply and blood flow restoration, leading to irreversible damage to the heart tissues (Lejay et al., 2016). The pathogenesis of MIRI is believed to be multifactorial, involving endothelial dysfunction, white blood cell infiltration and reactive oxygen species generation, cell swelling and microvascular obstruction, and mitophagy (Turer and Hill, 2010; Yang et al., 2019b). Several therapies, including anti-thrombolytic and anti-platelet agents, have been developed to manage MIRI, but their success has been limited (Kumar et al., 2021). There is a lack of effective treatment for MIRI in clinical settings (Zheng et al., 2021). Moreover, extensive research conducted over the last three decades has focused on clarifying the molecular mechanisms of MIRI, but intricate internal pathomechanisms are yet to be completely understood (Chi et al., 2017). Given the high prevalence of ischemic heart diseases, it is imperative to elucidate the precise mechanism of MIRI and to develop potential drug therapies, which are crucial to enhancing the prognosis of patients.

Dexmedetomidine (Dex) functions as a highly selective agonist of the α2-adrenoceptor, and possesses analgesic, sedative, and opioid-sparing properties, making it appropriate for both short-term and prolonged

sedation in an intensive care environment (Keating, 2015). Notably, Dex demonstrates efficacy as a cardioprotective agent against MIRI and inflammation (Xiong et al., 2021). The mechanism of action of Dex in MIRI has been extensively examined. For instance, Dex has been shown to protect the heart from MIRI-induced ferroptosis by activating nuclear factor erythroid 2-related factor 2 (Nrf2) via the adenosine 5'-monophosphate-activated protein kinase/glycogen synthase kinase-3 beta pathway, leading to beneficial effects on myocardial function (Wang et al., 2022). Dex mitigates MIRI in rats by stimulating the Nrf2/Sirtuin 3/superoxide dismutase 2 pathway (Hu et al., 2023). Dex up-regulates Beclin1 phosphorylation at the S295 site through the activation of the phosphatidylinositol 3-kinase/protein kinase B pathway and hinders autophagy-related 14-like protein -Beclin1- vacuolar protein sorting 34 complex interactions, thus protecting against MIRI (Li et al., 2021). Nonetheless, there is a scarcity of studies on the interplay between Dex and other autophagy-related genes in MIRI.

In recent decades, mitochondria have emerged as significant regulators that govern cardiac function and the viability of cardiomyocytes in both physiological and pathological states (Fuhrmann and Brune, 2017; Kozlov et al., 2017). Mitophagy, an evolutionarily conserved process that involves the repair of damaged mitochondria through the assistance of lysosomes, is crucial for regulating cell survival and mitochondrial quality, and is implicated in the pathogenesis of MIRI (Xiao et al., 2020; Cai et al., 2022). Mitophagy safeguards the microvascular structure and function in the presence of pathophysiological conditions linked to MIRI (Wang et al., 2020a). Autophagy is up-regulated in MIRI, and limited autophagy can attenuate MIRI-induced cardiomyocyte death (Li et al., 2018). Moreover, mitophagy mediated by hypoxia-inducible factor-1α (HIF-1α) plays a protective role against various diseases (Hu et al., 2020). MIRI-induced cardiomyocyte apoptosis is shielded by manipulating the mitophagy-mediated HIF-1α/Bcl-2/Adenovirus E1B 19 kDa-interacting protein 3 pathway (Zhu et al., 2020). Matsushima et al. illustrated the protective role of HIF-1α in preventing apoptosis in cardiac fibroblasts, suggesting its potential as a therapeutic target for cardiac remodeling following hypoxic injury (Matsushima et al., 2013). Dex treatment maintains the dynamic equilibrium of mitochondrial fusion/fission by modulating the HIF-1α/heme oxygenase-1 pathway (Shi et al., 2021). Furthermore, FUN14 domain-containing 1 (FUNDC1), a key mitochondrial membrane protein, regulates mitophagy and interacts with autophagy components to enhance mitophagy, thus maintaining mitochondrial homeostasis (Kuang et al., 2016; Lv et al., 2017). Impaired mitophagy linked to FUNDC1 is associated with various metabolic and cardiovascular disorders, and FUNDC1 also plays a crucial role in protecting cells from hypoxia-related damage (Zhang et al., 2017; Fu et al., 2018; Wang et al., 2020b). Activation of FUNDC1 represses mitochondrial apoptosis, mitigates oxidative stress, and enhances mitochondrial activity in cardiac cells during MIRI (Zhang et al., 2016). However, limited literature exists on the potential role of Dex in mitophagy during MIRI through the regulation of the HIF-1α/FUNDC1 axis. The purpose of this study was to investigate the role of Dex in mitophagy through regulating the HIF-1α/FUNDC1 axis in MIRI, and a new therapeutic target for MIRI.

## Materials and methods

### *Ethics statement*

The experiments underwent review and approval by the Animal Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University, and strict adherence to the approved protocols was maintained.

### *Development of an in vivo MIRI rat model*

Forty-two male Sprague-Dawley rats (twelve weeks old, weighing 185-240 g) were obtained from the Institute of Advanced Technology of the Chinese Academy of Sciences [SYXK (Guangdong) 2023-0351, Shenzhen, Guangdong, China]. The rats were housed in a standard specific pathogen-free animal facility at 24±2°C, 50±10% humidity, and a 12-hour (h) light-dark cycle.

