

RESEARCH ARTICLE

Carnitine Palmitoyltransferase 1A Alleviates Cardiac Injury and Mitochondrial Dysfunction in Diabetic Cardiomyopathy

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ABSTRACT

Diabetic cardiomyopathy (DCM) involves myocardial structural and functional remodeling, in which mitochondrial dysfunction and oxidative stress play central roles. Carnitine palmitoyltransferase 1A (CPT1A) may participate in diabetic cardiac injury, but its underlying mechanism remains unclear. A type 2 diabetes mouse model was established using a high-fat diet and low-dose streptozotocin, while H9c2 cardiomyocytes were exposed to high glucose and palmitic acid. CPT1A was overexpressed using AAV9 in mice and a lentiviral vector in cells. Cardiac injury, lipid metabolism, oxidative stress, and mitochondrial function were assessed through biochemical, histological, RT-qPCR and Western blot analyses. The regulatory relationship between FOXA1 and CPT1A was examined using gain- and loss-of-function approaches. CPT1A expression was reduced in diabetic myocardial tissues and high-glucose/palmitate-treated H9c2 cells. CPT1A overexpression attenuated cardiac injury, partially improved FBG, LDL, TC and TG levels, reduced cardiomyocyte hypertrophy and fibrosis-related gene expression, restored mitochondrial respiratory chain activity and ATP production, and alleviated oxidative stress. FOXA1 was identified as a potential upstream regulator of CPT1A. FOXA1 overexpression reduced cardiomyocyte injury, whereas FOXA1 knockdown aggravated mitochondrial dysfunction, which was partially reversed by CPT1A restoration. CPT1A alleviates diabetic cardiac injury by preserving mitochondrial function and limiting oxidative stress. The FOXA1–CPT1A axis may represent a potential therapeutic target for DCM.

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INTRODUCTION

Diabetic cardiomyopathy (DCM) refers to a specific form of diabetes-related heart disease characterized by myocardial dysfunction that develops independently of coronary artery disease or hypertension. It has become a significant contributor to the progression of heart failure in diabetic patients (Abdullah *et al.*, 2025). Progressive cardiac hypertrophy, myocardial fibrosis, metabolic abnormalities, and impaired contractile function are considered the major pathological features of this condition (Jia *et al.*, 2018). Despite advances in glucose-lowering therapies, the incidence of DCM

continues to increase, suggesting that hyperglycemia alone cannot fully explain its pathogenesis (Huang *et al.*, 2026a). A growing body of evidence has shown that mitochondrial dysfunction, in conjunction with heightened oxidative stress, plays a central role in driving the onset and progression of myocardial injury induced by diabetes.

As key intracellular organelles, mitochondria are crucial for preserving energy metabolism and redox equilibrium in cardiomyocytes (Huang *et al.*, 2026a; Huang *et al.*, 2026b). In diabetic states, excessive ROS production, impaired electron transport chain activity, and reduced ATP synthesis collectively contribute to cardiomyocyte dysfunction and pathological cardiac remodeling (Ye *et al.*,

2024; Huang *et al.*, 2026a). Accordingly, maintaining mitochondrial integrity is considered a promising therapeutic strategy for managing DCM.

Carnitine palmitoyltransferase 1A (CPT1A) is a critical enzyme anchored to the outer mitochondrial membrane that regulates mitochondrial fatty acid utilization and cellular energy metabolism (Liang, 2023; Ni *et al.*, 2026). Given the high dependence of cardiomyocytes on mitochondrial oxidative metabolism, CPT1A may be particularly relevant to cardiac energy homeostasis. Increasing evidence suggests that CPT1A is involved in metabolic disorders, particularly diabetes-associated complications (Liang, 2023; Xie *et al.*, 2024). However, its role in diabetic cardiomyopathy remains poorly understood. Given the dependence of cardiomyocytes on mitochondrial function and their vulnerability to oxidative stress and energy imbalance, CPT1A may play an important role in maintaining cardiac metabolic homeostasis under diabetic conditions.

Therefore, additional studies are warranted to ascertain whether CPT1A participates in the pathogenesis of diabetic cardiomyopathy (DCM) and to clarify its potential contribution to disease progression. Elucidating these aspects could provide fresh insights into the metabolic mechanisms governing diabetic cardiac dysfunction and aid in the identification of novel therapeutic targets.

MATERIALS AND METHODS

Animals: Male C57BL/6J mice (6 weeks old, n=36) were purchased from Beijing SPF Biotechnology Co., Ltd. and maintained under standard conditions with free access to food and water under a 12-h light/dark cycle. To establish the type 2 diabetes (T2D) model, mice were fed a high-fat diet (60% kCal fat) for 11 weeks and subsequently injected intraperitoneally with streptozotocin (STZ, 40 mg/kg; Cat. No. S0130, Sigma-Aldrich) dissolved in freshly prepared 0.1M citrate buffer (pH 4.5) for three consecutive days, whereas control mice received citrate buffer alone. Fasting blood glucose levels were measured using tail vein blood, and mice with glucose concentrations exceeding 16.7 mmol/L were considered diabetic. Diabetic mice continued receiving a high-fat diet throughout the experimental period. For the initial evaluation of the DCM model, mice were randomly assigned to the control and DCM groups (n=6 per group).

To induce cardiac-specific CPT1A overexpression, mice were intravenously injected with adeno-associated virus serotype 9 (AAV9) vectors encoding full-length CPT1A driven by the cTnT promoter, while control animals received empty vectors (1×10^{11} vg/mouse). For the CPT1A overexpression experiment, mice were randomly assigned to four groups (n=6 per group): Vector, CPT1A OE, DCM + Vector, and DCM + CPT1A OE. Mice in the CPT1A OE and DCM + CPT1A OE groups received AAV9 vectors encoding CPT1A, whereas mice in the Vector and DCM + Vector groups received empty AAV9 vectors. Vector administration was performed four weeks after STZ treatment. At the end of the experiment, fasting blood glucose was assessed, and heart tissues were collected following isoflurane anesthesia for subsequent analyses. All mice were

randomly assigned to the groups using a computer-generated randomization sequence.

Cell Culture: H9c2 rat cardiomyoblasts (Procell Life Science & Technology Co., Ltd., Wuhan, China; Cat. No. CL-0089) were cultured in DMEM (Cat. No. 11885084, Gibco) containing 10% FBS (Cat. No. 10099141, Gibco) and 1% penicillin/streptomycin (Cat. No. 15140122, Gibco) at 37°C with 5% CO₂. Cells were plated in six-well plates (1.5×10^5 cells/well) and serum-starved for 12 h before treatment. To establish an *in-vitro* diabetic cardiomyopathy model, cells were exposed to high glucose (33.3 mM) and palmitic acid (PA, 100 μ M; Cat. No. P0500, Sigma-Aldrich) for 72 h.

For CPT1A overexpression *in vitro*, H9c2 cells were transduced with a CPT1A-overexpressing lentiviral vector or the corresponding empty vector control. Cells were seeded and cultured to approximately 70% confluence before transduction. Lentiviral transduction was performed according to the manufacturer's instructions. After 48 h of transduction, CPT1A expression was verified by RT-qPCR and Western blot analysis.

Serum biochemical parameters: Blood samples and cardiac tissues were collected for analysis. Whole blood was centrifuged at 3000 rpm for 15 min to obtain serum, which was then aliquoted and stored at -20°C for subsequent biochemical assessments. Serum concentrations of creatine kinase-MB (CK-MB) (Cat. No. MAK039, Sigma-Aldrich) and lactate dehydrogenase (LDH) (Cat. No. MAK066, Sigma-Aldrich) were quantified using commercial assay kits according to the manufacturer's instructions with a microplate reader.

