Research Article

ΔnFGF1 Protects β-Cells against High Glucose-Induced Apoptosis via the AMPK/SIRT1/PGC-1α Axis

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1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by persistent hyperglycemia in the context of insulin resistance [1, 2]. Insulin resistance and pancreatic β-cell dysfunction are considered the central characteristics of T2DM [3, 4]. Long-term high blood glucose can induce the loss of β-cells, considered a key step in the development of diabetes [5]. Therefore, an effective strategy to prevent diabetes mellitus may be the reduction of pathological β-cell apoptosis induced by high blood glucose levels.

The fibroblast growth factor (FGF) family consists of 22 members that play important roles in regulating the function of endocrine-relevant tissues and various metabolic processes [6–9]. The biological activities of FGFs are regulated by FGF receptors, and multiple FGFs may share the same FGF receptors to modulate cellular activity [10, 11]. FGF21 increases insulin secretion in diabetic islets and protects β-cells against apoptosis through extracellular signal-regulated kinase 1/2 (Erk1/2) and AKT pathways [12]. As a recently found metabolic modulator, FGF1 has been reported to improve insulin resistance and β-cell insulin secretion in diabetic mouse models [13, 14]. However, the underlying mechanisms of these effects to counter glucolipotoxicity-induced β-cell dysfunction remain unclear.

Wild-type FGF1 (FGF1 WT) has mitogenic activity, limiting...
its therapeutic potential, but a nonmitogenic FGF1 (ΔnFGF1) lacking N-terminal residues 1–27 of FGF1\(^{WT}\) exhibits no apparent proliferative activity [15]. The goals of this study were to investigate the roles of ΔnFGF1 on glucose control and its protective effect against islet β-cell apoptosis after chronic administration in a diabetes model and determine the underlying mechanisms of these effects.

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is highly expressed in metabolically active tissues and plays a crucial role in whole-body energy homeostasis [16–18]. AMPK dysregulation has been implicated in metabolic disorders like insulin resistance and T2DM [19–21]. AMPK activation may mediate the effect of FGF1 to protect against nonalcoholic fatty liver disease (NAFLD) in T2DM mice [22], and FGF4 improves blood glucose through activation of the AMPK pathway [23], implicating the AMPK pathway in the protective effects of FGF treatment on pancreatic islet β-cells.

In this study, we explored the glucose-lowering effects of ΔnFGF1 in insulin resistance mouse models. Our results show that ΔnFGF1 significantly inhibits β-cell apoptosis and improves β-cell function through the AMPK/SIRT1/PGC-1α pathway. Overall, these results indicate the therapeutic potential of ΔnFGF1 to prevent high glucose-induced β-cell apoptosis for T2DM treatment.

2. Materials and Methods

2.1. Cell Culture and Treatments. Mouse pancreatic β-cell line MIN6 (Keygen Biotech, Nanjing, China) cells were cultured in RPMI1640 medium (Gibco) accompanied with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin. Cells were passaged every three days. Culture media was changed every 24 h.

For intracellular signaling studies, MIN6 cells were starved for 12 h followed by incubation with or without ΔnFGF1 (50 ng/mL) for 1 h. Cells were then exposed to media containing normal glucose (NG, 11.1 mM) as a control or high glucose (HG, 33 mM)+palmitic acid (PA, 0.5 mM) for 24 h and then lysed to detect protein expression by Western blot. For inhibitor experiments, MIN6 cells were also starved for 12 h and treated with AMPK inhibitor Compound C (10 μM, Selleck Chemicals, S7306) or SIRT1 inhibitor EX-527 (10 μM, MedChemExpress, HY-15452) or PGC-1α inhibitor SR-18292 (10 μM, MedChemExpress, HY-101491) for 1 h and then incubated in NG or HG+PA or HG+PA+ΔnFGF1 for 24 h and lysed to detect protein expression by Western blot. For siRNA knockdown experiments, MIN6 cells were seeded and grown in six-well plates for 24 h to achieve 70% confluence. Cell transfection was performed with the transfection reagent Lipofectamine 3000 in accordance with the manufacturer’s instructions. A 24 h transfection of AMPK siRNA (Santa Cruz Biotechnology, sc45313) was followed by starvation and treatment as described above.

