Never in mitosis gene A related kinase-6 deficiency deteriorates diabetic cardiomyopathy via regulating Heat shock protein 72

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Research Article

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Abstract

NIMA (never in mitosis, gene A)-related kinase-6 (NEK6) a cell cycle regulatory gene was found to regulate cardiac hypertrophy. However, it's role in diabetic cardiomyopathy (DCM) have not been fully elucidated. This research was designed to illustrate the effect of NEK6 involved in DCM. Here we used a streptozotocin (STZ) induced mice diabetic cardiomyopathy model and NEK6 knockout mice to explore the role and mechanism of NEK6 on DCM. NEK6 knockout mice and wild type littermates were subjected to STZ injection (50mg/kg/d for five constitute days) to induce DCM model. As a result, 4 months after final STZ injection, DCM mice revealed cardiac hypertrophy, fibrosis and systolic and diastolic dysfunction. NEK6 deficiency caused deteriorated cardiac hypertrophy, fibrosis as well as cardiac dysfunction. Moreover, we observed serve inflammation and oxidative stress in NEK6 deficiency mice heart under DCM pathology. We used an adenovirus to overexpress NEK6 in neonatal rat cardiomyocytes, and found that NEK6 ameliorated high glucose induced inflammation, and oxidative stress. Mechanismly, we found that NEK6 increased phosphorylation of heat shock protein 72 (HSP72) and enhanced the protein level of PGC-1α, NRF2. Co-IP assay experiment confirmed that NEK6 interacted with HSP72. When we silence HSP72 the anti-inflammation and anti-oxidative stress effects of NEK6 were blurred. In summary, NEK6 could protect diabetic induced cardiomyopathy via interacting with HSP72 and promoting the HSP72/PGC-1α/NRF2 signaling.

Introduction

Diabetic cardiomyopathy is a special form of heart disease, which occurs independently of other heart diseases, such as coronary artery disease and hypertension[1]. The main characteristics of diabetic cardiomyopathy are heart tissue insulin resistance, compensatory hyperinsulinemia and hyperglycemia, which promote cardiac remodeling and cardiac function deterioration[2]. Study found that an increased in 1% of HbA1c result in 30% increased risk of heart failure in patients with type 1 diabetes, which independent of hypertension, smoking and obesity[3]. In the early stage of diabetic cardiomyopathy, metabolic disorders contribute to compensatory changes in cardiac structure and function. However, persistent metabolic disorders can promote cardiac hypertrophy, myocardial fibrosis and cardiac diastolic dysfunction, and eventually lead to systolic dysfunction, resulting in heart failure[4]. The pathophysiological changes include impaired autophagy, increased cardiomyocyte death, activation of renin angiotensin aldosterone system (RAAS), oxidative stress and so on[5]. For decades, hypoglycemic drugs reduce the mortality of diabetic patients, but the heart complications caused by diabetes are increasing gradually. Most hypoglycemic drugs have been proven to increase the risk of cardiovascular disease. Therefore, it is necessary to further explore the development mechanism of diabetic cardiomyopathy and find therapeutic targets.

NIMA related kinase family is a mitotic protein found by researchers through genetic screening of cell division cycle mutants in filamentous fungus Aspergillus nidus[6]. Nek6, a serine / threonine kinase, is one of its family members, which is closely related to cell cycle, cell division and apoptosis. Xu have found that NEK6 was associated with cardiac collagen volume fraction (CCVF) in hypertrophic

cardiomyopathy [7]. However, Bian found that NEK6 could attenuate pressure overload induced cardiac hypertrophy via inhibiting AKT signaling[8]. These indicate NEK6 may participate the pathological process of DCM. In this study, we used a NEK6 knockout mice to elucidate the functional role of NEK6 on DCM and found that NEK6 could protect diabetic induced cardiac hypertrophy, fibrosis as well as dysfunction via promoting HSP72 activation.

Methods

Animals

NEK6 knockout mice were came from the European Mouse Mutant Archive (EMMA: 02372) and raised at the SPF Laboratory Animal Center of Zhengzhou University. NEK6 knockout mice and their wild type littermates (aged 8-10 week, 23.5-27.5mg) were injected with streptozotocin (STZ, intraperitoneal injection, i.p.50mg/kg for 5 consecutive days) as previous study described[9]. One week after final injection, mice were subjected to fast blood glucose (FBG) test. Diabetes was defined asFBG ≥ 16.6 mmol/L. 16 weeks after final STZ injection, mice were sacrificed and hearts were collected. Control mice were received same volume of solution. All animal experiments were approved by the Institutional Animal Care and Use Committee of Zhengzhou University (Zhengzhou, China). To overexpress NEK6 (or knockdown HSP72) in heart, mice were subjected to AAV9-NEK6 or AAV9-shHSP72 injection at ten weeks after the final STZ injection. Then, 16 weeks after the final STZ injection, the mice were sacrificed and their hearts were removed.

AAV9 construction and viral delivery

The AAV9-NEK6 and the control AAV9-NC were purchased from the Vigene Bioscience Company (Jinan, China). The AAV9-shHSP72 and the scramble RNA (AAV9-shRNA) were constructed by Vigene Bioscience (Shanghai, China). Ten weeks after the STZ injection, both the DCM mice and the sham mice were randomly assigned to receive either 60–80 μL AAV9-NEK6/ AAV9-shHSP72 (n = 12) or AAV9-NC/AAV9-shRNA (n = 12) at 5.0–6.5 × 10^{13} GC/ml in sterile PBS at 37 °C by injection into the retroorbital venous plexus as described in a previous study[9].

Echocardiographic evaluation

Transthoracic echocardiography was performed as previously described[10, 11]. Isoflurane (1.5%) was used to anaesthetize the mice, and echocardiography was performed with a 10-MHz linear-array ultrasound transducer to obtain M-mode echocardiography data. The left ventricle (LV) end-diastolic dimension (LVEDd) and LV end-systolic dimension (LVESd) were obtained, and the LV ejection fraction (LVEF) and LV fractional shortening (LVFS) values were calculated. A total of 10 mice from each group were subjected to transthoracic echocardiography.

Triphenyltetrazolium chloride, PSR staining, Immunofluorescence staining

Hematoxylin & eosin (HE) staining was used to valuate cross section area as previous described[8]. Image-Pro Plus 6.0 was used to analyze 10 sections from each heart and 6 hearts from each group. PSR staining was used to show collagen volume. For the fibrosis area calculation, Image-Pro Plus 6.0 was used to analyze 6 sections from each heart and 6 hearts from each group.

Oxidative stress assessment

The activities of manganese superoxide dismutase, superoxide dismutase 2 (MnSOD), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and malondialdehyde (MDA) in heart tissues and cardiomyocytes were detected by corresponding kits purchased from Beyotime (Shanghai, China) according to the manufacturers' instructions. The level of reactive oxygen species (ROS) was measured according to a previous study [12] using 2 , 7 -dichlorofluorescin diacetate (DCFH-DA) and an ELISA plate reader (Synergy HT, BioTek, Vermont, USA).

Cardiomyocyte isolation and culture

Neonatal rat cardiomyocyte (NRCM) culture was performed as previously described[10, 11]. Briefly, the hearts of Sprague-Dawley rats (1-3 days old) were quickly removed, and ventricles were preserved and digested with 0.125% trypsin-EDTA (Gibco) 4 times for 15 min each time. Digestion was halted with DMEM-F12 supplemented with 15% fetal bovine serum (FBS, Gibco, USA). After 5 digestion reactions, the cells were collected and incubated in a 100-mm dish with DMEM-F12 supplemented with 15% FBS. After 90 minutes, the cell culture medium was collected, and NRCMs in the upper layer of the cell medium were removed and seeded onto a 6-well plate to exclude the non-cardiac myocytes adhered to the bottom of the 100-mm dish. NRCMs were identified by α-actin staining.