Biochemical indicators, including fasting blood glucose (FBG), low-density lipoprotein (LDL) (Cat. No. E-BC-K020, Tiangen Biotech Co., Ltd.), total cholesterol (TC) (Cat. No. E-BC-K019, Tiangen Biotech Co., Ltd.) and triglycerides (TG) (Cat. No. E-BC-K018, Tiangen Biotech Co., Ltd.), were determined using commercial kits.

Real-time quantitative polymerase chain reaction (RT-qPCR): Total RNA was extracted with TRIzol reagent (Cat. No. 15596026, Invitrogen) and cDNA was generated using the SuperScript™ III First-Strand Synthesis SuperMix kit (Cat. No. 18080400, Invitrogen). Quantitative real-time PCR was conducted on a Roche LightCycler 480 II system using SYBR Green Master Mix (Cat. No. 4913914001, Roche) and the primers listed in Table 1. Relative gene expression was normalized to β -actin.

Table 1: Sequences of primers applied in qRT-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
CPT1A	GGCATAAACGCAGAGCAT TCCTG	CAGTGTCCATCCTCTGAGT AGC
Nppa	TACAGTGC GG TGTCCAAC ACAG	TGCTTCCTCAGTCTGCTCA CTC
Nppb	TCCTAGCCAGTCTCCAGA GCAA	GGTCCTTCAAGAGCTGTCT CTG
Myh7	GCTGGAAGATGAGTGCTC AGAG	TCCAAACCAGCCATCTCCT CTG
Col1a1	CCTCAGGGTATTGCTGGA CAAC	CAGAAGGACCTTGTTCG CAGG
Tgfb1	TGATACGCCTGAGTGGCT GTCT	CACAAGAGCAGTGAGCGC TGAA
FOXA1	GCCTTACTCTACATCTC GCTC	CTGCTGGTTCTGGCGGTA ATAG
β -actin	CACCATTGGCAATGAGCG GTTC	AGGTCTTTGCGGATGTCCA CGT

Western blot analysis: Proteins from cardiac tissues and cultured cells were extracted using RIPA lysis buffer (Cat. No. P0013B, Beyotime Biotechnology) containing protease and phosphatase inhibitors. Protein concentrations were measured with a BCA Protein Assay Kit (Cat. No. P0012S, Beyotime Biotechnology). Equal amounts of protein (30 µg) were separated on Bolt™ Bis-Tris Mini Protein Gels (Cat. No. NW04122BOX, Thermo Fisher Scientific) and transferred to PVDF membranes (Cat. No. IPVH00010, MilliporeSigma). After blocking with 5% BSA (Cat. No. A1933, Sigma-Aldrich) or skim milk, membranes were incubated with primary antibodies overnight at 4°C. The primary antibodies used were anti-CPT1A (Cat. No. 66039-3-Ig, Proteintech, 1:10000), anti-FOXO1 (Cat. No. 53528, 1:1000, Cell Signaling Technology), anti-β-MyHC (1:1000, Proteintech, Cat. No. 22280-1-AP), anti-IL-1β (1:1000, Proteintech, Cat. No. 26048-1-AP), anti-cleaved caspase-3 (1:1000, Cell Signaling Technology, Cat. No. 9664), and anti-β-actin (Cat. No. 66009-1-Ig, 1:10000, Proteintech). Membranes were then incubated with HRP-conjugated secondary antibodies (Cat. No. SA00001-2 or Cat. No. SA00001-1, Proteintech). Protein bands were visualized using ECL reagent (Cat. No. 34580, Thermo Fisher Scientific) and ImageJ software was used for densitometric analysis with β-actin as the loading control.

Histological examination: Heart tissues were fixed in 4% paraformaldehyde (Cat. No. P1110, Solarbio Life Sciences), paraffin-embedded, and sectioned into 5µm slices. Histological alterations were assessed by hematoxylin and eosin staining using an H&E Staining Kit (Cat. No. C0105S, Beyotime Biotechnology), followed by imaging with an Olympus Corporation BX53 microscope.

Cardiomyocyte size was evaluated on tissue sections stained with fluorescein isothiocyanate (FITC)-conjugated wheat germ agglutinin (WGA) (Cat. No. W11261, Invitrogen) for membrane labeling and 4',6-diamidino-2-phenylindole (DAPI) (Cat. No. D1306, Invitrogen) for nuclei. Images were acquired by fluorescence microscopy and cross-sectional area was quantified using ImageJ software. At least 100 cardiomyocytes per mouse were analyzed.

ROS production was evaluated by dihydroethidium (DHE) staining. Briefly, frozen heart sections were incubated with a DHE fluorescent probe (10µM; Cat. No. D7008, Sigma-Aldrich) for 30 min in a humidified chamber protected from light. After incubation, the sections were rinsed with phosphate-buffered saline (PBS) and fluorescence images were acquired using a Leica Microsystems DMI8 fluorescence microscope. Signal intensity was then quantified with ImageJ software.

Immunohistochemistry: During immunohistochemical staining, cardiac tissue sections were first incubated with 10% H₂O₂ for 30min to quench endogenous peroxidase activity. Non-specific binding was subsequently blocked using 10% normal goat serum for 1 h at room temperature. The sections were then exposed to primary antibodies against CPT1A and incubated at 4°C for 24h. Immunodetection was carried out using the Histostain-Plus Bulk kit (Bioss, Inc.), with 3,3'-diaminobenzidine (DAB) serving as the chromogenic substrate. Images were acquired using an Olympus Corporation BX51

microscope with a C-5050 digital camera after PBS washing. Brown cytoplasmic staining was defined as positive. For quantification, ≥50 cardiomyocytes per field were analyzed in six randomly selected fields per section.

Immunofluorescence staining: Cultured H9c2 cardiomyocytes were fixed with 4% paraformaldehyde (Cat. No. P1110, Solarbio Life Sciences) for 30min at room temperature, permeabilized using 0.1% Triton X-100 (Cat. No. T8787, Sigma-Aldrich) for 15min and blocked with 5% bovine serum albumin (BSA; Cat. No. A1933, Sigma-Aldrich) for 1h. Cells were then incubated overnight at 4°C with anti-α-actinin primary antibody (Cat. No. ab68167, Abcam), followed by Alexa Fluor® 594-conjugated goat anti-mouse IgG (Cat. No. A-11005, Invitrogen) for 1h in the dark after PBS washing. Nuclei were counterstained with DAPI (Cat. No. D1306, Invitrogen).

Intracellular ROS in H9c2 cardiomyocytes was detected using a dihydroethidium (DHE) fluorescent probe (10µM; Cat. No. D7008, Sigma-Aldrich). After 30min incubation at 37°C in the dark, cells were washed with PBS and imaged under a Nikon Corporation Eclipse 80i fluorescence microscope. Fluorescence intensity was quantified using ImageJ software.

Mitochondrial ROS detection and JC-1 staining:

Mitochondrial ROS production was assessed by co-staining with MitoTracker™ Green FM and MitoSOX™ Red. Briefly, H9c2 cells were incubated with MitoTracker™ Green FM (200nM; Cat. No. M7514, Invitrogen) at 37°C for 30min to label mitochondria. After washing with warm PBS, cells were incubated with MitoSOX™ Red mitochondrial superoxide indicator (5µM; Cat. No. M36008, Invitrogen) at 37°C for 10min in the dark. Following PBS washing, fluorescence images were captured using a Leica Microsystems DMI8 fluorescence microscope. Green fluorescence represented total mitochondrial distribution, whereas red fluorescence represented mitochondrial superoxide production. Merged images were generated to visualize the localization of mitochondrial ROS. Fluorescence intensity of MitoSOX Red was quantified using ImageJ software and normalized to the control group.