Rats were fed adaptively for 1 week. After anesthesia with 100 mg/kg ketamine and xylazine (Rompun 10 mg/kg, i.p.), rats were subjected to endotracheal intubation (Cheng et al., 2016). A suction lancet was inserted into the trachea through the mouth, and the rats were subjected to mechanical ventilation at a rate of 80 breaths/min to maintain normal respiration, with a tidal volume of 8 mL/kg. The limbs of the rats were connected to electrocardiogram leads. Then, a left thoracotomy was performed, and an 8-0 prolene suture was passed from the left anterior descending (LAD) coronary artery to the lower part of the left atrium and tied. Observation of persistent ST-segment elevation confirmed that the occlusion was successful. After a 30-minute ischemia period, the ligature was released and reperfusion was performed for 120 minutes. The rib cage of the rats was closed with a 6-0 prolene suture and the endotracheal tube was removed to resume spontaneous respiration. The rats in the control group (n=6) underwent the same procedures without occlusion and were continuously monitored for 150 minutes.

A total of 53 rats were used for surgery, among which one rat was excluded due to ventilator malfunction, and 10 rats died during or after surgery (three rats died during ischemia, three died within the first few minutes after reperfusion, and four died within two hours after reperfusion). Finally, 36 rats were available for analysis 24 hours after reperfusion.

The rats were grouped as follows (six rats per group): (1) Normal sham group: control rats; non-

occlusion rats; (2) Model group: MIRI rats; (3) Dex group: rats were injected with Dex 25 min after LAD ligation; (4) Dex + Topotecan group: rats were injected with Topotecan for 14 consecutive days prior to MIRI, followed by the injection of Dex 25 min after LAD ligation; (5) Dex + vehicle group: rats received injections of an equal volume of dimethyl sulfoxide (DMSO) to Topotecan for 14 consecutive days before MIRI, and an injection of Dex 25 min after LAD ligation; (6) Dex + Topotecan + overexpressing negative control (oe-NC) group: rats were injected intraperitoneally with Topotecan and lentiviral (Lv)-oe-NC for 14 consecutive days before MIRI and administered Dex 25 min after LAD ligation; (7) Dex + Topotecan + oe-FUNDC1 group: rats were injected intraperitoneally with Topotecan and Lv-oe-FUNDC1 (a lentiviral vector overexpressing FUNDC1) for 14 consecutive days before MIRI and administered with Dex 25 min after LAD ligation.

Color Doppler echocardiography was implemented the day after completion of the interventions. Heart tissues were procured after the sacrifice of the rats using 0.5% pentobarbital sodium (200 mg/kg) (P3761, Sigma-Aldrich, St Louis, MO, USA), and later utilized for hematoxylin-eosin (HE) staining, Masson staining, immunohistochemistry (IHC), western blot, and immunofluorescence assays.

### *Drug administration*

The rats were injected with Dex dissolved in DMSO i.v. (10 μg/kg, HY-12719, MCE, Monmouth Junction, NJ, USA) (Cheng et al., 2016), and were intraperitoneally injected with Topotecan dissolved in DMSO (1.25 mg/kg/time/d, #14129, Cayman Chemical, Ann Arbor, MI, USA) (Kunimi et al., 2019). Furthermore, Lv-oe-NC and Lv-oe-FUNDC1 were dissolved in phosphate buffer saline (PBS) and intraperitoneally injected into rats (once a week, Packgene Biotech, Guangzhou, Guangdong, China) (Xie et al., 2019).

### *Color doppler echocardiography*

Cardiac hemodynamic parameters such as left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and maximum rate of change in left ventricular pressure (± (dP/dt)max) were assessed after 24h MIRI by ultrasound Doppler (KJ-2V2M, Kejin Industrial, Nanjing, Jiangsu, China) (Zhou et al., 2017).

### *Histopathological examination*

After fixation in 4% paraformaldehyde (PFA) for 24h, the samples were embedded in paraffin, followed by cutting into sections (4 μm thickness). Thereafter, sections were subjected to HE staining and Masson staining as per the protocols. Five fields of view were randomly selected to observe histopathological changes under a microscope (Olympus, Tokyo, Japan) (Xu et al., 2021; Gao et al., 2023).

### *Western blot*

The collected tissues were homogenized, and then lysis buffer was added (AR0107, Boster, Wuhan, Hubei, China) to extract proteins, with the protein concentration determined using the bicinchoninic acid kit (AR1189, Boster). Afterward, the protein samples, with an appropriate amount of loading buffer, were heated via a boiling water bath for 5 min for denaturation. Following the loading in the wells, the denatured protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis to separate the proteins, which were subsequently transferred onto polyvinylidene fluoride membranes. Next, membranes were blocked in 3% bovine serum albumin for 2h before the addition of primary antibodies (Table 1) overnight at 4°C. Then, membranes were cultivated with a horseradish peroxidase (HRP)-labeled secondary antibody (1:2000, BA1054, Boster) at room temperature in the dark for 1h and subjected to development with the enhanced chemiluminescence working solution (AR1191, Boster). With glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as an internal reference, gray-scale quantification of bands from Western blot was performed using Image Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD, USA). The experiment was conducted thrice.

### *Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)*

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and later reverse transcribed to complementary DNA using PrimeScript RT reagent kit (Takara, Dalian, Liaoning, China) (TaqMan primers and probes used for the assay were from TaKaRa). qPCR was performed on the ABI PRISM 7900 Sequence Detection System of SYBR Green II (Takara). The PCR program was established with an initial pre-denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 20 s, and extension at 72°C for 35 s. With β-actin as the internal reference, data were analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences (synthesized by Sangon Biological Engineering Technology, Shanghai, China) are listed in Table 2.