2.2. Cell Counting Kit-8 Assay. MIN6 cells were seeded into a 96-well plate (3000 cells/well) and serum starved in RPMI1640 medium for 24 h. The MIN6 cells were then exposed to 50 ng/mL ΔnFGF1 or FGF1\(^{WT}\) for 24 h or exposed to 33 mM HG and 0.5 mM PA in the presence or absence of 50 ng/mL ΔnFGF1 for 24 h. Cell Counting Kit-8 (CCK-8, Jiancheng Bioengineering Institute, China) was used to assess cell viability according to the manufacturer’s instructions. In brief, cells treated as described above were incubated with 10 μL of CCK-8 solution at 37°C for 1 h. The optical density (OD) value was detected by a microplate reader (Thermo, USA).

2.3. TUNEL Labeling. MIN6 cells were seeded at a total concentration of 3 × 10\(^4\) on a glass coverslip in six-well plates for 24 h, followed by treatment with 33 mM HG and 0.5 mM PA in the presence or absence of 50 ng/mL ΔnFGF1 for 24 h. Then, cells were washed with sterile PBS twice, fixed using 4% paraformaldehyde (PFA) for 15 min, and then permeabilized with 0.2% Triton X-100 for 5 min. Subsequently, each slide was incubated with a terminal deoxynucleotidyl transferase- (TdT-) labeled reaction mix at 37°C for 60 min in the dark. Cell nuclei were counterstained with DAPI for 10 min. Finally, fluorescent images were captured by Leica SP8 confocal microscopy using the FITC channel (Leica, Wetzlar, Germany).

2.4. Flow Cytometry Analysis. MIN6 cells were seeded at a total concentration of 3 × 10\(^4\) and cultured in six-well plates. Apoptosis of MIN6 cells was detected using an Annexin V-FITC Apoptosis Detection Kit (A211, Vazyme, Shanghai, China). In brief, treated and untreated cells were washed with cold PBS and resuspended in 500 μL binding buffer and then incubated with 5 μL of Propidium Iodide (PI) and 5 μL of Annexin V-FITC in the dark for 10 min. The fluorescence intensity was measured using a flow cytometer (Beckman, USA) within 1 h and monitoring 1 × 10\(^5\) cells per sample. Apoptosis was analyzed using FlowJo VX10.

2.5. Animal Models. Eight-week-old male db/db (C57BLKS/J Leprdb/db) mice and normal db/m mice were purchased from GemPharmatech Co. Ltd. (Nanjing, China) and allowed to adapt to the new environment for no less than 7 days. All animals were fed with basal rodent chow and tap water ad libitum and housed at 22–24°C. The animal study was approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

10-week-old db/db mice received every other day intraperitoneal injection of ΔnFGF1 protein (0.5 mg/kg) for eight weeks, whereas nondiabetic db/m and db/db control mice were injected with the equal amount of 0.9% saline. Body weights were recorded before injection every two days, and glucose tests were performed weekly. Random nonfasted blood glucose was measured in mouse tail venous blood using an automatic glucose monitor (Roche, Germany). Finally, mice in each group were sacrificed and the pancreatic tissues were collected for further analysis.

2.6. Hematoxylin and Eosin (H&E) Staining. After dehydration and hydration, tissue sections were stained with H&E (Beyotime Biotech, Nantong, China) and captured by light microscopy (Nikon, Tokyo, Japan) to evaluate histological
changes. The pancreatic islet areas were measured by ImageJ (National Institutes of Health, USA).

2.7. Histology. For pancreatic tissue immunohistochemical (IHC) staining, tissue sections were deparaffinized, rehydrated, antigen recovered, and permeabilized with 0.2% Triton X-100 at room temperature for 15 min. After blocking with 0.5% bovine serum albumin (BSA, Sigma), sections were probed with anti-PCNA antibody (Santa, SC-25280, dilution: 1:100) at 4°C overnight and then incubated with anti-mouse IgG horseradish peroxidase- (HRP-) conjugated secondary antibody at room temperature for 1 h. Subsequently, sections were reacted with diaminobenzidine (DAB) for 3 min, and then, the reactions were quenched in double-distilled water, counterstained with hematoxylin, and examined by light microscopy (Nikon, Japan).