Mitochondrial membrane potential ($\Delta\psi_m$) was evaluated using the JC-1 assay. Cells were incubated with JC-1 dye for 20min at 37°C and then washed with phosphate-buffered saline (PBS). JC-1 monomer (green, 535nm) and aggregate (red, 570nm) fluorescence were imaged using a Leica Microsystems DMI8 fluorescence microscope.

Measurement of ATP: Isolated hearts were homogenized in 2.5% (v/v) trichloroacetic acid (TCA; Cat. No. T6399, Sigma-Aldrich) at a ratio of 500µL per 20 mg tissue. After centrifugation, 400µL of supernatant was neutralized by adding 80µL of 1M Tris buffer (Cat. No. T1503, Sigma-Aldrich). ATP levels were measured using a luciferin-luciferase-based ATP Bioluminescent Assay Kit (Cat. No. MAK473, Sigma-Aldrich) following the manufacturer's protocol.

Extraction of mitochondrial proteins: Cells were washed in ice-cold PBS (Cat. No. P1020, Solarbio Life

Sciences) and centrifuged at 600×g for 5min at 4°C and the supernatant was discarded. The pellet was resuspended in 1mL mitochondrial isolation buffer from a Cell Mitochondria Isolation Kit (Cat. No. C3601, Beyotime Biotechnology) and incubated on ice for 15min, followed by homogenization. After centrifugation at 1000×g for 10 min at 4°C, the supernatant was further spun at 11000×g for 10 min at 4°C. The final supernatant was collected as the cytosolic fraction, and the pellet was retained as the mitochondrial fraction.

Measurement of Complex I, II, III and IV activities:

Mitochondrial respiratory chain complexes I–IV activities were measured in isolated cardiac mitochondria using commercial microplate assay kits according to the manufacturers' instructions. Complex I activity was detected using a Complex I Enzyme Activity Microplate Assay Kit (Cat. No. ab109721, Abcam), Complex II using a Complex II Enzyme Activity Microplate Assay Kit (Cat. No. ab109908, Abcam), Complex III using the MitoTox™ OXPHOS Complex III Activity Assay Kit (Cat. No. ab109905, Abcam), and Complex IV activity was determined using a Complex IV Rodent Enzyme Activity Microplate Assay Kit (Cat. No. ab109911, Abcam). Absorbance was read on a microplate reader, and activities were normalized to mitochondrial protein concentration.

Statistical analysis: All *in-vitro* experiments were independently repeated at least three times. *In-vivo* studies were performed with 6 mice per group. Results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Comparisons between groups were assessed by two-way ANOVA, while multiple-group differences were analyzed using one-way ANOVA followed by Bonferroni's post hoc test when appropriate. Statistical significance was defined as $P < 0.05$.

RESULTS

CPT1A expression is reduced in DCM tissues and HG/HP-treated H9c2 cardiomyocytes: RT-qPCR and Western blot analyses demonstrated that CPT1A expression at both mRNA and protein levels was markedly downregulated in the myocardial tissues of the DCM group compared with the normal control group (Fig. 1A–B). H&E staining revealed myocardial structural disorder in DCM tissues, while immunohistochemical staining demonstrated reduced CPT1A expression in the DCM group (Fig. 1C).

In vitro experiments further showed that HG/HP treatment markedly decreased CPT1A mRNA and protein expression in H9c2 cardiomyocytes compared with the NG group (Fig. 1D–E). A mannitol-treated group was included as an osmotic control to determine whether the observed effects of HG/HP were attributable to hyperosmolarity rather than the specific metabolic effects of high glucose. No obvious difference in CPT1A protein expression was observed between the NG and mannitol-treated groups, suggesting comparable expression levels under both conditions (Fig. 1F). To further explore the

functional role of CPT1A in H9c2 cardiomyocytes, a CPT1A overexpression model was generated via lentiviral transduction. RT-qPCR and Western blot analyses demonstrated that CPT1A mRNA and protein levels were markedly upregulated in CPT1A-overexpressing cells compared with those transfected with the empty vector control. These results confirmed the successful transduction and effective overexpression of the target gene (Fig. 1G–H).

CPT1A overexpression alleviates cardiac injury and improves mitochondrial function in DCM mice: To investigate the role of CPT1A in DCM, a CPT1A overexpression model was established in mice. RT-qPCR together with Western blot analysis verified that CPT1A expression was effectively upregulated in both the CPT1A OE and DCM + CPT1A OE groups compared with their corresponding vector control groups (Fig. 2A–B).

Metabolic profiling revealed that DCM+Vector mice exhibited marked metabolic dysregulation compared with Vector mice, as evidenced by significantly elevated FBG, LDL, TC, and TG levels. Notably, CPT1A overexpression did not significantly alter metabolic parameters in non-diabetic mice, but partially reduced the elevated FBG, LDL, TC, and TG levels in DCM mice (Fig. 2C–F).

Relative to the Vector group, mice in the DCM + Vector group showed markedly elevated serum levels of LDH and CK-MB, suggesting pronounced myocardial damage. Importantly, overexpression of CPT1A significantly attenuated the increases in LDH and CK-MB in DCM mice (Fig. 2G–H). Histological examination revealed that cardiomyocyte size was markedly enlarged in DCM + Vector mice relative to Vector controls, while overexpression of CPT1A significantly alleviated cardiomyocyte hypertrophy in DCM mice (Fig. 2I). In addition, DHE staining demonstrated elevated ROS production in DCM hearts compared with controls, which was significantly reversed by CPT1A overexpression (Fig. 2J–K). Consistently, mRNA levels of cardiac hypertrophy and fibrosis-related genes (*Nppa*, *Nppb*, *Myh7*, *Col1a1*, *Tgfb1*) were significantly upregulated in DCM+Vector mice compared with Vector mice, while CPT1A overexpression markedly suppressed their expression in DCM mice (Fig. 2L).

Furthermore, mitochondrial function was significantly impaired in DCM+Vector mice, as evidenced by reduced Complex I–IV activities and ATP production compared with Vector mice. Notably, CPT1A overexpression significantly restored mitochondrial respiratory chain activities and ATP levels in DCM mice (Fig. 2M–Q).

Overall, these findings indicate that CPT1A overexpression alleviates DCM-induced cardiac injury, oxidative stress, and mitochondrial dysfunction.

CPT1A overexpression alleviates HG/HP-induced cardiomyocyte injury and mitochondrial dysfunction:

To investigate the role of CPT1A in cardiomyocyte injury induced by HG/HP conditions, CPT1A overexpression was established *in vitro*. RT-qPCR and Western blot results showed that CPT1A expression was markedly downregulated in the HG/HP + Vector group relative to the Vector control group. In contrast, CPT1A

overexpression markedly restored CPT1A expression levels under HG/HP stimulation (Fig. 3A–B).

Compared with the Vector group, HG/HP treatment significantly increased LDH release, indicating enhanced cardiomyocyte injury. Importantly, CPT1A overexpression markedly reduced LDH levels in HG/HP-

treated H9c2 cardiomyocytes (Fig. 3C). Similarly, hypertrophy-related genes (*Nppa*, *Nppb*, *Myh7*) were significantly upregulated in the HG/HP+Vector group compared with the Vector group, whereas CPT1A overexpression significantly reversed these changes under HG/HP conditions (Fig. 3D).