**Table 1.** Antibodies used in Western blot.

| Antibody | Catalog number and Company | Dilution ratio |
|---|---|---|
| p62 | ab91526, ABcam | 2 μg/mL |
| Becline-1 | ab207612, ABcam | 1/2000 |
| LC3I/II | #2775, Cell Signaling Technology | 2 μg/mL |
| PINK1 | ab186303, ABcam | 1:1000 |
| Parkin | ab77924, ABcam | 2 μg/mL |
| GAPDH | ab181602,ABcam | 1/1000 |

### *Immunohistochemistry (IHC)*

Paraffin-embedded tissues were kept under high temperature and high pressure for 2 min and then blocked with goat serum (SL038, Solarbio, Beijing, China) for 20 min at room temperature. Next, sections were incubated with anti-HIF-1α (1:20, ab114977, Abcam, Cambridge, UK) or anti-FUNDC1 (1:200, ab272627, Abcam) overnight at 4°C, followed by incubation with secondary antibody goat anti-rabbit immunoglobulin G (IgG) HRP (1:1000, ab214050, Abcam) at 37°C for 60 min. Subsequently, sections underwent diaminobenzidine staining (DA1016, Solarbio) and hematoxylin re-staining (H8070, Solarbio) before observation and imaging using a microscope (Olympus). Finally, Image Pro Plus 6.0 (Media Cybernetics) was utilized for quantitative analysis (Wan et al., 2019).

### *Dual-luciferase reporter gene assay*

The binding sites between HIF-1α and the FUNDC1 promoter were predicted using the JASPAR database (https://jaspar.genereg.net/). The pGL3-FUNDC1-wild type (FUNDC1-WT) and pGL3-FUNDC1-mutant (FUNDC1-MUT) reporter vectors were constructed by amplifying FUNDC1 promoter sequence fragments via PCR and cloning into the pGL3-Basic vector (Promega, Madison, WI, USA). The vectors and HIF-1α-overexpressing plasmid were co-transfected into H9c2 cells using the Lipofectamine™ 2000 kit (11668019, Invitrogen). After 48h, luciferase activity was detected using a dual-luciferase reporter system (Promega).

### *Chromatin immunoprecipitation (ChIP) assay*

A Z-Magna ChIP Assay Kit (17-10086, Millipore, Billerica, MA, USA) was used to perform the ChIP assay (Jin et al., 2022). In short, heart tissues were homogenized to produce a tissue homogenate, which was subsequently cross-linked with 1% PFA/PBS at room temperature for 10 min. The unreacted PFA was neutralized with 10× glycine. Thereafter, samples underwent sonication in lysis buffer to yield DNA fragments ranging from 200 to 1000 bp, which were subjected to immunoprecipitation with 5 μg HIF-1α (1:100, 36169S, Cell Signaling Technology, Beverly, MA, USA) or rabbit IgG (1:100, 2729S, Cell Signaling Technology) antibody. RT-qPCR was used to determine the relative expression of FUNDC1 messenger RNA (mRNA). The specific primer sequences of the FUNDC1 promoter region were as follows: Forward: TGG TGTGCAGGATTTTTATTCCA, Reverse: AACTCTC TCTTCCAGTCGATCTGT.

### *Statistical analysis*

Data were analyzed using GraphPad Prism 8.01 (GraphPad Software, San Diego, CA, USA) and confirmed by the Kolmogorov-Smirnov test to be normally distributed, with the results expressed as mean ± standard deviation (SD). Data comparisons among multiple groups were carried out using one-way analysis of variance (ANOVA), followed by a post-hoc test utilizing Tukey's multiple comparison test. The level of significance was $p<0.05$.

## Results

### *Dex promoted mitophagy and mitigated myocardial injury in MIRI rats*

Color Doppler echocardiography demonstrated elevated LVEDP ($p<0.001$), reduced LVSP, and decreased amplitude of change in ± (dP/dt)max ($p<0.001$) in the Model group compared with the Sham group (Fig. 1A). As reflected by HE and Masson staining results, rats in the Model group exhibited disordered muscle fibers and increased fracture necrosis, with the formation of fibrosis area (blue) versus the Sham group (Fig. 1B), indicating that rats in the Model group had myocardial injury and severe myocardial dysfunction. Western blot assay was carried out to determine the levels of typical mitophagy proteins [PTEN-induced kinase 1 (PINK1), Parkin, microtubule-associated protein 1 light chain 3 (LC3 II/I), Beclin-1 and p62]. The results showed that relative to rats in the Sham group, the rats in the Model group displayed decreased PINK1, Parkin, LC3 II/I ratio and Beclin-1 protein levels ($p<0.01$), and raised p62 expression ($p<0.01$) (Fig. 1C) in myocardial tissues. Conversely, reduced myocardial injury, normalized myocardial morphology, decreased LVEDP ($p<0.01$), increased LVSP, raised amplitude of change in ±(dP/dt)max ($p<0.01$), elevated PINK1, Parkin, LC3 II/I ratio and Beclin-1 protein levels ($p<0.05$), and abated p62 expression ($p<0.05$) (Fig. 1A-C) were observed in Dex-treated MIRI rats. The results suggested that Dex promoted myocardial mitophagy and ameliorated myocardial injury in MIRI rats.