For immunofluorescence staining, samples were treated as for IHC analysis until sections were incubated with primary antibodies against insulin (Proteintech, 15848-1-AP, dilution: 1:100), cleaved caspase 3 (Cell Signaling, 9664, dilution: 1:200), SIRT1 (Proteintech, 13161, dilution: 1:1000), and PGC-1α (Proteintech, 66369, dilution: 1:200) overnight at 4°C. Next, the slides were incubated with goat anti-mouse (Abcam, ab150113, dilution: 1:1000) or goat anti-rabbit (Abcam, ab150077, dilution: 1:1000) antibodies for 1 h at room temperature. Sections were stained with DAPI to detect the nuclei, and the stained tissues were imaged by Leica SP8 confocal microscopy.

2.8. Western Blot Analysis. Briefly, 30 μg of total protein extracts from MIN6 cells was separated by SDS-PAGE and transferred to PVDF membranes (Merck Millipore, IPVH00010). The primary antibodies used are as follows: phospho-AMPK (Cell Signaling, 2535S, dilution: 1:1000), total AMPK (Cell Signaling, 5831S, dilution: 1:1000), cleaved caspase 3 (Abmart, MB0711, dilution: 1:1000), Bax (Santa, SC-493, dilution: 1:1000), Bcl-2 (Abmart, T30056, dilution: 1:1000), PGC-1α (Abmart, T56630, dilution: 1:1000), SIRT1 (Proteintech, 13161, dilution: 1:1000), and β-actin (Cell Signaling, 3700, dilution: 1:1000). Secondary antibodies (Cell Signaling, 7074 or 7076, dilution: 1:3000) were used to detect the immunoreactive bands at room temperature for 1 h. To visualize the immunoreactivity bands, the membranes were treated with an EasySee Western Blot Kit (Transgene Biotech, China) and densitometric analysis was carried out using ImageJ.

2.9. Statistical Analysis. Statistical analysis was performed using GraphPad Prism version 8.0. All data are presented as mean values ± SEM. For the comparison of two groups, unpaired Student’s t-test (two-tailed) was performed. One-way ANOVA was used to compare more than two groups. A value of p < 0.05 was considered statistically significant.

3. Results

3.1. ΔnFGF1 Ameliorates Diabetes in db/db Mice. To evaluate the protective effects of long-term treatment with ΔnFGF1 on type 2 diabetes, db/db mice received every other day intraperitoneal injection of ΔnFGF1 (0.5 mg/kg) for eight weeks (Figure 1(a)). Compared with saline-treated db/m mice, the blood glucose level was markedly increased in db/db mice from day 6 but restored to near normal level after ΔnFGF1 treatment (Figures 1(b) and 1(c)). The db/db mice treated with ΔnFGF1 exhibited effectively reduced food intake and water intake, with maintenance of a continuous upward trend in the saline-treated mice (Figures 1(d) and 1(e)), suggesting that ΔnFGF1 treatment significantly improved polydipsia and polyphagia caused by diabetes. As a consequence, the db/db mice treated with ΔnFGF1 showed notably reduced body weight compared with db/db mice treated with saline (Figures 1(f) and 1(g)). Thus, these data suggest that ΔnFGF1 suppressed body weight gain and blood glucose levels of db/db mice.

3.2. ΔnFGF1 Relieves β-Cell Apoptosis in db/db Mice. Given that β-cell apoptosis is the major risk factor for diabetes, we next investigated whether ΔnFGF1 protected against pancreatic β-cell apoptosis. We found markedly decreased areas of pancreatic islets in db/db mice injected with saline for 8 weeks compared to that in db/m mice, with the areas significantly increased nearly to normal level in ΔnFGF1-treated db/db mice (Figure 2(a)). Caspases are crucial mediators of apoptotic process, and cleaved- (C-) caspase 3 is frequently activated cell death protease that catalyzes the specific cleavage of numerous crucial cellular proteins [24, 25]. To further investigate whether ΔnFGF1 countered β-cell apoptosis in db/db mice, we performed coimmunostaining of insulin and C-caspase 3 in pancreatic islets. Compared with the saline-treated db/m mice, the numbers of insulin/C-caspase 3 double-positive β-cells were significantly increased in the saline-treated db/db mice. Conversely, ΔnFGF1 treatment notably decreased the numbers of double-positive pancreatic β-cells (Figure 2(b)). Additionally, there was no significant increase in immunostaining for proliferating cell nuclear antigen (PCNA) in pancreatic islets of db/db mice treated with ΔnFGF1 compared with that of db/db mice treated with saline (Figure 2(c)). Collectively, these data suggest that ΔnFGF1 protects against diabetes by inhibiting β-cell apoptosis without affecting cell proliferation in db/db mice.