Cells were transfected with adenovirus (Ad-) to overexpress NEK6 (Ad-NEK6, MOI = 50, Vigene Bioscience, Jinan China). Then, the cells were stimulated with 33 mmol/L glucose using 5.5 mmol/L normal glucose as a control. Cells were transfected with NRF2 siRNA to knock down the NRF2 (Santa Cruz, sc-156128).

ELISA detection of inflammatory cytokines

Tumor necrosis factor α (TNFα), interleukin (IL)-1 from mouse hearts as well as cardiomyocytes were detected with ELISAs purchased from BioLegend (430901, 432604). An ELISA instrument (Synergy HT, BioTek, United States) was used to measure the absorbance.

Western blot and qPCR

Total protein was isolated from heart tissues, NRCMs then subjected to SDS-PAGE (50 μg per sample). After transfer onto Immobilon membranes (Millipore, Billerica, MA, USA), proteins were incubated overnight at 4°C with primary antibodies against PGC-1α purchased from Abcam (1:1000 dilution), NRF2 (1:1000 dilution) and GAPDH (1:1000 dilution) purchased from (Cell Signaling Technology (1:1000 dilution); HSP72 (1:1000 dilution), phosphorylated (P)-HSP72(1:1000 dilution) purchased from Enzo Life Sciences. Blots were developed with enhanced chemiluminescence (ECL) reagents (Bio-Rad, Hercules, CA,

USA) and captured by a ChemiDoc MP Imaging System (Bio-Rad). GAPDH served as an internal reference protein.

Total RNA (2μg per sample) from frozen mouse heart tissue and cardiomyocytes was reverse transcribed into cDNA using the oligonucleotide (DT) primer and the transcript first strand cDNA synthesis kit (Roche). Then, a light Cycler 480 instrument (software version 1.5, Roche) and the SYBR green PCR master mix (Roche) was used to perform RT-PCR. All genes were normalized using GAPDH.

Co-immunoprecipitation assays

Cultured NRCMs were lysed in immunoprecipitation buffer. For immunoprecipitation, 10 μL protein A/G-agarose beads and 1 μg antibody were incubated with each sample (500 μL) overnight at 4 °C. After washing with immunoprecipitation buffer, the eluted proteins were immunoblotted with the indicated primary antibodies.

Statistical analysis

All data are expressed as the mean ± SD. Differences among groups were analyzed by two-way analysis of variance followed by Tukey's post hoc test. Comparisons between two groups were analyzed by an unpaired Student's t-test. P values less than 0.05 indicated statistical significance.

Results

The expression level of NEK6 in diabetic cardiomyopathy

We explore the expression level of NEK6 during the process of DCM. As a result, we found that protein and mRNA level of NEK6 was down-regulated in heart tissue after 4 months of STZ injection (Figure 1A and B). We also detected the protein level of NEK6 in cardiomyocytes stimulated with high glucose (HG). A decreased expression level of NEK6 was observed in cardiomyocytes exposed to HG (Figure 1C and D).

NEK6 knockout deteriorates cardiac hypertrophy and fibrosis during DCM

We then used a NKE6 knockout mice to explore the functional role of NEK6 on DCM. As shown in figure 1A, the fast blood glucose was increased in two DCM group when compared with the corresponding control group; while body weight was decreased in two DCM group. No significant difference was observed in fast blood glucose and body weight between WT and KO DCM- mice. Four months after STZ injection, hearts were removed, as shown in figure 2B, heart weight to body weight ratio (HW/BW) and lung weight to body weight ratio (LW/BW) were increased in DCM group; NEK6 knockout increased HW/BW, LW/BW ratios when compared with WT-DCM mice. H&E staining was used to detect cross section area (CSA) about cardiomyocytes. As shown in figure 2C, CSA was increase in DCM mice, while NEK6 deficiency increased this change in DCM mice. Cardiac collagen volume was evaluated by PSR staining, DCM induced enhanced left ventricular (LF) collagen volume, while NEK6 deficiency deteriorated collagen deposition. We also detected transcription level of those fibrosis markers: collagen I, collagen III,

connective tissue growth factor (CTGF), which was proved to further increased in NEK6 knockout mice heart during DCM (Figure 2E).

NEK6 deficiency aggressive DCM induced cardiac dysfunction

Cardiac function was assessed by echocardiography, 4 months after final STZ injection, the LV end diastolic diameter (LVEDd), and systolic diameter (LVESd) were increased in two DCM groups. LV ejection fraction (LVEF) and fractional shortening (LVFS) were decreased when compared with the control group. These indicate our DCM model was successfully established with impaired cardiac systolic and diastolic funciton. NEK6 knockout induced increased LVEDd and dropped LVEF, LVFS, indicating deteriorating cardiac dysfunction (Figure 3). Pressure volume loop result also showed that E/A ratio, maximum rate of left ventricular pressure rise/decay (dp/dt max, dp/dt min) were decreased in DCM heart; while NEK6 knockout further enhance this dysfunction (Figure 3C and D).

NEK6 knockout increased cardiac inflammation and oxidative stress

We further evaluated inflammation and oxidative stress as these two factors are the main feature of DCM pathology. The pro-inflammatory factors including TNFα, IL-1 and IL-6 were increased in mice heart under DCM, while NEK6 deficiency increased the level of those pro-inflammatory factors in heart tissue (Figure 4A). The ROS level was also increased in DCM mice hearts with a remarkable increased in NEK6 deficiency heart tissue. Malondialdehyde (MDA), an intermediate product of lipid metabolism, was also elevated, while the anti-oxidase such as SOD2, Gpx activity was reduced in DCM heart tissue. These changes were deteriorated in NEK6 deficiency heart tissue (Figure 4 B and C).

NEK6 overexpress in cardiomyocytes attenuates high glucose induced inflammation and oxidative stress

Cardiomyocytes were transfected with NEK6 to overexpress NEK6 (Figure 5A). Cells were also exposed to HG. We evaluated the role of NEK6 on cardiomyocytes inflammation and oxidative stress under HG stimulation. As expected, cells in HG group showed increased release of TNFα as well as IL-1, IL-6; while NEK6 overexpression could ameliorate these pro-inflammatory factors release (Figure 5B). The ROS level in cardiomyocytes was increased as well as the level of MDA after HG stimulation, while the activity of anti-oxidases such as SOD2 and Gpx were reduced. NEK6 overexpression could reduce cell ROS and MDA level and increase SOD, Gpx activity (Figure 5C and D).

NEK6 interacts with HSP72 and increases HSP72 phosphorylation

Previous study has found that NEK6 could affect HSP72[6]. We assessed the level of HSP72 in DCM heart tissue as well as cardiomyocytes. As shown in figure 6A, the total level of HSP72 was unchanged in the two DCM mice heart. However, the phosphorylated HSP72 was down-regulated in DCM mice hearts, while NEK6 knockout further decrease the phosphorylation of HSP72 (Figure 6A). We also detected the down-stream targets NRF2 and PGC1-α. As expected, the level of PGC1-α and NRF2 was down-regulated in DCM hearts while further reduced in NEK6 –KO mice hearts (Figure 6A and B). In NEK6 overexpressed cardiomyocytes, cells in HG group showed reduced level of phosphorylated HSP72, NRF2 and PGC1-α.