**Table 2.** Primer sequences.

| Gene | Forward 5'-3' | Reverse 5'-3' |
|---|---|---|
| *FUNDC1* | AAGAGGCGAAGGGCAAGAATAA | GGTGCTTCACAGTCATTTCCAGA |
| *HIF-1α* | GAACGTCGAAAAGAAAAGTCTCG | CCTTATCAAGATGCGAACTCACA |
| *β-actin* | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |

### *Dex promoted mitophagy by up-regulating HIF-1α, thereby ameliorating myocardial injury in MIRI rats*

As demonstrated by RT-qPCR and IHC results, HIF-1α expression was elevated in the myocardial tissues of rats in the Model group compared with those in the Sham group ($p<0.01$), and the level was further boosted by Dex treatment ($p<0.05$) (Fig. 2A-B). Subsequently, the MIRI rats were treated with Dex and Topotecan simultaneously. In contrast to the Dex group, the Dex + Topotecan group exhibited reduced HIF-1α levels in myocardial tissues ($p<0.05$) (Fig. 2A-B), reduced PINK1, Parkin, LC3 II/I ratio and Beclin-1 protein levels ($p<0.05$), and raised p62 expression ($p<0.01$) (Fig. 2E). Meanwhile, after co-treatment with Dex and Topotecan, the MIRI rats displayed augmented LVEDP, diminished LVSP, and reduced amplitude of change in ±(dP/dt) max ($p<0.05$) (Fig. 2C), along with myocardial infarction and myocardial dysfunction (Fig. 2D). Overall, Dex promoted mitophagy by up-regulating HIF-1α to ameliorate myocardial injury in MIRI rats, and HIF-1α silencing partially annulled the ameliorative effects of Dex on myocardial injury and mitophagy in MIRI rats.

### *Dex facilitated transcriptional expression of FUNDC1 by modulating HIF-1α*

The binding sites between HIF-1α and FUNDC1 were predicted using the JASPAR database (Fig. 3A). With regard to the results of the dual-luciferase reporter gene assay, dual-luciferase activity was notably reduced after co-transfection of pcDNA3.1-HIF-1α and FUNDC1-WT into H9c2 cells ($p<0.001$) (Fig. 3B), whereas there was no substantial change in the dual-luciferase activity after co-transfection of H9c2 cells with pcDNA3.1-HIF-1α and FUNDC1-MUT plasmids ($p>0.05$) (Fig. 3B). ChIP assay results revealed that the HIF-1α enrichment level in the FUNDC1 promoter

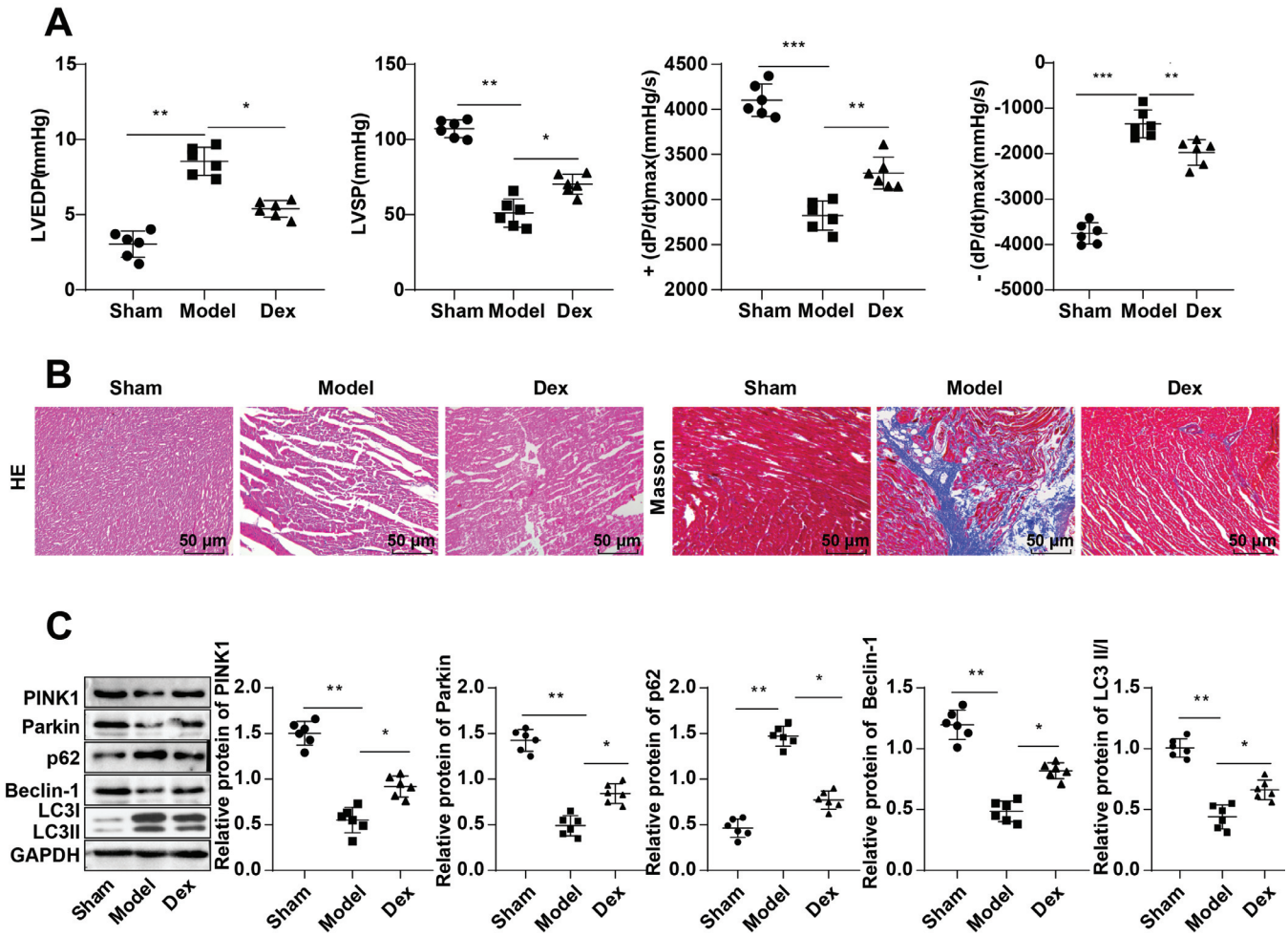


**Fig. 1.** Dex facilitated mitophagy and ameliorated myocardial injury in MIRI rats. **A.** The detection of Color Doppler echocardiography. **B.** HE staining and Masson staining to assess histopathological damage of myocardial tissues. **C.** Western blot to determine PINK1, Parkin, LC3 II/I, Beclin-1 and p62 protein levels. n=6. Data are depicted as the mean ± SD. Data comparisons among multiple groups are performed using one-way ANOVA, followed by Tukey's test. *$p<0.05$, **$p<0.01$.