3.3. ΔnFGF1 Protects MIN6 Cells against Glucolipotoxicity-Induced Apoptosis. Long-term hyperglycemia and high levels of circulating free fatty acids (FFAs) are related to pancreatic β-cell dysfunction [26]. To further evaluate the effect of ΔnFGF1, we next explored the damage effects of hyperglycemia and/or hyperlipidemia on MIN6 cells. Exposure to both 33 mM high glucose (HG) and 0.5 mM palmitic acid (PA) resulted in a notable decrease in antiapoptotic protein Bcl-2 expression compared with the untreated group (Figure 3(a)). Based on the established cell apoptosis model, we asked if ΔnFGF1 could inhibit cell apoptosis. Western blot analysis showed that the expression levels of proapoptotic protein C-caspase 3 and Bax significantly decreased in a dose-dependent manner in ΔnFGF1-treated MIN6 cells, with an optimal concentration of ΔnFGF1 at 50 mg/mL (Figure 3(b)).
To mimic in vivo hyperglycemia and hyperlipidemia conditions, MIN6 cells were treated with 0.5 mM PA and 33 mM HG for 24 h and cell viability was detected by CCK-8 assays. As shown in Figure 3(c), HG and PA combined treatment significantly decreased cell viability and ΔnFGF1 treatment notably increased cell survival. Importantly, compared with the increased cell proliferation in the wild-type FGF1- (FGF1WT-) treated group, ΔnFGF1 treatment showed no effect on cell proliferation (Figure 3(d)).

To confirm this effect of ΔnFGF1 was due to cell apoptosis, we next performed TUNEL assay to directly examine cell apoptosis. We found that ΔnFGF1 alleviated HG+PA-induced apoptosis of MIN6 cells as evidenced by decreased numbers of TUNEL-positive cells (Figures 3(e) and 3(g)). In parallel, immunofluorescence staining showed that ΔnFGF1 treatment markedly reduced C-caspase 3 expression induced by glucolipotoxicity in MIN6 cells (Figures 3(f) and 3(g)). Consistent with the immunostaining results, Western blot analysis revealed that treatment of ΔnFGF1 notably reversed the HG+PA-induced increased Bax and C-caspase 3 expression and decreased Bcl-2 (Figure 3(h)). Taken together, these results demonstrate that ΔnFGF1 can effectively inhibit glucolipotoxicity-induced apoptosis in MIN6 cells.

3.4. ΔnFGF1 Induces the Activation of the AMPK/SIRT1/PGC-1α Signaling Pathway. Numerous studies have demonstrated that FGF1 can activate the AMPK signaling pathway, which plays important pleiotropic roles in cellular responses to metabolic stress [27]. Activated AMPK further stimulates SIRT1 and subsequently increases the PGC-1α expression, thus playing a central regulatory role in energy metabolism [28]. We next evaluated the AMPK/SIRT1/PGC-1α signaling pathway in MIN6 cells after ΔnFGF1 treatment. We