NEK6 overexpression attenuated these alterations (Figure 6C and D). To assess whether NEK6 could interact with HSP72, we performed co-IP assay, as shown in figure 6E, NEK6 had interaction role with HSP72. These data indicate that NEK6 may interact with HSP72 the promoting its' activation then regulating the down-stream nti-oxidative pathway.

HSP72 silence abrogates anti-inflammation and anti-oxidative stress effects of NEK6

To confirm the mechanism of NEK6 on HSP72, we generated a HSP72 siRNA to knockdown HSP72 (Figure 7A and B). Cardiomyocytes were transfected with both Ad-NEK6 and HSP72 siRNA and exposed to HG. HG induced remarkable pro-inflammatory factors release (TNFα, IL-1, IL-6) and oxidative stress (increased ROS, MDA, decreased SOD2, Gpx activity). HSP72 silence caused a deteriorated inflammation response and oxidative stress. However, cells with both Ad-NEK6 and HSP72 siRNA revealed the same extend of inflammation response and oxidative stress as the cells in HSP72 siRNA group (Figure 7C-E). These data indicate that NEK6 couldn't exert any protection in cardiomyocytes without HSP72.

NEK6 overexpress in mice hearts inhibits cardiac hypertrophy and fibrosis but HSP72 knockout blurs these effects

To confirm the mechanism of NEK6 on HSP72 in vivo, mice were injection with AAV9-NEK6 to overexpress NEK6 in mice heart and mice injected with AAV9-HSP72 to overexpress HSP72 in mice hearts (Figure 8A). NEK6 overexpress led to increased HSP72 phosphorylation; while HSP72 knockdown reduced the enhanced HSP72 phosphorylation level induced by NEK6 overexpress (Figure 8A). The fast blood glucose increased in three DCM groups and body weight was decreased in three DCM groups (Figure 8B). No significant difference was observed in fast blood glucose and body weight among these three DCM groups. Four months after STZ injection, HW/BW and LW/BW ratio were increased in DCM groups; NEK6 overexpressing reduced HW/BW, LW/BW ratios when compared with DCM group; while HSP72 knockdown blurred this result. CSA and Cardiac collagen volume were increase in DCM mice, NEK6 overexpressing decreased these changes in DCM mice; while HSP72 knockdown counteracted these results by NEK6 overexpress (Figure 8D and E).

NEK6 overexpress in mice improves cardiac function in DCM while HSP72 knockout blurs these effects

Cardiac function was assessed by echocardiography, 4 months after final STZ injection, the LVEDd was increased, LVEF and LVFS were decreased in three DCM groups when compared with the control group. NEK6 overexpress induced increased LVEF and LVFS, indicating improved cardiac function (Figure 9A). Pressure volume loop result also showed that E/A ratio, dp/dt max, dp/dt min were also increased NEK6 overexpressing DCM heart; while HSP72 knockdown counteracted these results induced by NEK6 overexpress (Figure 9A and B). We further evaluated inflammation and oxidative stress. The pro-inflammatory factors were inhibited in mice with AAV9-NEK6 injection, while HSP72 knockdown increased the level of those pro-inflammatory factors in heart tissue (Figure 9C). The MDA level was also increased in DCM mice hearts with a remarkable decreased in NEK6 overexpressing heart tissue. The anti-oxidase such as SOD2, Gpx activity was increased in NEK6 overexpressing DCM heart tissue. However,

these changes were deteriorated in mice with AAV9-shHSP72 injection (Figure 9D). All these data suggest that HSP72 knockdown counteracts the protective effects of NEK6 overexpress in heart.

Discussion

Diabetes mellitus is a worldwide epidemic affecting 463 million people worldwide, and its incidence rate has increased annually[13]. In recent years, despite the great progress in the treatment of diabetes, diabetes related cardiovascular diseases, have become the main causes of disability and death[13]. In addition, diabetes increases the incidence rate and severity of coronary artery disease and myocardial infarction, increase the development of heart failure. The study found that the risk of cardiovascular disease related death or hospitalization due to heart failure increased by 75% in diabetic patients[14]. The risk of HF in diabetics is two times of non-diabetic patients[14]. DCM occurs independently of other heart diseases, such as coronary artery disease and hypertension[1]. In this study, we found that NEK6 was down-regulated in T1DM induced cardiomyopathy heart tissue and cardiomyocytes. NEK6 knockout deteriorated cardiac dysfunction, cardiac hypertrophy, fibrosis as well as inflammation response, and oxidative stress. Besides, we also found that NEK6 overexpression could attenuate high glucose induced inflammation and oxidative stress. NEK6 may become a new therapeutic target for diabetic cardiomyopathy.

Oxidative stress is a characteristic pathological feature of diabetic cardiomyopathy. It was found that the occurrence of cardiac dysfunction in T1DM animal model was significantly correlated with the increase of ROS level in cardiomyocytes[15]. STZ induced diabetic cardiomyopathy is characterized by increased lipid peroxidation, increased, nitrotyrosine and carbonyl protein content, and and glutathione reduced[1, 15, 16]. The increase of oxidative stress in STZ model is considered to be related to the decrease of electron transfer chain enzyme activity[16]. Other diabetic animal models, including transgenic OVE26 mice, insulin dependent spontaneous diabetes mellitus wistar rats, Akita diabetic mice and four oxazine induced diabetic mice, showed marked increase in lipid peroxidation, tyrosine nitration and protein carbonylation[15]. In addition, redox imbalance was significantly associated with impaired mitochondrial respiration[17]. In this study, we found that ROS was increased in STZ induced DCM; the anti-oxidases were reduced in T1DM-DCM mice heart, which indicates a redox imbalance. In this study we found that NEK6 deficiency further increase this redox imbalance. Previous study found that in human cancer cells NEK6 could inhibit ROS generation by targeting P53[18]. We also found that NEK6 restored the redox balance in cardiomyocytes exposed to HG. Thus, NEK6 may exert protection in DCM via balancing redox system.

Heat shock protein 72 (HSP72) is an important member of the heat shock protein family[6]. Its expression is low in healthy cells, but it increases under stress. Studies have shown that HSP72 has antioxidant effect and can regulate different signal molecules to participate in cell oxidative stress and apoptosis[19, 20]. Laura O'Regan reported that NEK6 could activate HSP72 and increase mitotic progress[6]. In this study, we found that NEK6 increased the phosphorylation activation of HSP72 and increased the NRF2 expression level. NEK6 also interacted with HSP72, which may promote it's activation. Nrf2 normally

trans-locates into the nucleus in the cytoplasm under cell stress and directly binds to the gene regulatory region of antioxidant response elements, including many antioxidant and detoxifying enzymes, ECT transporters, and mitochondria biogenesis proteins[21]. Paula reported that after high intensity interval training, intramuscular express high levels of HSP72 and PGC-1α, which is associated with high mitochondria biogenesis[22]. In this study, we also found the level of PGC-1α was up-regulated by NEK6. Thus we imply that NEK6 may interact with HSP72 promoting HSP72-NRF2- PGC-1α pathway. In the reversion experiments, HSP72 knockdown abolished the protection effects of NEK6 overexpression both in vivo and vitro, which confirmed our hypothesis.

In conclusion, NEK6 knockout deteriorated cardiac dysfunction, cardiac hypertrophy, fibrosis as well as inflammation response, and oxidative stress. NEK6 overexpression could attenuate high glucose induced inflammation and oxidative stress. By regulating HSP72-NRF2- PGC-1α pathway, NEK6 may become a new therapeutic target for diabetic cardiomyopathy.