region in the myocardial tissues of the Model group was dramatically higher than that in the Sham group ($p$<0.01) and was higher in the Dex group than in the Model group ($p$<0.01), but lower in the Dex + Topotecan group than in the Dex + vehicle group ($p$<0.01) (Fig. 3C). RT-qPCR and IHC assays were conducted to measure FUNDC1 expression, which was found to be up-regulated in the myocardial tissues of the Model group

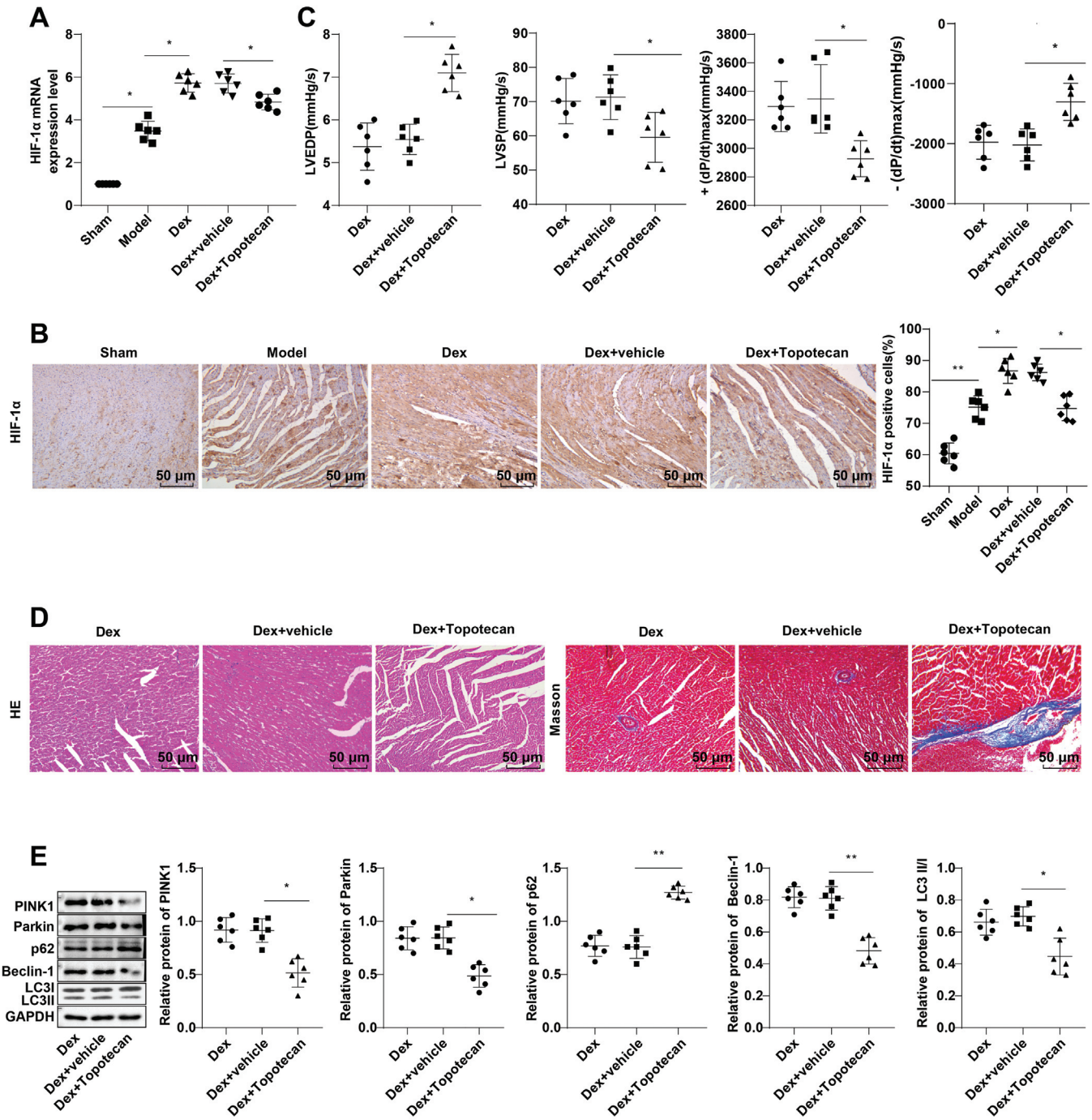


**Fig. 2.** Dex alleviated myocardial injury in MIRI rats by promoting mitophagy via HIF-1α regulation. **A.** RT-qPCR to measure FUNDC1 mRNA levels. **B.** IHC to determine HIF-1α expression in myocardial tissues. **C.** Color Doppler echocardiography to measure rat heart rate. **D.** HE staining and Masson staining to assess pathological damage in myocardial tissues. **E.** Western blot to determine PINK1, Parkin, LC3 II/I, Beclin-1 and p62 protein levels. n=6. Data are presented as mean ± SD. Data comparisons among multiple groups are performed using one-way ANOVA and *post-hoc* tests using Tukey's multiple comparison test. *$p$<0.05, **$p$<0.01.

versus the Sham group ($p$<0.01). The FUNDC1 level in the Dex group was elevated in comparison with the Model group ($p$<0.01), and it was observed to decrease in the Dex + Topotecan group versus the Dex + vehicle group ($p$<0.01) (Fig. 3D-E). Taken together, Dex promoted the transcriptional expression of FUNDC1 by regulating HIF-1α.