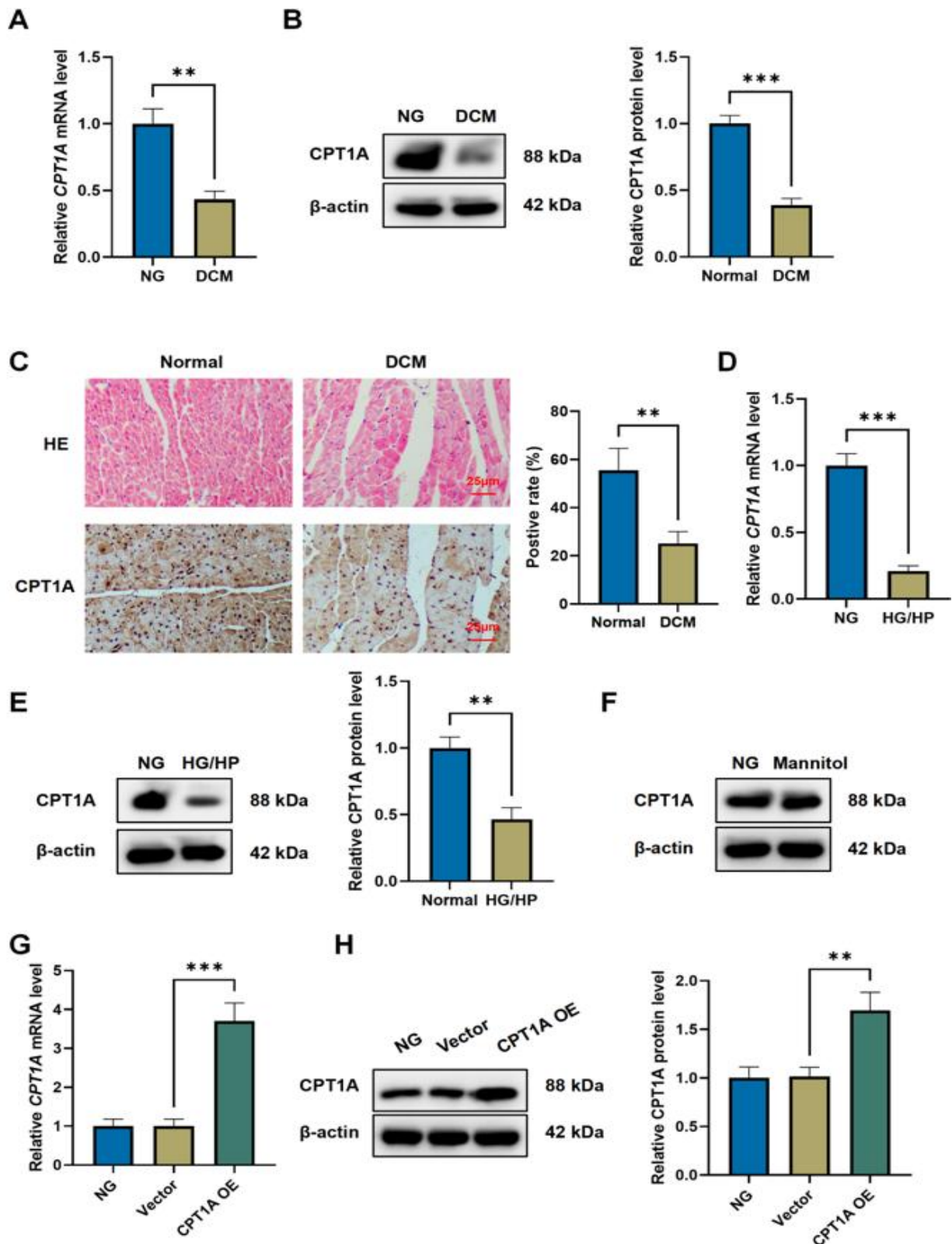


Fig. 1: CPT1A was downregulated in myocardial tissues and H9c2 cardiomyocytes under diabetic conditions. A–B: CPT1A mRNA and protein levels in myocardial tissues. C: H&E and CPT1A immunohistochemistry in myocardial tissues. D–E: CPT1A mRNA and protein levels in NG and HG/HP-treated H9c2 cardiomyocytes. F: CPT1A protein levels in NG and Mannitol-treated H9c2 cardiomyocytes. G–H: CPT1A mRNA and protein levels after CPT1A overexpression. Data were calculated as means±SD. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

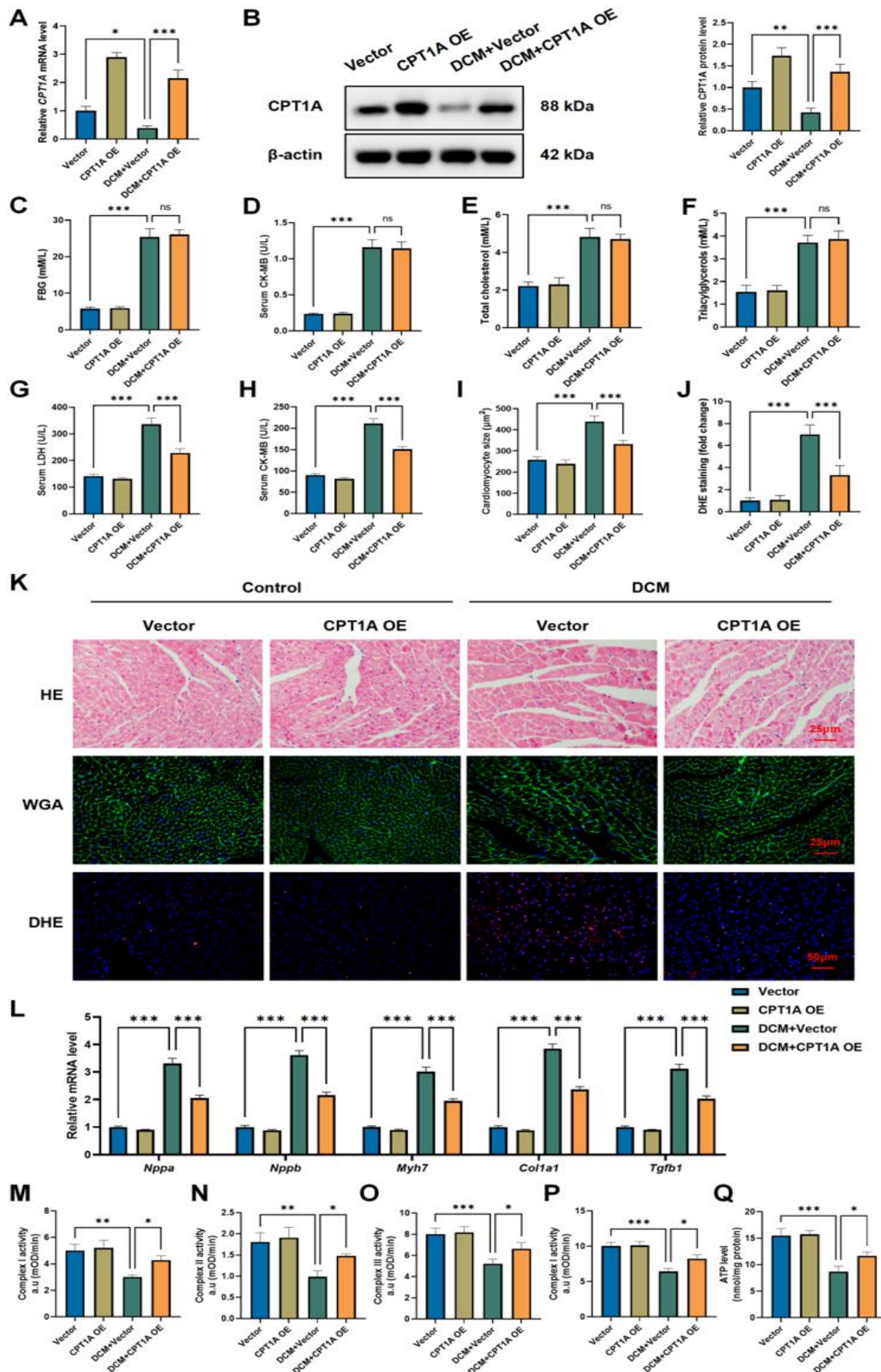


Fig. 2: CPT1A overexpression improves cardiac injury and mitochondrial dysfunction in DCM mice. Mice were divided into Vector, CPT1A OE, DCM+Vector, and DCM+CPT1A OE groups. A–B: CPT1A mRNA and protein expression. C–F: Serum metabolic parameters including FBG, LDL, TC, and TG. G–H: Serum LDH and CK-MB levels. I: Cardiomyocyte cross-sectional area (μm^2). J: DHE staining (fold change). K: Representative staining of HE, WGA, DHE. L: mRNA expression of cardiac hypertrophy and fibrosis markers (*Nppa*, *Nppb*, *Myh7*, *Col1a1*, *Tgfb1*). M–Q: Mitochondrial Complex I–IV activities (a.u., mOD/min) and ATP levels (nmol/mg protein). Data were calculated as means $\pm$ SD. * P <0.05, ** P <0.01, *** P <0.001.