Declarations

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Author contribution

Shuangyin Shao and Lu Gao had the idea for this review. Lu Gao, Lili Xiao and Meng Jia provided advice on experimental design and contributed critical suggestions for revision and finalization of the manuscript. Lili Xiao, Meng Jia, Guojun Zhao, Rui Yao and Xiaofang Wang contributed to the literature search and critical appraisal of the studies. Chuyang Zhang contributed suggestions for data analyses part. revised and finalized the manuscript. All authors have given critical suggestions for the final version of the manuscript.

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Availability of data and materials

The data that support the fndings of this study are available from the corresponding author upon reasonable request.

Declarations Ethics approval and consent to participate

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Zhengzhou University.

Consent for publication

All authors consent to the publication of the article in Journal of Molecular Medicine.

Competing interests

The authors declare no competing interests

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Figures

Figure 1

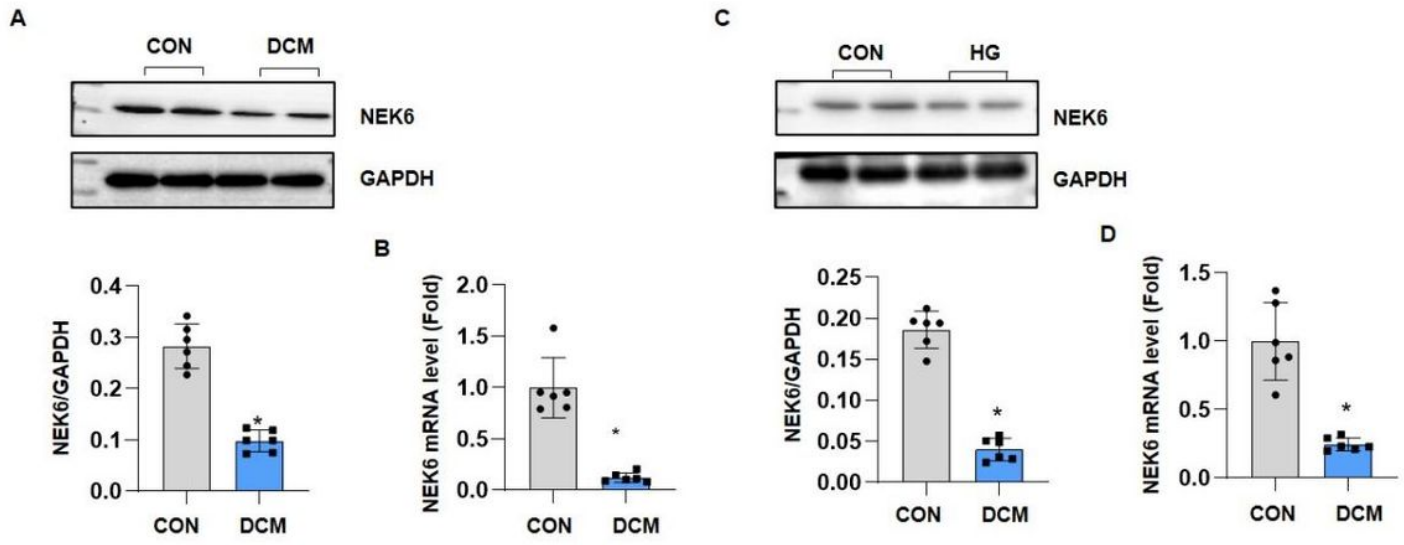

Figure 1

The expression level of NEK6 in diabetic cardiomyopathy

A. Protein level of NEK6 in diabetic cardiomyopathy(DCM) mice heart (n=6). B. mRNA level of NEK6 in DCM mice hearts (n=6). C. Protein level of NEK6 in cardiomyocytes exposed to high glucose (HG) (n=6). B. mRNA level of NEK6 in cardiomyocytes exposed to HG (n=6). * P 0.05 *vs*. CON group

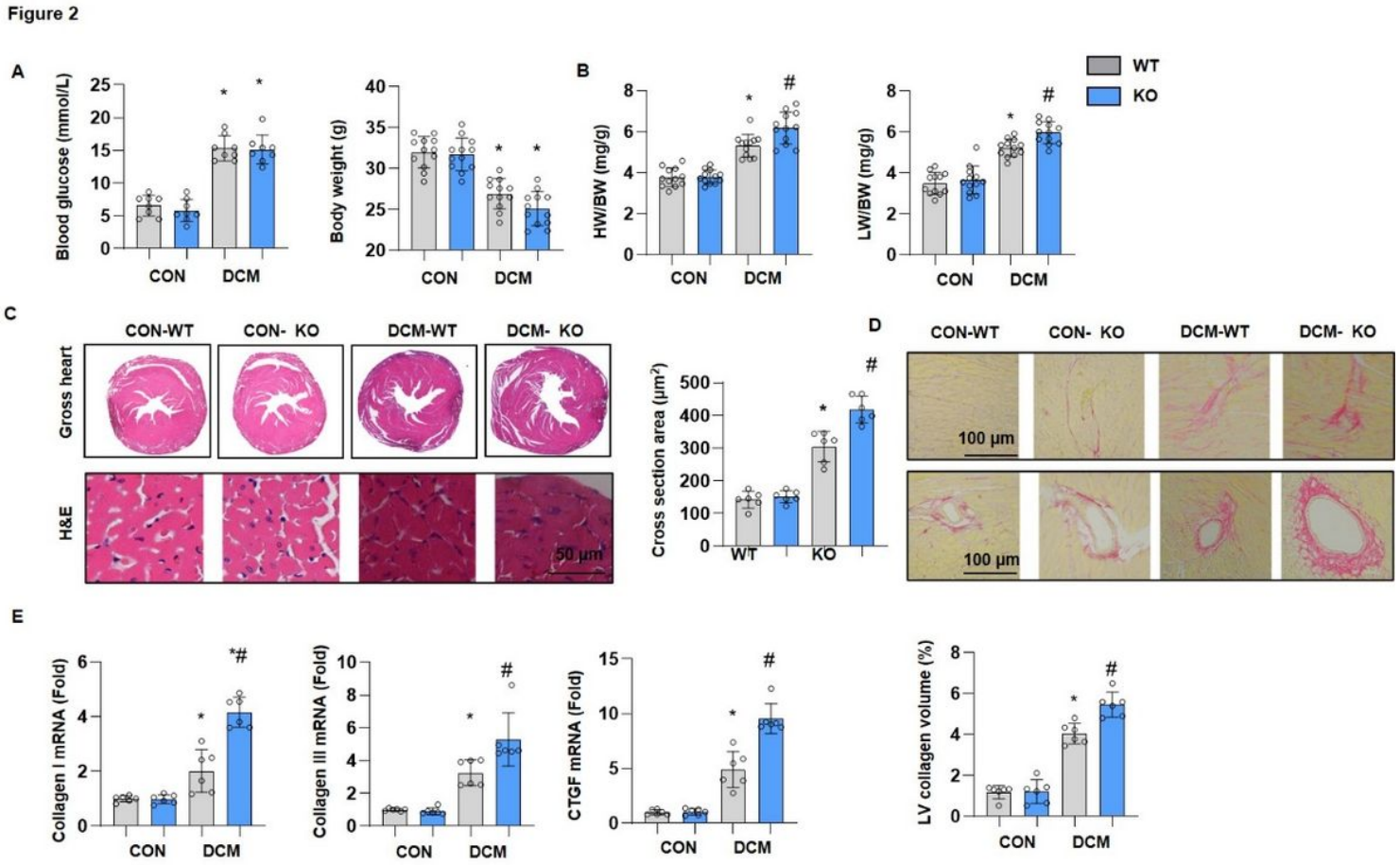

Figure 2

NEK6 knockout deteriorates cardiac hypertrophy and fibrosis during DCM

A. Blood glucose and body weight after 4 months of final STZ injection (n=8). B. Heart weight to body weight ratio (HW/BW), lung weight to body weight ratio (LW/BW) (n=12). C. H&E staining and cross section area in heart tissue (n=6). D. PSR staining and LV collagen volume in heart tissue (n=6). E. mRNA level of fibrosis markers (n=6). * P 0.05 *vs*. WT-CON group; # P 0.05 *vs*. WT-DCM group