### *Interference with FUNDC1 partially reversed the ameliorative effects of Dex on myocardial injury and mitophagy in MIRI rats*

Next, in MIRI rats treated with Dex, Topotecan, and Lv-oe-FUNDC1, FUNDC1 ($p$<0.05) (Fig. 4A-B), PINK1, Parkin, LC3 II/I ratio and Beclin-1 ($p$<0.05)

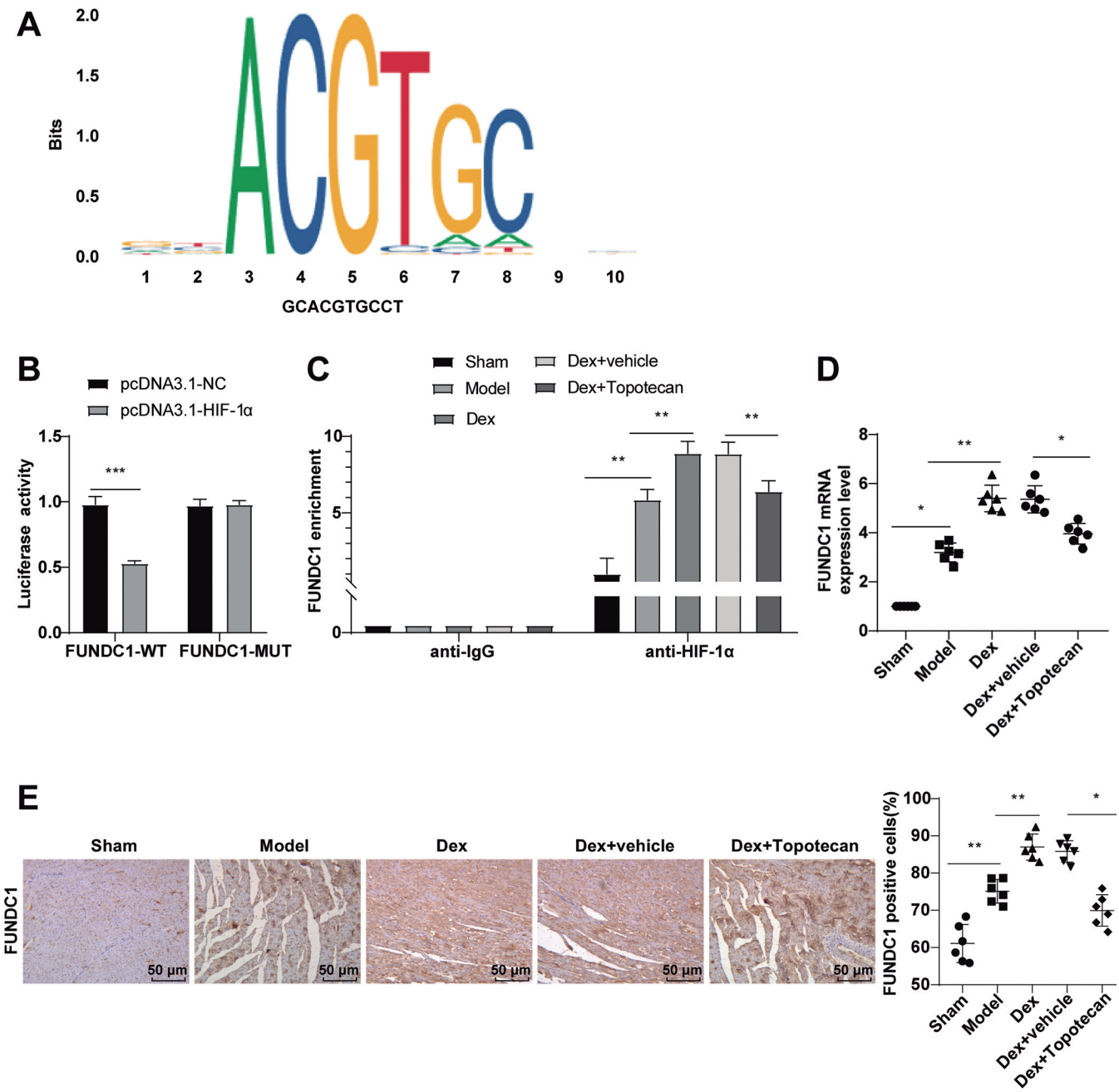


**Fig. 3.** Dex promoted FUNDC1 transcriptional expression by regulating HIF-1α. **A.** The binding sites between HIF-1α and the FUNDC1 promoter were predicted by the JASPAR database. **B.** Dual-luciferase assay was adopted for the verification of the binding relationship between HIF-1α and the FUNDC1 promoter region. **C.** ChIP assay to determine the enrichment level of HIF-1α in the FUNDC1 promoter region. **D.** RT-qPCR to determine FUNDC1 mRNA level. **E.** FUNDC1 levels were measured by IHC assay. n=6. Data were expressed as mean ± SD, and one-way ANOVA was utilized for data comparisons among multiple groups, followed by Tukey's test. *$p$<0.05, **$p$<0.01, ***$p$<0.001.