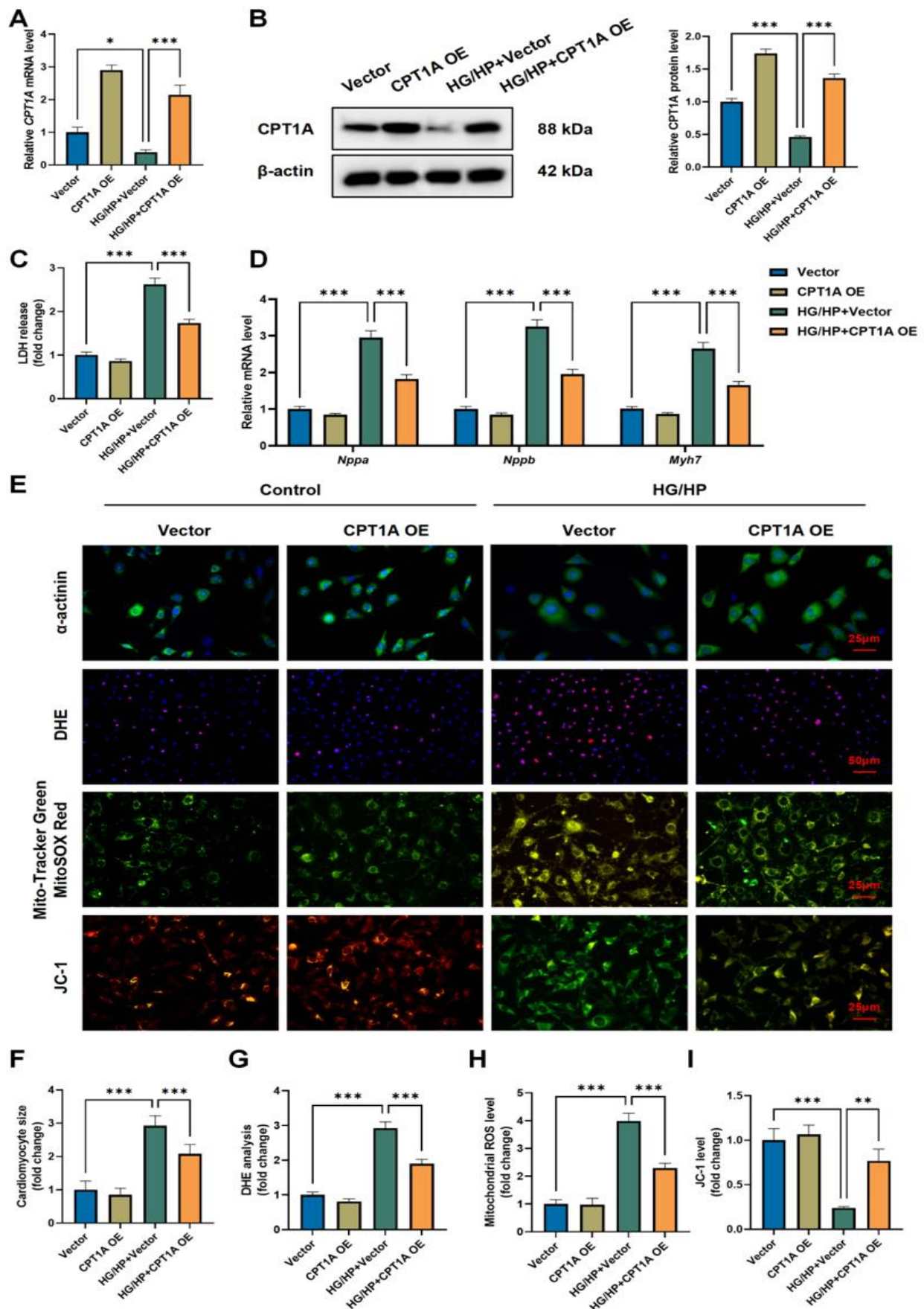


Fig. 3: CPT1A overexpression alleviates HG/HP-induced cardiomyocyte injury and mitochondrial dysfunction. Cells were divided into Vector, CPT1A OE, HG/HP+Vector and HG/HP+CPT1A OE groups. A–B: CPT1A mRNA and protein expression. C: LDH release. D: mRNA expression of hypertrophy-related genes (*Nppa*, *Nppb*, *Myh7*). E: Representative staining of α -actinin, DHE, MitoTracker Green, MitoSOX, and JC-1. F: Cardiomyocyte size. G: Intracellular ROS levels (DHE fluorescence, fold change). H: Mitochondrial ROS levels (MitoSOX fluorescence, fold change). I: Mitochondrial membrane potential (JC-1 ratio, fold change). Data were calculated as means $\pm$ SD. * P <0.05, ** P <0.01, *** P <0.001.

HG/HP exposure was observed to induce an increase in cardiomyocyte size, as demonstrated by α -actinin staining, whereas CPT1A overexpression significantly alleviated this hypertrophic effect (Fig. 3E, F). DHE staining showed that HG/HP significantly increased intracellular ROS production, which was suppressed by CPT1A overexpression (Fig. 3E, G). MitoSOX™ Red staining further demonstrated that HG/HP promoted mitochondrial ROS generation, whereas this increase was significantly attenuated by overexpression of CPT1A (Fig. 3E, H). Subsequently, mitochondrial membrane potential was evaluated by JC-1 staining analysis. HG/HP treatment resulted in a reduction of red JC-1 aggregate fluorescence accompanied by an increase in green monomer fluorescence, indicating mitochondrial depolarization. In contrast, CPT1A overexpression restored mitochondrial membrane potential under HG/HP conditions (Fig. 3E, I).

Taking together, these results indicate that overexpression of CPT1A confers a protective effect on H9c2 cardiomyocytes exposed to HG/HP conditions.

FOXA1 is downregulated under diabetic conditions and may regulate cardiac injury-related gene expression: To explore the mechanism underlying CPT1A downregulation under diabetic conditions, transcriptional regulators of CPT1A were predicted using the ChIPBase database. FOXA1 was identified as a potential transcription factor enriched in the promoter region of CPT1A. Bioinformatic analysis further revealed putative FOXA1 binding motifs and three potential binding sites within the CPT1A promoter region (Fig. 4A–B).

To validate FOXA1 expression under diabetic conditions, FOXA1 levels were assessed in both tissues and cells. The mRNA and protein levels of FOXA1 were markedly downregulated in myocardial tissues from the DCM group compared with normal control samples (Fig. 4C–D). Similarly, FOXA1 expression was significantly decreased in H9c2 cardiomyocytes treated with HG/HP relative to the NG group (Fig. 4E–F). To further confirm the role of FOXA1, FOXA1 was overexpressed in H9c2 cardiomyocytes. RT-qPCR and Western blot analyses verified successful FOXA1 overexpression (Fig. 4G–H).