Figure 3

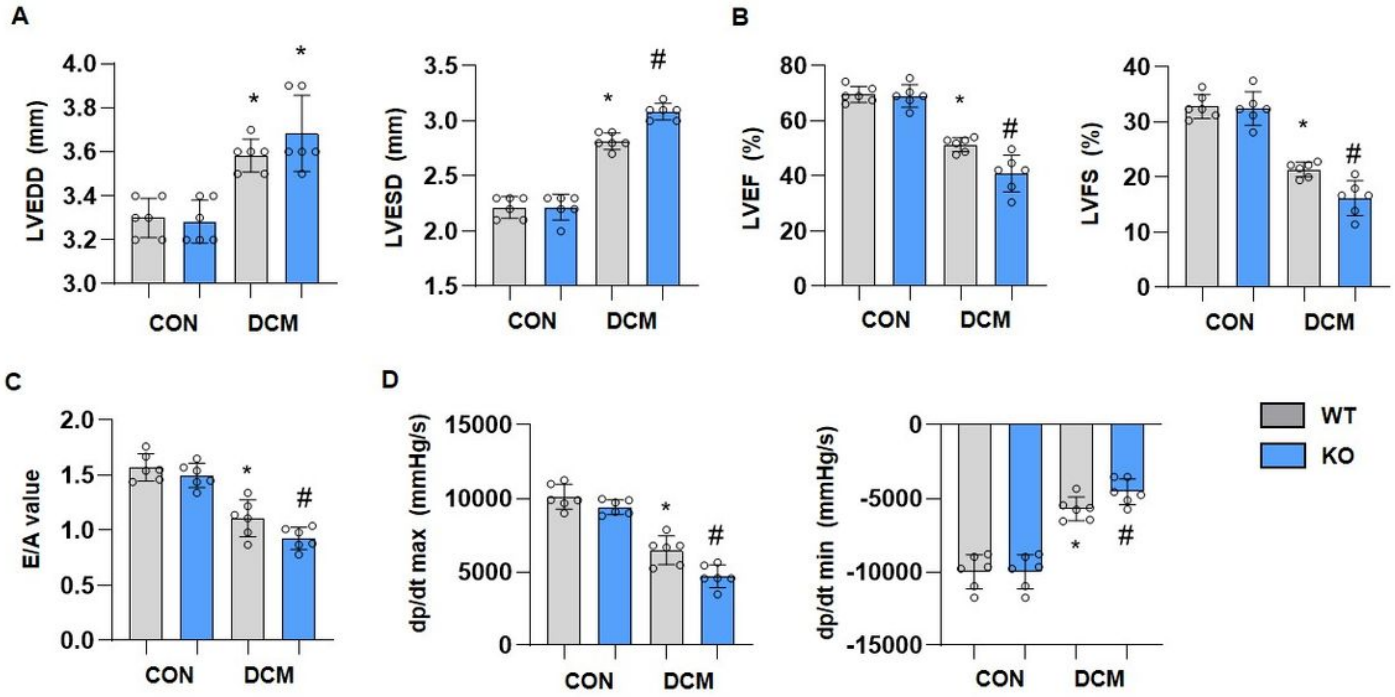

Figure 3

NEK6 deficiency aggressive DCM induced cardiac dysfunction

A and B. Echocardiography data after 4 months of final STZ injection (n=6). C and D. Pressure loop volume data after 4 months of final STZ injection (n=6). * P 0.05 *vs*. WT-CON group; # P 0.05 *vs*. WT-DCM group

LV end diastolic diameter (LVEDd), LV systolic diameter (LVESd), LV ejection fraction (LVEF), LV fractional shortening (LVFS), E/A ratio, maximum rate of left ventricular pressure rise/decay (dp/dt max, dp/dt min)

Figure 4

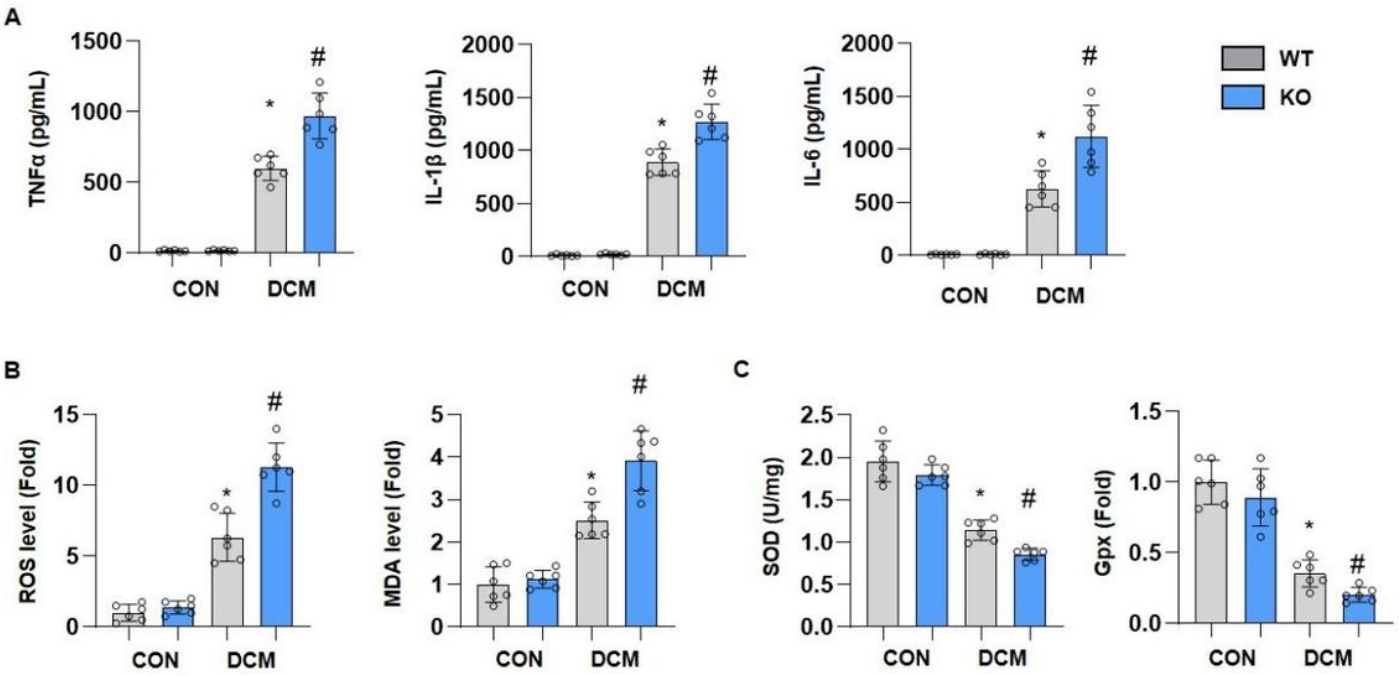

Figure 4

NEK6 knockout increased cardiac inflammation and oxidative stress

A. Pro-inflammatory factors in heart tissue after 4 months of final STZ injection (n=6). B. ROS level and MDA level in heart tissue after 4 months of final STZ injection (n=6). C. SOD2 and Gpx activity in heart tissue after 4 months of final STZ injection (n=6).* P 0.05 *vs*. WT-CON group; # P 0.05 *vs*. WT-DCM group