protein levels were elevated, and p62 expression was decreased ($p$<0.05) (Fig. 4C) in contrast to those treated with Dex, Topotecan, and Lv-oe-NC. Meanwhile, rats in the Dex + Topotecan + oe-FUNDC1 group had a decrease in LVEDP ($p$<0.01), an enhancement in LVSP, and an increase in the change amplitude of ±(dP/dt)max ($p$<0.05) (Fig. 4D) relative to those in the Dex + Topotecan + oe-NC group, along with a decrement in

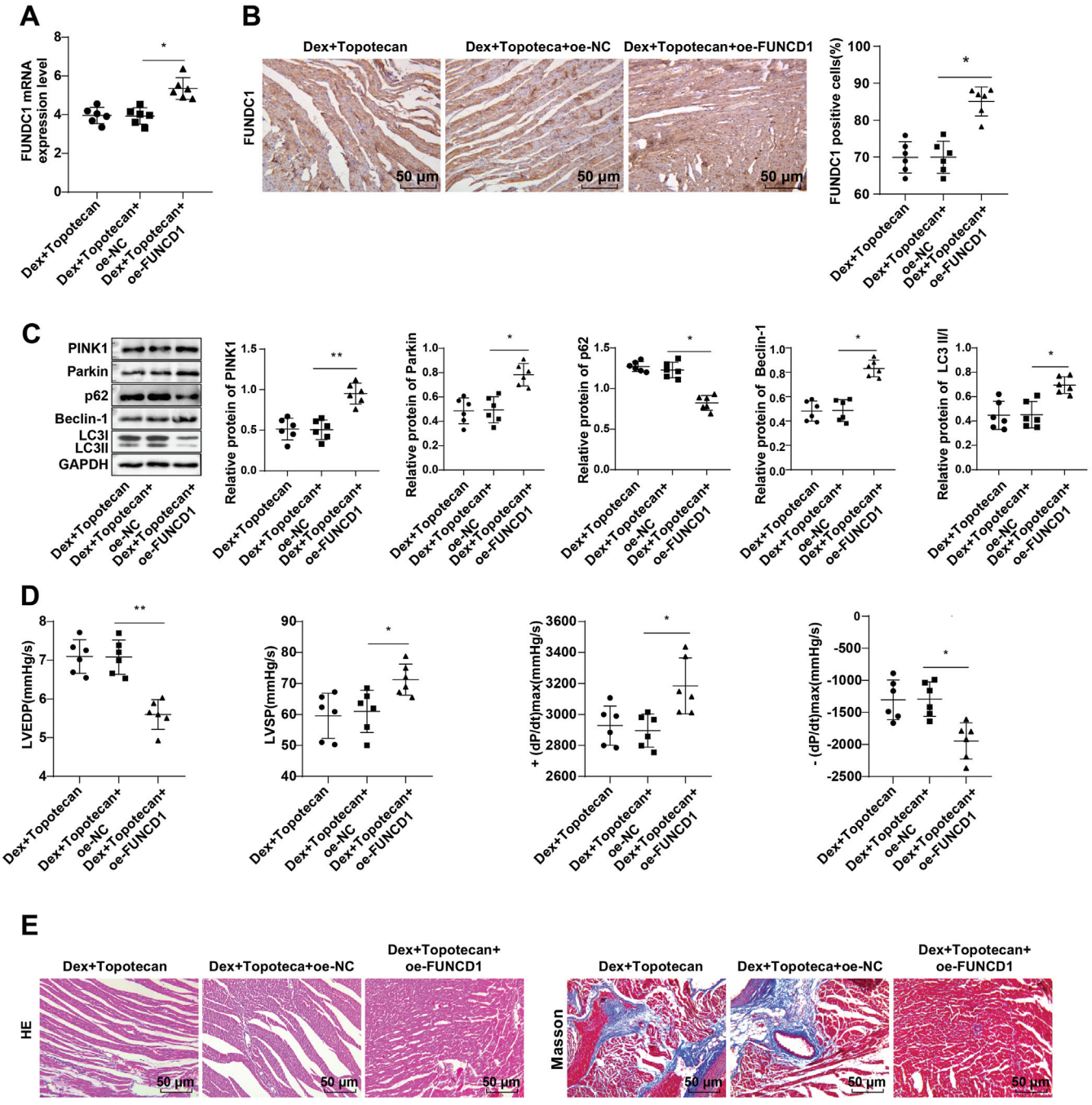


**Fig. 4.** Interference with FUNDC1 partially diminished the ameliorative effects of Dex on myocardial injury and mitophagy in MIRI rats. **A.** RT-qPCR to determine FUNDC1 mRNA levels. **B.** IHC to determine FUNDC1 levels. **C.** Western blot to measure PINK1, Parkin, LC3 II/I, Beclin-1 and p62 protein levels. **D.** Color Doppler echocardiography to measure rat heart rate. **E.** HE staining and Masson staining to evaluate histopathological damage of myocardial tissues. n=6. All experiments were independently repeated thrice. Data are presented in the form of mean ± SD. One-way ANOVA was conducted for data comparisons among multiple groups, and Tukey's multiple comparison test for post hoc tests. *$p$<0.05, **$p$<0.01.

myocardial injury and a normalized myocardial morphology (Fig. 4E). The results described above revealed that Dex promoted mitophagy to ameliorate myocardial injury in MIRI rats by regulating the HIF-1α/FUNDC1 axis.

## Discussion

Evidence has shown that Dex exhibits a protective effect against MIRI (Chen et al., 2023). Hence, this study explored the mechanism of Dex in MIRI by regulating the HIF-1α/FUNDC1 axis.