FOXA1 overexpression mitigated HG/HP-induced cardiomyocyte injury, which was accompanied by reduced expression of hypertrophic marker genes, including *Nppa*, *Nppb* and *Myh7* (Fig. 4I). Moreover, FOXA1 overexpression markedly reduced the protein levels of β -MyHC, IL-1 β and cleaved caspase-3 in H9c2 cardiomyocytes exposed to HG/HP, indicating that FOXA1 may function as a protective factor against diabetes-associated cardiac hypertrophy, inflammatory responses, and apoptosis (Fig. 4J). Collectively, these findings indicate that FOXA1 may act as an upstream regulatory molecule involved in the pathogenesis of diabetic cardiomyocyte injury.

FOXA1 knockdown aggravates HG/HP-induced cardiomyocyte injury through suppression of CPT1A: To further clarify the regulatory interaction between FOXA1 and CPT1A, FOXA1 expression in H9c2 cardiomyocytes was suppressed using siRNA. RT-qPCR and Western blot assays showed that both FOXA1 mRNA

and protein levels were significantly reduced in the FOXA1 siRNA group relative to the control siRNA group, indicating successful gene silencing (Fig. 5A–B).

Protein analysis showed that HG/HP exposure markedly reduced both FOXA1 and CPT1A expression compared with the NG group. FOXA1 silencing further decreased CPT1A protein expression under HG/HP conditions, whereas CPT1A overexpression restored CPT1A levels without affecting FOXA1 expression (Fig. 5C).

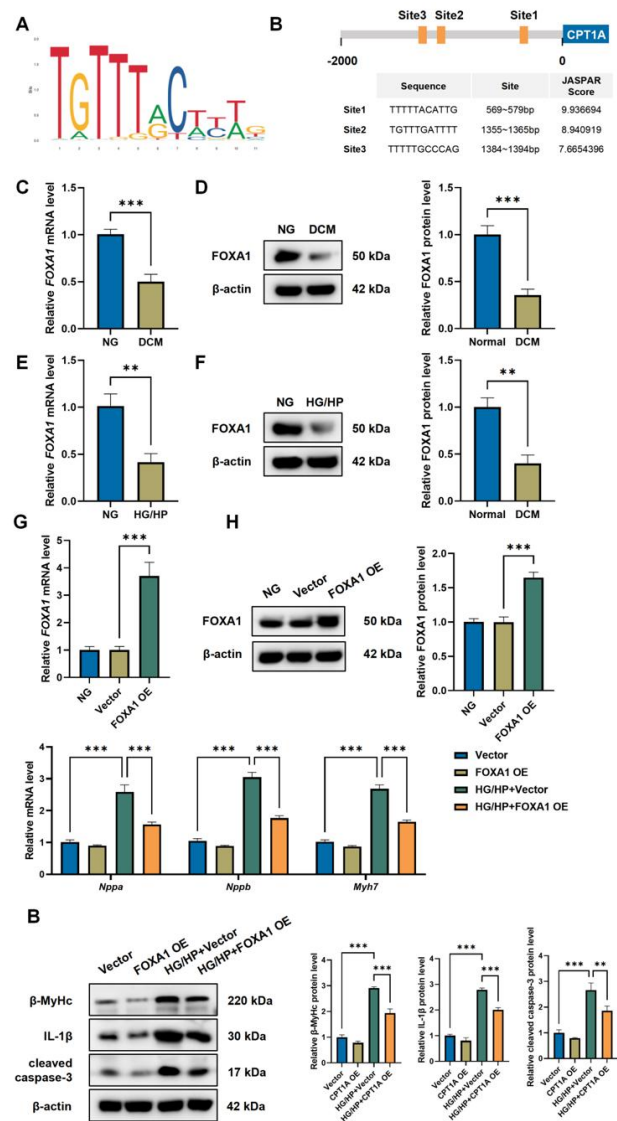


Fig. 4: FOXA1 is downregulated under diabetic conditions and regulates cardiac injury-related gene expression. A–B: Bioinformatic prediction of FOXA1 binding sites in the CPT1A promoter region (ChIPBase database analysis). C–D: FOXA1 mRNA and protein expression in Normal and DCM myocardial tissues. E–F: FOXA1 mRNA and protein expression in NG and HG/HP-treated H9c2 cardiomyocytes. G–H: FOXA1 mRNA and protein expression after FOXA1 overexpression. I: mRNA expression of hypertrophy-related genes (*Nppa*, *Nppb*, *Myh7*). J: Protein expression of β -MyHC, IL-1 β and cleaved caspase-3. Data were calculated as means $\pm$ SD. * P <0.05, ** P <0.01, *** P <0.001

Functional assays demonstrated that HG/HP significantly increased LDH release, which was further elevated following FOXA1 knockdown. Notably, CPT1A overexpression attenuated the increase in LDH induced by

FOXA1 silencing under HG/HP conditions (Fig. 5D). Similarly, HG/HP stimulation increased the expression of hypertrophy-related genes, including *Nppa*, *Nppb*, and *Myh7*. FOXA1 knockdown further enhanced these changes, whereas CPT1A overexpression markedly reversed the elevated expression of these genes in FOXA1-deficient cells (Fig. 5F).

Overall, these findings suggest that FOXA1 protects against HG/HP-induced cardiomyocyte injury, at least in part, through regulation of CPT1A.

FOXA1 knockdown aggravates HG/HP-induced oxidative stress and mitochondrial dysfunction in association with reduced CPT1A expression: To further determine whether FOXA1 is involved in cardiomyocyte injury through CPT1A, oxidative stress and mitochondrial function were evaluated following FOXA1 silencing and CPT1A restoration under HG/HP conditions. α -actinin

staining showed that HG/HP exposure induced cardiomyocyte hypertrophy, while FOXA1 silencing further increased cardiomyocyte size. This effect was markedly attenuated by CPT1A overexpression (Fig. 6A, B). DHE staining demonstrated that FOXA1 knockdown further enhanced HG/HP-induced intracellular ROS accumulation, whereas CPT1A overexpression largely reversed this increase (Fig. 6A, C). Similarly, MitoSOX staining revealed elevated mitochondrial ROS production following FOXA1 knockdown under HG/HP conditions, which was reduced after CPT1A restoration (Fig. 6A, D). In addition, JC-1 staining revealed that FOXA1 knockdown exacerbated the HG/HP-induced reduction in mitochondrial membrane potential, as evidenced by diminished red fluorescence alongside enhanced green fluorescence intensity. CPT1A overexpression partially restored mitochondrial membrane potential in FOXA1-deficient H9c2 cardiomyocytes (Fig. 6A, E).