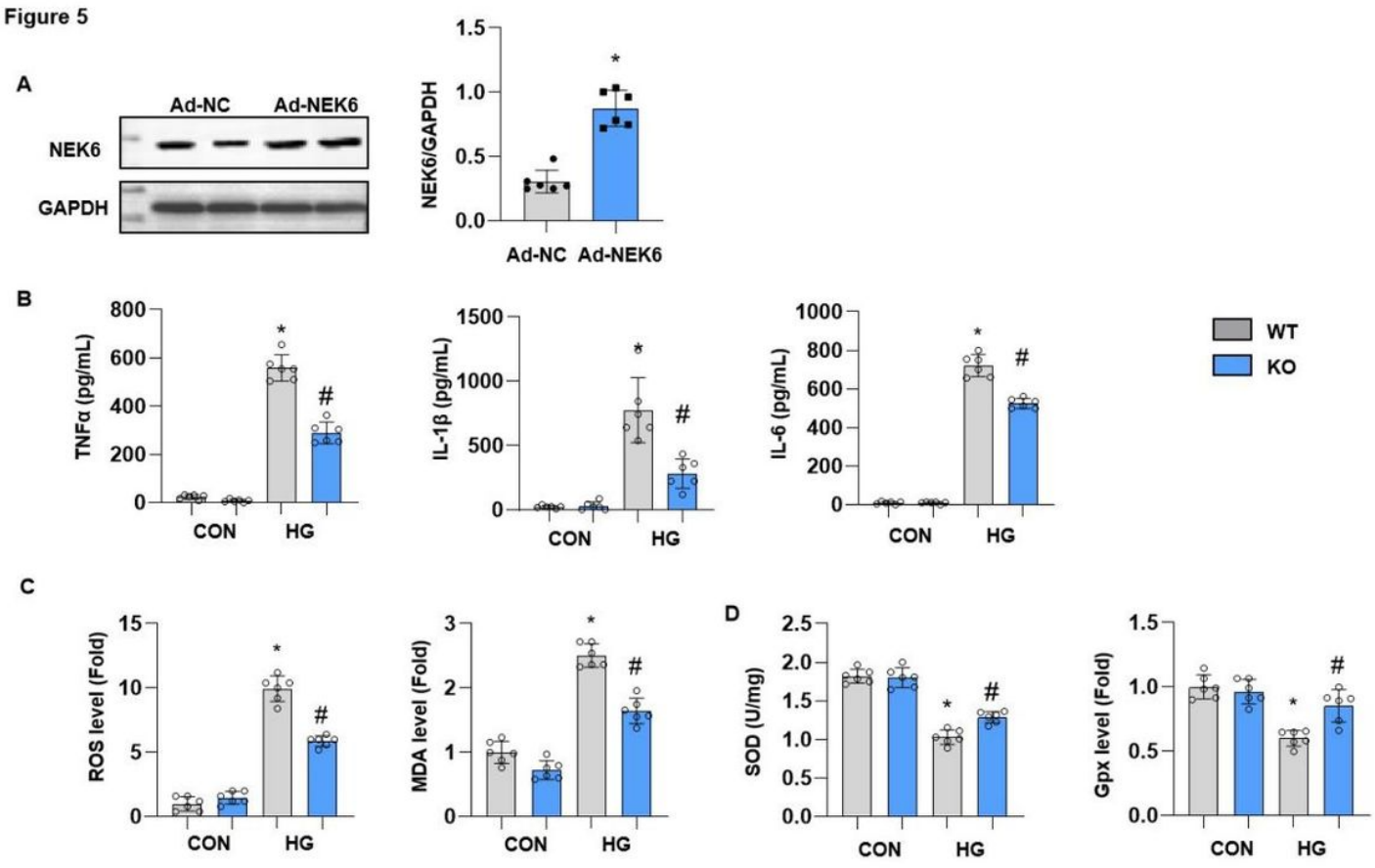

Figure 5

NEK6 overexpress in cardiomyoctes attenuates high glucose induced inflammation and oxidative stress

Cardiomyocytes were transfected with Ad-NEK6. A. Protein level of NEK6 after transfection (n=6). B. Pro-inflammatory factors in cardiomyocytes after exposed to high glucose (n=6). C. ROS level and MDA level in cardiomyocytes (n=6). D. SOD2 and Gpx activity in in cardiomyocytes (n=6). * P 0.05 *vs*. Ad-NC-CON group; # P 0.05 *vs*. Ad-NC- HG group