**Figure 1:** The glucose-lowering effect of ΔnFGF1 in db/db mice. (a) The 10-week-old db/db mice received every other day intraperitoneal injection of ΔnFGF1 (0.5 mg/kg) or saline for eight weeks, with age-matched nondiabetic db/m littermates served as the normal control group. (b, c) Random nonfasted blood glucose and changes, (d, e) average food intake and water intake per mouse, and (f, g) body weight alteration. All data are presented as mean values ± SEM. n = 6 mice per group. * p < 0.05, ** p < 0.01, and *** p < 0.001. n.s.: no significance.
Figure 2: ΔnFGF1 relieves β-cell apoptosis in db/db mice. (a) H&E staining of pancreas tissue from mice sacrificed after intraperitoneal (i.p.) injection of ΔnFGF1 or saline for 8 weeks (left panel). The black dotted lines indicate the borders of pancreatic islets.Pancreatic islet areas were quantified using ImageJ (right panel). (b) Coimmunofluorescence staining of insulin (green) and C-caspase 3 (red) in pancreatic islets. DAPI was used to stain nuclei. The white arrows indicate insulin/C-caspase 3 double-positive β-cells. (c) Representative PCNA-positive cells in pancreas sections (left panel). The black dotted lines indicate the borders of pancreatic islets. PCNA-positive cells were quantified using ImageJ (right panel). All data are presented as mean values ± SEM. n = 5 mice per group. ***p < 0.001; n.s.: no significance.
found that ΔnFGF1 significantly increased AMPK phosphorylation in HG+PA-induced MIN6 cells (Figure 4(a)). The ΔnFGF1 treatment group also exhibited increased expression of SIRT1 and PGC-1α (Figure 4(b)). Furthermore, we examined the expression of SIRT1 and PGC-1α in pancreatic tissues and found higher protein levels of SIRT1 and PGC-1α in islets of ΔnFGF1-treated db/db mice than of db/db control mice (Figures 4(c)–4(e)). Therefore, we speculate that ΔnFGF1 modulates the AMPK/SIRT1/PGC-1α axis.

3.5. ΔnFGF1 Inhibits Glucolipotoxicity-Induced Apoptosis in MIN6 Cells via AMPK/SIRT1/PGC-1α Signaling. To determine whether the inhibitory effect of ΔnFGF1 on cell
apoptosis resulted from ΔnFGF1-induced activated AMPK signaling, we used the AMPK inhibitor Compound C (CC) to inhibit AMPK activity of MIN6 cells. Western blot result revealed that ΔnFGF1 increased the expression of p-AMPK, SIRT1, and PGC-1α, while CC abolished the ΔnFGF1-induced expression increase of these proteins (Figure 5(a)). Next, we investigated the effect of AMPK inhibition on apoptosis of islet β-cells and found that ΔnFGF1 treatment significantly increased the antiapoptotic protein expression of Bcl-2 and decreased the proapoptotic protein expression of Bax and C-caspase 3. Conversely, the antiapoptotic effects induced by ΔnFGF1 were impeded by CC treatment (Figure 5(b)). To further confirm that ΔnFGF1 inhibits cell apoptosis through AMPK signaling, we used siRNA to knockdown AMPK expression in MIN6 cells. We found that AMPK siRNA treatment notably inhibited the activation of SIRT1 and PGC-1α signaling by ΔnFGF1 (Figure 5(c)). In parallel, the antiapoptotic signaling induced by ΔnFGF1 was inhibited by AMPK siRNA (Figure 5(d)). Moreover, flow cytometry assay showed that ΔnFGF1 significantly alleviated glucolipotoxicity-induced apoptosis of cells, whereas cotreatment with AMPK inhibitor CC counteracted this effect of ΔnFGF1 (Figure 5(e)), suggesting the importance of AMPK in the regulation of apoptosis in MIN6 cell.

To confirm that ΔnFGF1 protects β-cell apoptosis by activating AMPK/SIRT1/PGC-1α signaling, we used EX-527 (SIRT1 inhibitor) and SR-18292 (PGC-1α inhibitor) to inhibit the activities of SIRT1 and PGC-1α, respectively. As expected, Western blot results showed that EX-527 abolished the increased expression of SIRT1 and PGC-1α and antiapoptotic effects induced by ΔnFGF1 (Supplementary Figure S1A). Similar results were also obtained after cotreatment with SR-18292 (Supplementary Figure S1B). All these results suggest that ΔnFGF1 inhibits apoptosis of MIN6 cells through activating the AMPK/SIRT1/PGC-1α signaling pathway.

4. Discussion

Stable β-cell numbers are critical in insulin resistance and secretion, with significant reductions in β-cell functions in T2DM [29–31]. In this study, we determined that ΔnFGF1, a nonmitogenic truncation of FGF1, effectively reduces blood glucose in T2DM mice. More importantly, ΔnFGF1 can protect β-cells from apoptosis induced by high glucose levels through AMPK/SIRT1/PGC-1α signaling in vivo and in vitro.