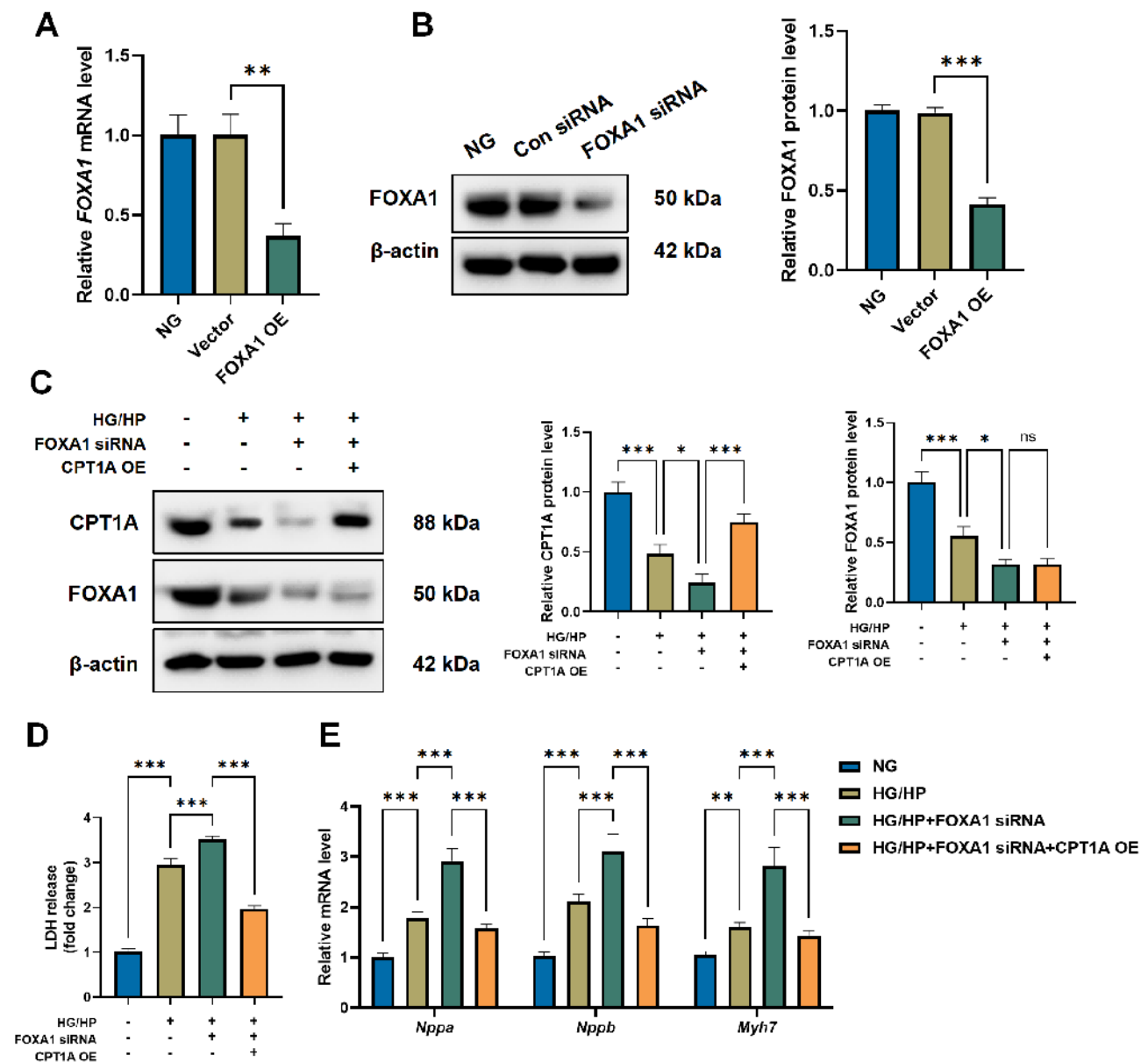


Fig. 5: CPT1A overexpression reverses FOXA1 silencing-mediated aggravation of HG/HP-induced cardiomyocyte injury. A–B: FOXA1 mRNA and protein expression in NG, Con siRNA and FOXA1 siRNA groups. Cells were divided into NG, HG/HP, HG/HP+FOXA1 siRNA and HG/HP+FOXA1 siRNA+CPT1A OE groups. C: Protein expression of FOXA1 and CPT1A. D: LDH release. E: mRNA expression of hypertrophy-related genes (*Nppa*, *Nppb*, *Myh7*). Data were calculated as means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

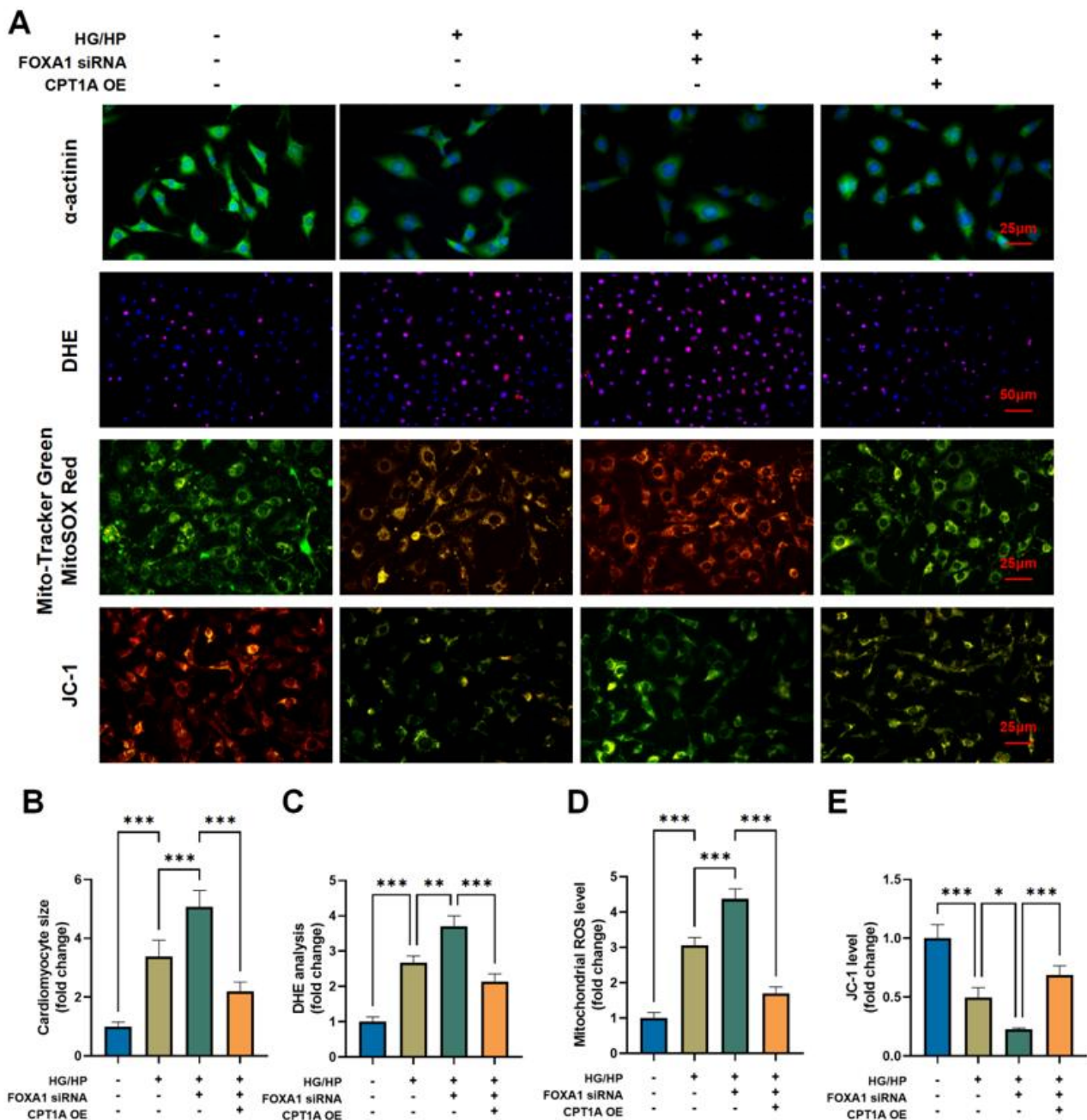


Fig. 6: FOXAI deficiency aggravates HG/HP-induced oxidative stress and mitochondrial dysfunction through CPT1A regulation. Cells were divided into NG, HG/HP, HG/HP+FOXAI siRNA and HG/HP+FOXAI siRNA+CPT1A OE groups. A: Representative staining of α -actinin, DHE, MitoTracker Green, MitoSOX and JC-1. B: Cardiomyocyte size. C: Intracellular ROS levels (DHE fluorescence, fold change). D: Mitochondrial ROS levels (MitoSOX fluorescence, fold change). E: Mitochondrial membrane potential (JC-1 ratio, fold change). Data were calculated as means $\pm$ SD. * P <0.05, ** P <0.01, *** P <0.001.