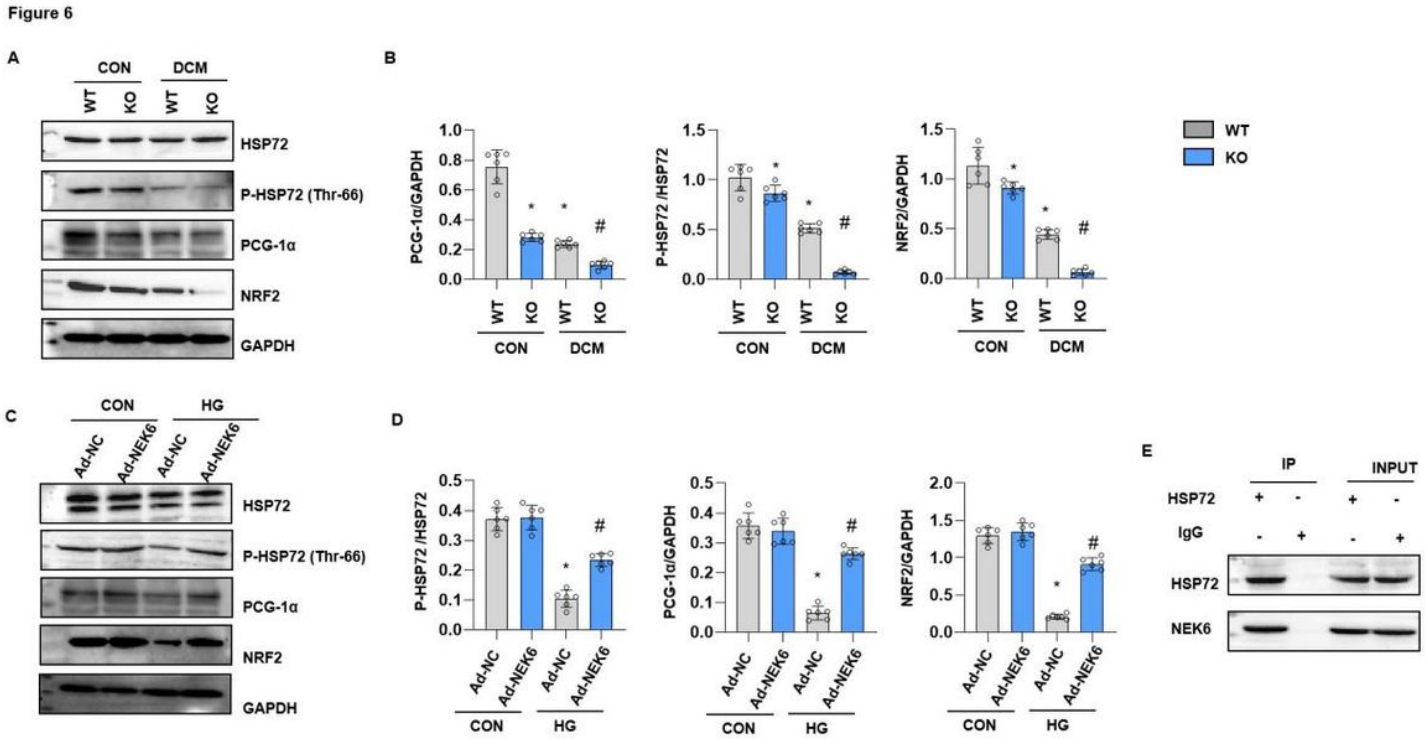

Figure 6

NEK6 interacts with HSP72 and increases HSP72 phosphorylation

A and B. Protein level of total HSP72, P-HSP72, PGC-1α, NRF2 in heart tissue after 4 months of final STZ injection (n=6). * P 0.05 *vs*. WT-CON group; # P 0.05 *vs*. WT-DCM group. C and D. Protein level of total HSP72, P-HSP72, PGC-1α, NRF2 in cardiomyocytes transfected with Ad-NEK6 (n=6). * P 0.05 *vs*. Ad-NC-CON group; # P 0.05 *vs*. Ad-NC- HG group. E. Co-IP assay about HSP72 and NEK6 in cardiomyocytes.

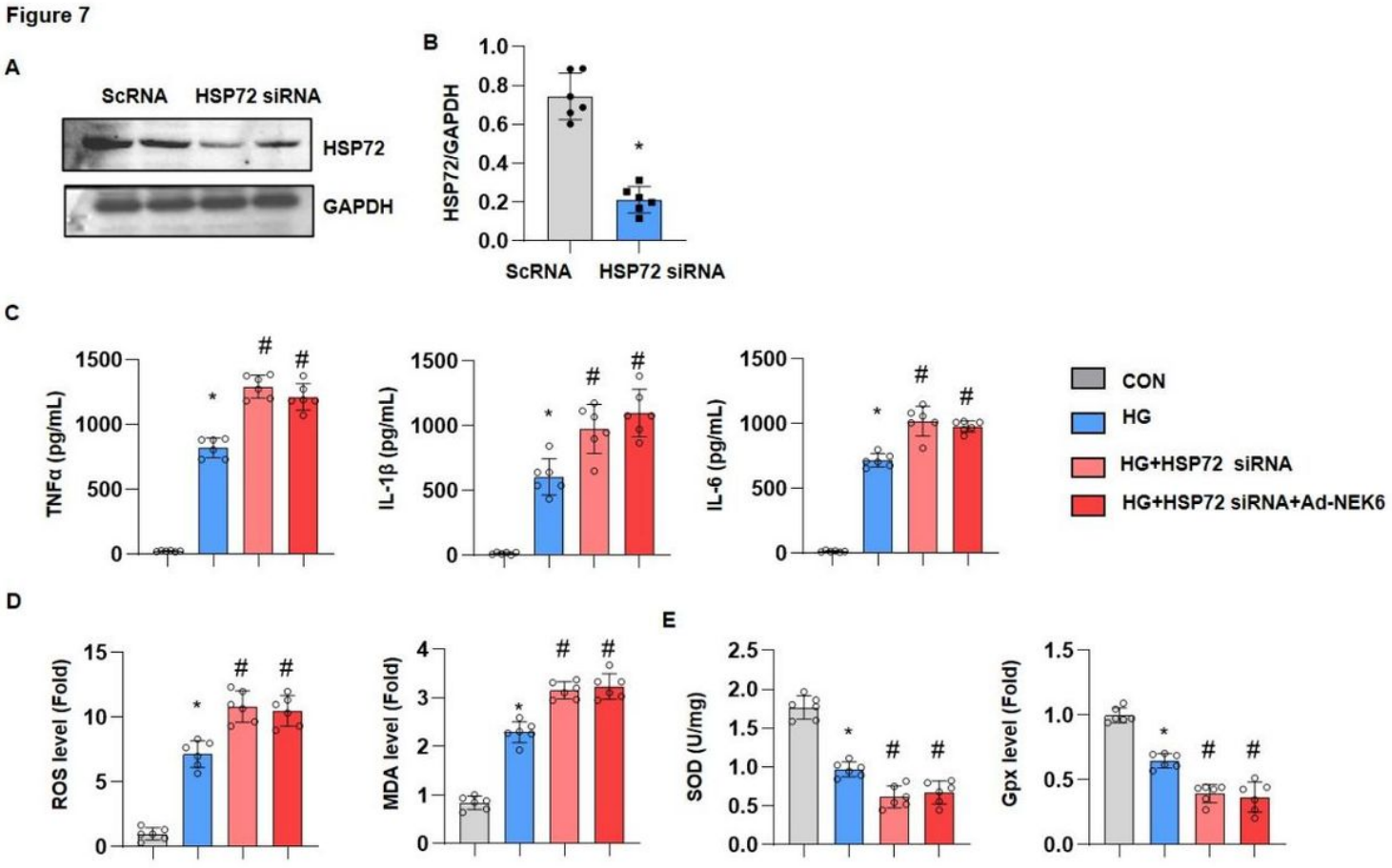

Figure 7

HSP72 silence abrogates anti-inflammation and anti-oxidative stress effects of NEK6

Cardiomyocytes were transfected with Ad-NEK6 and HSP72 siRNA. A and B. Protein level of NEK6 after HSP72 siRNA transfection (n=6). * P 0.05 *vs*. ScRNA group. C. Pro-inflammatory factors in cardiomyocytes after exposed to high glucose (n=6). D. ROS level and MDA level in cardiomyocytes (n=6). E. SOD2 and Gpx activity in cardiomyocytes (n=6). * P 0.05 *vs*. CON group; # P 0.05 *vs*. HG group.