Dex preconditioning decreases the size of myocardial infarction, enhances cardiac function, suppresses the formation of autophagosomes, and preserves mitochondrial structural integrity (Chen et al., 2023). Also, Dex has been demonstrated to decrease the no-reflow area of ischemic myocardia, enhance cardiac function, and safeguard myocardium from IRI, as shown by higher +(dP/dt) max values following a 120-min reperfusion period in rabbits treated with Dex (Ren et al., 2018). As demonstrated by Zhang et al., Dex augments autophagy by increasing autophagy and mitophagy-related protein levels (p62, LC3 II/I ratio, Beclin-1, PINK1), leading to the elimination of impaired mitochondria in a rat model of intestinal IRI (Zhang et al., 2021). The combination of Dex with propofol mitigates histopathological injury, decreases myocardial infarction, improves cardiac function, and elevates expression levels of Beclin-1 and LC3II/LC3I in MIRI rats (Yang et al., 2023a). Similarly, this study investigated the effect of Dex treatment in MIRI-induced rats and revealed that Dex-treated rats exhibited reduced myocardial injury, normalized myocardial morphology, and raised levels of PINK1, Parkin, LC3 II/I ratio and Beclin-1 proteins, and abated p62 expression, suggesting that Dex promoted myocardial mitophagy and ameliorated myocardial injury in MIRI rats.

Left ventricular developed pressure (LVDP) and LVEDP are related to decreased infarct size in remote ischemic conditioning (Chen et al., 2022). Relative to the MIRI group, Dex partially counteracts the reductions in LVDP (LVDP = LVSP - LVEDP), ± dp/dtmax, and rate pressure product, as well as the increase in shock index (= heart rate/LVDP) except for -dp/dtmax at 140 min, suggesting that Dex pretreatment mitigates hemodynamic disorders and diminishes infarct sizes after MIRI ex vivo (Wang et al., 2023). In a similar light, this study also explored the effects of Dex in MIRI and discovered that Dex-treated rats had decreased LVEDP, increased LVSP, and elevated ±(dP/dt) max, indicating that Dex treatment reinstated the cardiac hemodynamic parameters of MIRI rats.

HIF-1α is up-regulated in MIRI rats and shows more pronounced elevation in brain tissues following Dex treatment, suggesting that Dex may further enhance the level of HIF-1α (Yang et al., 2023b). Unsurprisingly, our study results also revealed further raised HIF-1α levels in Dex-treated MIRI rats. The deletion of tubular HIF-1α hinders mitophagy and exacerbates renal tubular apoptosis (Fu et al., 2020). It's noteworthy that knockdown of HIF-1α represses the onset of mitophagy in adipose-derived stem cells when exposed to hypoxic environments (Zhang et al., 2023). The promotion of Pink1/Parkin-mediated mitophagy improves cardiac function and prevents cardiac mitochondrial dysfunction against myocardial infarction-caused chronic heart failure (Guan et al., 2023). Furthermore, it has been documented that elevated HIF expression facilitates mitophagy to mitigate MIRI (Liu et al., 2023). HIF-1α upregulation facilitates mitophagy, consequently mitigating hypoxia-reoxygenation injury in cardiomyocytes (Yang et al., 2019a). Intriguingly, our study probed into the role of Dex in myocardial injury by HIF-1α and found that Dex promoted mitophagy by upregulating HIF-1α to ameliorate myocardial injury in MIRI rats; moreover, inhibition of HIF-1α partially reversed the ameliorative effects of Dex on myocardial injury and mitophagy in MIRI rats.

FUNDC1 has the capability to preserve mitochondrial function through the stimulation of cardiomyocyte mitophagy, thus protecting myocardial injury resulting from IR (Yu et al., 2019). Furthermore, FUNDC1 functions as a downstream regulator of HIF-1α in mitophagy within renal tubular cells (Zhang et al., 2024). Therefore, our study confirmed the binding relationship between HIF-1α and FUNDC1. In addition, we innovatively unveiled that Dex up-regulated FUNDC1 in MIRI rats, and that Dex boosted HIF-1α enrichment levels in the FUNDC1 promoter region in myocardial tissues of MIRI rats. Moreover, FUNDC1 enhances reactive oxygen species generation, leading to the upregulation of HIF-1α activity in the context of hypoxic pulmonary hypertension (Liu et al., 2022). Interestingly, our study demonstrated that Dex promoted trans-criptional expression of FUNDC1 by regulating HIF-1α. FUNDC1 knockdown results in exacerbated mitochondrial and cardiac dysfunction following myocardial infarction and markedly reduces mitophagy (Xu et al., 2022). Activation of FUNDC1 enhances the mitochondrial activity of cardiac cells after IRI, suppresses oxidative stress, and subdues mitochondrial apoptosis in cardiomyocytes (Zhang et al., 2016). Innovatively, our study investigated the effects of Dex in MIRI rats by HIF-1α/FUNDC1 and showed that Dex promoted mitophagy to ameliorate myocardial injury in MIRI rats by modulating the HIF-1α/FUNDC1 axis.

To our knowledge, Dex promoted mitophagy by upregulating HIF-1α to facilitate the transcriptional expression of FUNDC1, thereby ameliorating myocardial injury in MIRI rats. However, this study had the following limitations: (1) it was mainly conducted in an animal model (MIRI rats), and the effect of Dex on myocardial injury via the HIF-1α/FUNDC1 axis has not been verified at the clinical level; (2) although Dex has been identified to promote the transcriptional expression of FUNDC1 by upregulating HIF-1α, how Dex regulates the upstream targets of HIF-1α and the downstream

targets of FUNDC1 remains unexplored in-depth; (3) it has been mentioned that Dex may ameliorate myocardial injury by regulating oxidative stress, but the exact mechanism remains incompletely elucidated. In the future, further research will be conducted to explore the perspective of Dex in regulating the upstream targets of HIF-1α, the downstream targets of FUNDC1, and other associated pathways.

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