Administration of ΔnFGF1 to db/db mice resulted in a notable decrease of blood glucose. Additionally, insulin secretion and apoptosis of islet β-cells were dramatically improved in ΔnFGF1-treated db/db mice. Immunofluorescence staining revealed significant more insulin-positive pancreatic β-cells in the ΔnFGF1-treated db/db mice compared with that in the db/db control mice. Interestingly, ΔnFGF1 treatment of db/db mice for 8 weeks did not increase the number of PCNA-positive islet cells. As a crucial cellular energy sensor, AMPK regulates energy metabolism and promotes glucose uptake through the modulation...
The apoptotic rate was calculated as the percentage of Annexin V-positive cells divided by the total number of cells. (f) Schematic diagram illustrating the model of ΔnFGF1-mediated inhibition of pancreatic β-cell apoptosis in T2DM. All data are presented as mean values ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001. n.s.: no significance.
of metabolic cell signaling molecules [32–34]. Previous studies identified the activation of the AMPK/SIRT1 pathway as a potential target to inhibit apoptosis in diabetic mice models [35, 36]. We tested the effects of nFGF1 treatment on this pathway using MIN6, a pancreatic β-cell line, and found that treatment with nFGF1 significantly inhibited glucolipotoxicity-induced apoptosis, and consistent with previous reports, we observed a reduction in phosphorylated- (p-) AMPK and Sirtuin-1 (SIRT1) expression. We demonstrated that nFGF1 can attenuate glucolipotoxicity-induced apoptosis of MIN6 cells via activation of the AMPK/SIRT1 signaling pathway, indicating that nFGF1 promotes SIRT1 signaling via AMPK activation. AMPK can promote mitochondrial synthesis via the direct phosphorylation of PGC-1α [37]. SIRT1 also acts upstream of PGC-1α [38]. Our results showed that nFGF1 acts to phosphorylate AMPK and increase SIRT1 and PGC-1α expression. We also found that nFGF1 can directly inhibit proapoptotic protein C-caspase 3 and Bax as well as increase antiapoptotic protein Bcl-2 in HG+PA-treated MIN6 cells. Further, the use of an AMPK inhibitor, Compound C, abolished the protective effects of nFGF1. Overall, our results revealed that nFGF1 induces AMPK phosphorylation to increase SIRT1 and PGC-1α expression and inhibit apoptosis in pancreatic β-cells (Figure 5(f)).

In summary, this study uncovered a crucial role of nFGF1 to inhibit β-cell apoptosis and promote cell survival in diabetes. Long-term application of nFGF1 significantly reduced circulating blood glucose and increased the number of β-cells in db/db mice, by promoting insulin biosynthesis and increasing β-cell survival. Further investigation of the mechanism revealed that nFGF1 inhibits β-cell apoptosis via activating AMPK/SIRT1/PGC-1α signaling to improve β-cell function. Our results illustrate the effectiveness of nFGF1 as a potential treatment for high glucose-induced β-cell apoptosis and T2DM.

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
All authors declared no competing interests.

Authors’ Contributions
J.S. and Q.C. contributed to the conception and design of the study; X.C. and Z.J. were involved in the experimental design and performed the animal experiments; S.Z., Y.D., W.X., and B.P. carried out the cell experiments; J.L. performed the statistical analysis; Q.C. wrote the first draft of the manuscript; J.S. and J.Z. reviewed the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version. Qiong Che, Xinwei Chen, and Zhenyu Jia contributed equally to this work.

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Supplementary Materials
Figure S1: inhibition of the activity of SIRT1 and PGC-1α blocks the protective effects of nFGF1 on β-cell apoptosis. MIN6 cells were exposed to NG (11.1 mM) and HG+PA (33 mM HG+0.5 mM PA) in the presence or absence of nFGF1 with or without 10 μM EX-527 (Sirt1 inhibitor) and 10 μM SR-18292 (PGC-1α inhibitor) for 24 h. (A) The protein expression of Sirt1, PGC-1α, Bcl-2, Bax, and C-caspase 3 analyzed by Western blot (left panel) and quantitated using ImageJ (right panels) (n = 3). (B) The protein expression of PGC-1α, Bcl-2, Bax, and C-caspase 3 analyzed by Western blot (left panel) and quantitated using ImageJ (right panels) (n = 3). All data are presented as mean ± SEM. NG: normal glucose; HG: high glucose; PA: palmitic acid. "p < 0.05, **p < 0.01, and ***p < 0.001. n.s.: no significance. (Supplementary Materials)

References