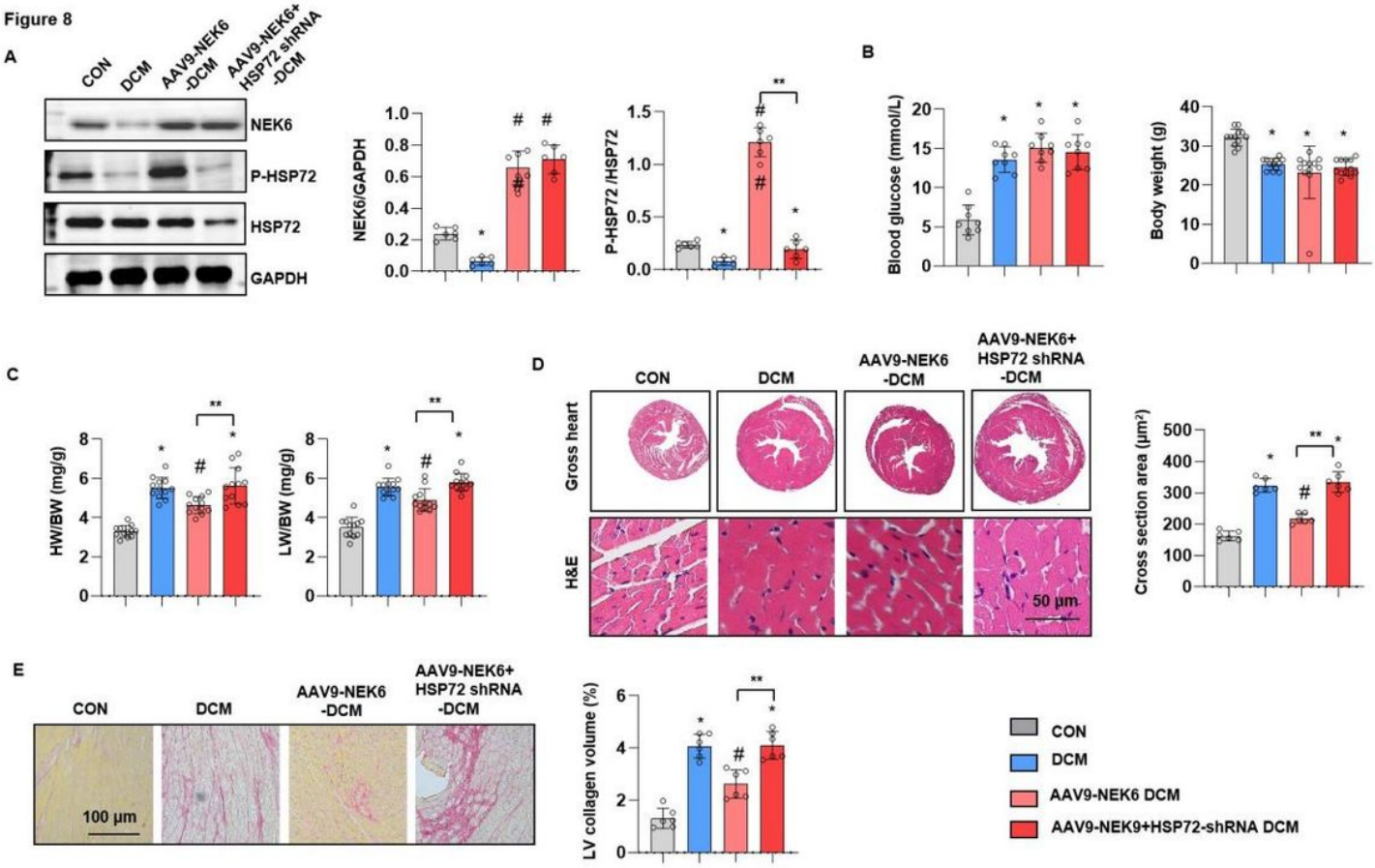

Figure 8

NEK6 overexpress in mice hearts inhibits cardiac hypertrophy and fibrosis but HSP72 knockout blurs these effects

A. Protein level of NEK6 and phosphorylated-HSP72, total HSP72 in mice heart injected with AAV9-NEK6 and HSP72 shRNA(n=6). B. Blood glucose and body weight after 4 months of final STZ injection (n=8). B. Heart weight to body weight ratio (HW/BW), lung weight to body weight ratio (LW/BW) (n=12). C. H&E staining and cross section area in heart tissue (n=6). D. PSR staining and LV collagen volume in heart tissue (n=6). * P 0.05 *vs*. CON group; # P 0.05 *vs*. DCM group

Figure 9

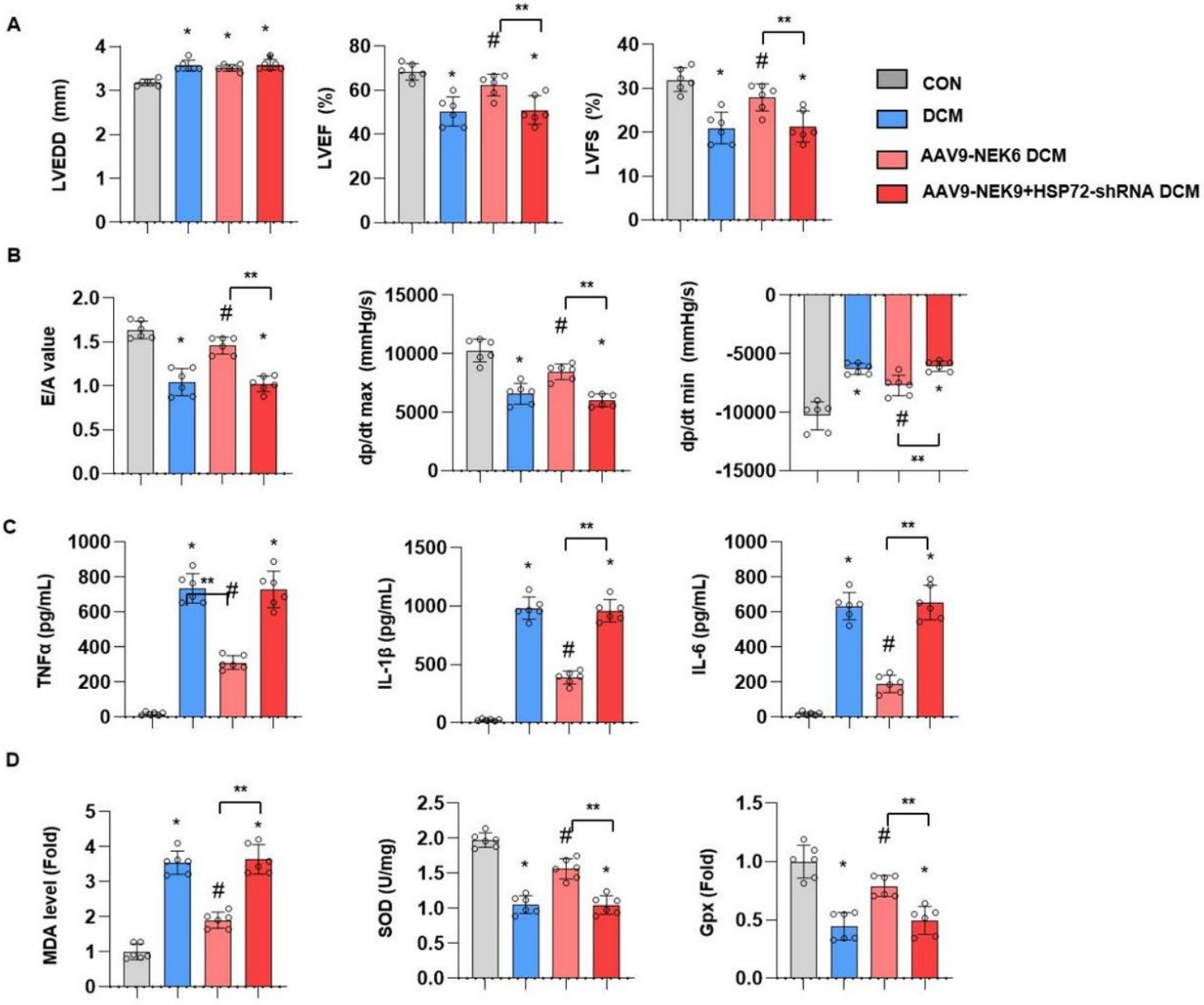

Figure 9

NEK6 overexpress in mice improves cardiac function in DCM while HSP72 knockout blurs these effects

A. Echocardiography data after 4 months of final STZ injection (n=6). B. Pressure loop volume data after 4 months of final STZ injection (n=6). C. Pro-inflammatory factors in heart tissue after 4 months of final STZ injection (n=6).D. MDA level, SOD2 and Gpx activity in heart tissue after 4 months of final STZ injection (n=6).* P 0.05 *vs*. CON group; # P 0.05 *vs*. DCM group

Supplementary Files

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- Keymessages.docx