Collectively, these findings suggest that FOXAI knockdown aggravates HG/HP-induced oxidative stress and mitochondrial dysfunction, at least in part, in association with reduced CPT1A.

DISCUSSION

DCM is characterized by progressive myocardial remodeling, metabolic disturbances, oxidative stress and mitochondrial dysfunction, occurring independently of clinically apparent coronary artery disease or hypertension (Salvatore *et al.*, 2021). Because cardiomyocytes have a high demand for continuous ATP production, disruption of mitochondrial metabolic homeostasis is considered an

important contributor to DCM progression (Gollmer *et al.*, 2020; Huang *et al.*, 2026a). Under diabetic conditions, excessive glucose and lipid exposure disrupt mitochondrial homeostasis, leading to ROS accumulation, impaired respiratory chain activity, and myocardial injury (Dabravolski *et al.*, 2022; Pan *et al.*, 2024).

CPT1A, an important mitochondrial membrane-associated protein, is crucial for maintaining cellular energy metabolism and has recently been implicated in the pathogenesis and progression of cardiovascular disorders (Liang, 2023; Ni *et al.*, 2026). Although CPT1A is traditionally recognized as a rate-limiting enzyme in fatty acid transport, emerging studies suggest that CPT1A may also influence mitochondrial quality control,

oxidative stress responses, and cellular adaptation to metabolic stress (Li *et al.*, 2023; Liu *et al.*, 2025). Previous investigations have demonstrated that abnormal expression of CPT1A is strongly associated with impaired mitochondrial respiratory activity and aggravated oxidative stress-mediated injury in diabetic tissues (Peng *et al.*, 2023; Xiang *et al.*, 2024). In cardiac tissue, dysregulated CPT1A activity has been linked to myocardial metabolic remodeling and energy imbalance, both of which contribute to DCM progression (Tanabe *et al.*, 2025). In the present study, CPT1A expression was significantly decreased in DCM myocardial tissues and HG/HP-treated cardiomyocytes. Importantly, CPT1A restoration markedly attenuated cardiac injury and pathological remodeling in DCM mice, while also reducing ROS accumulation and improving mitochondrial function. These findings suggest that reduced CPT1A expression may contribute to diabetic cardiac injury by impairing mitochondrial metabolic homeostasis and increasing susceptibility to oxidative stress.

Oxidative stress is widely recognized as a key pathological hallmark of DCM (De Geest and Mishra, 2022). Excessive ROS production contributes to cardiomyocyte injury and mitochondrial dysfunction under diabetic conditions (Xu *et al.*, 2023; Xiong *et al.*, 2025). Previous studies have indicated that increased ROS production contributes to mitochondrial membrane depolarization and dysfunction of the respiratory chain in diabetic cardiac tissues (Kaludercic and Di Lisa, 2020; Pan *et al.*, 2024). In the present study, overexpression of CPT1A markedly attenuated intracellular and mitochondrial ROS accumulation in both DCM mice and HG/HP-treated cardiomyocytes, indicating that CPT1A may exert protective effects against oxidative stress-induced injury.

Mitochondrial dysfunction is a key pathogenic mechanism in DCM (Zhou *et al.*, 2023). In the present study, CPT1A restoration improved mitochondrial Complex I-IV activity and ATP production in diabetic hearts, suggesting that CPT1A may contribute to maintaining mitochondrial respiratory efficiency. Furthermore, overexpression of CPT1A restored mitochondrial membrane potential in HG/HP-treated cardiomyocytes, supporting the protective role of CPT1A in maintaining mitochondrial function under diabetic stress.

Cardiomyocyte hypertrophy and fibrosis are important pathological features of DCM and are strongly correlated with mitochondrial impairment and excessive oxidative stress (Huang *et al.*, 2026a). Increased expression of hypertrophic markers such as Nppa, Nppb and Myh7 often reflects pathological cardiac remodeling under diabetic conditions (Han *et al.*, 2025; Lu *et al.*, 2025). Previous reports suggest that oxidative injury promotes activation of pro-hypertrophic signaling pathways and extracellular matrix remodeling (Capece *et al.*, 2026). In this study, overexpression of CPT1A decreased the expression levels of hypertrophy- and fibrosis-associated genes, indicating that maintenance of mitochondrial function may indirectly inhibit pathological cardiac remodeling.

To further elucidate the upstream regulatory mechanism of CPT1A, FOXA1 was identified as a candidate transcription factor that may bind to the CPT1A

promoter region (Gan *et al.*, 2024). FOXA1 belongs to the fork head box transcription factor family and has been implicated in metabolic regulation, differentiation, and stress adaptation (Bernardo and Keri, 2012; Gan *et al.*, 2024). Although FOXA1 has been extensively studied in cancer metabolism and endocrine regulation, its role in diabetic cardiac injury remains poorly understood. Our results demonstrated that FOXA1 expression was decreased in diabetic conditions, consistent with the reduction observed for CPT1A. FOXA1 overexpression attenuated HG/HP-induced cardiomyocyte injury, whereas FOXA1 knockdown aggravated cellular damage. Importantly, CPT1A restoration partially rescued the detrimental effects induced by FOXA1 knockdown, including increased LDH release, hypertrophic gene expression, ROS accumulation, and mitochondrial dysfunction. These findings suggest that FOXA1 may exert cardioprotective effects, at least in part, through regulation of CPT1A. Similar transcriptional regulatory mechanisms have been reported in other metabolic diseases, where fork head family transcription factors modulate mitochondrial homeostasis and oxidative stress responses. Therefore, the FOXA1-CPT1A axis may represent a potentially underexplored regulatory mechanism in DCM.

Several limitations should be acknowledged. First, although FOXA1 binding sites were predicted using bioinformatic analysis, direct validation of FOXA1 binding to the CPT1A promoter by chromatin immunoprecipitation or luciferase reporter assays were not performed. Second, although the present study demonstrated the regulatory role of the FOXA1-CPT1A axis in cardiomyocyte injury, the downstream signaling pathways linking CPT1A to mitochondrial protection and redox balance were not fully elucidated. Third, all mechanistic studies were conducted using cellular and animal models; thus, additional validation in human DCM specimens is necessary to further enhance the clinical applicability of these results. In conclusion, our findings suggest that CPT1A is downregulated under diabetic conditions and that restoration of CPT1A alleviates oxidative stress, mitochondrial dysfunction, and cardiomyocyte injury. Furthermore, FOXA1 may act as a potential upstream regulator of CPT1A, contributing to mitochondrial protection under diabetic stress. These findings support a potential FOXA1-CPT1A regulatory axis in DCM and suggest its potential as a therapeutic target for diabetic cardiomyopathy.

Authors contribution: DC: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing—original draft; FW: Methodology, investigation, validation, and data curation; YX: Investigation, formal analysis, and visualization; CL: Investigation, validation, and data curation; MT: Methodology, interpretation of the results, and writing—review and editing; JL: Conceptualization, supervision, project administration and writing—review and editing; XZ: Conceptualization, supervision, project administration, interpretation of the results and writing—review and editing; All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Statement: All animal experiments were approved by the Animal Ethics Committee of South Zhejiang Institute of Radiation Medicine and Nuclear Technology Applications (Approval No. ZFY20260490) and were conducted in accordance with institutional guidelines and the international standards for animal care and use to ensure ethical treatment of animals.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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