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Nicotinamide Mononucleotide Restores Pancreatic β-Cell Function in Type 2 Diabetes through SIRT6-HIF-1α-Mediated Mitochondrial Stress Adaptation and Glycolytic Reprogramming

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Nicotinamide Mononucleotide Restores Pancreatic β-Cell Function in Type 2 Diabetes through SIRT6-HIF-1α-Mediated Mitochondrial Stress Adaptation and Glycolytic Reprogramming

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Abstract

Type 2 diabetes mellitus (T2DM) is characterized by progressive pancreatic β-cell dysfunction, in which mitochondrial impairment represents a central pathogenic event. However, effective strategies to restore mitochondrial resilience and preserve β-cell function remain limited. Nicotinamide mononucleotide (NMN), a precursor of NAD^+, has emerged as a metabolic intervention capable of improving mitochondrial homeostasis; however, its regulatory mechanisms in diabetic β-cell failure remain incompletely understood.

Here, we demonstrate that mitochondrial adaptive responses are insufficiently activated during hyperglycemia-induced β-cell stress, resulting in mitochondrial dysfunction and impaired cellular survival. Pharmacological activation of NAD^+ metabolism by NMN markedly alleviated high glucose-induced mitochondrial injury in INS1 β-cells, characterized by reduced mitochondrial reactive oxygen species accumulation, restoration of mitochondrial membrane potential, and increased ATP production. Mechanistically, NMN selectively enhanced mitochondrial stress adaptation programs and promoted glycolytic metabolic remodeling through activation of the SIRT6-HIF-1α signaling axis. Genetic inhibition of HIF-1α abolished NMN-mediated restoration of glycolytic capacity, mitochondrial homeostasis, and cytoprotection, indicating that HIF-1α is essential for NMN-induced metabolic adaptation.

Consistently, long-term NMN administration improved glucose metabolism, increased circulating insulin levels, and preserved pancreatic islet integrity in db/db diabetic mice. Furthermore, NMN restored SIRT6 expression and enhanced HIF-1α-dependent glycolytic responses in diabetic pancreatic tissues.

Collectively, our findings identify a previously unrecognized SIRT6-HIF-1α-mediated mitochondrial adaptive pathway underlying NMN-mediated β-cell protection and provide new insights into metabolic strategies for preventing β-cell failure in T2DM.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both, posing a significant global health burden[1]. Type 2 diabetes mellitus (T2DM), the most prevalent form, arises from a

combination of insulin resistance in peripheral tissues and progressive pancreatic β-cell dysfunction[2]. Pancreatic β-cells are the primary source of insulin, and their mass, morphology, and functional capacity are critical for maintaining glucose homeostasis[3, 4]. A hallmark of T2DM is the progressive loss of β-cell mass and function, driven by multiple stressors, including oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, glucotoxicity, lipotoxicity, islet inflammation, fibrosis, and amyloid deposition[5-7]. Despite advances in T2DM management, no clinical therapies effectively prevent or reverse β-cell loss, leaving many patients reliant on exogenous insulin, which significantly impacts quality of life[8].

Recent studies have implicated mitochondrial dysfunction as a central contributor to β-cell failure in T2DM[9]. In both T2DM patients and animal models, β-cell mitochondria exhibit morphological abnormalities (e.g., swelling, network fragmentation, reduced mitochondrial mass) and functional impairments (e.g., decreased membrane potential, impaired oxidative phosphorylation, and diminished ATP production)[10, 11]. Mitochondria are critical for energy production, converting pyruvate from glycolysis into ATP via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation[12]. In β-cells, this process regulates glucose-stimulated insulin secretion (GSIS)[13]. Mitochondrial dysfunction disrupts GSIS by reducing ATP production and increasing reactive oxygen species (ROS), exacerbating oxidative stress and further impairing mitochondrial integrity[14].

Cells possess sophisticated mitochondrial quality-control mechanisms to maintain organelle integrity during stress. Among these mechanisms, the mitochondrial unfolded protein response (UPR^{mt}) functions as an adaptive signaling pathway.[16]. The UPR^{mt} is a stress response pathway that signals mitochondrial distress to the nucleus, upregulating chaperone proteins and metabolic genes to restore mitochondrial function[17, 18]. Specifically, UPR^{mt} enhances glycolysis and amino acid catabolism to sustain ATP production while suppressing TCA cycle and oxidative phosphorylation genes to promote cell survival [19, 20]. However, clinical antioxidant therapies targeting oxidative stress have shown limited efficacy in restoring β-cell function, suggesting that repairing mitochondrial damage is critical[21]. Recent studies have highlighted NAD^+ metabolism as a critical regulator of mitochondrial function and stress adaptation. Nicotinamide mononucleotide (NMN), a key NAD^+ precursor, can restore cellular NAD^+ availability and improve mitochondrial homeostasis in various disease models.

Nevertheless, the molecular mechanisms by which NMN regulates mitochondrial adaptation and protects pancreatic β-cells under diabetic conditions remain unclear.

Sirtuin proteins are NAD^+-dependent enzymes that regulate cellular metabolism, mitochondrial function, and stress responses. Among them, SIRT6 has emerged as an important regulator of glucose metabolism and mitochondrial homeostasis. Meanwhile, hypoxia-inducible factor-1α (HIF-1α) acts as a master transcriptional regulator of glycolytic metabolism and enables cells to adapt to mitochondrial stress by promoting glycolytic ATP production.

Based on these observations, we hypothesized that NMN may preserve β-cell function by activating a SIRT6-HIF-1α-dependent adaptive metabolic program. In this study, we investigated the protective effects of NMN in high glucose-stressed β-cells and db/db diabetic mice and identified a previously unrecognized role of the SIRT6-HIF-1α axis in regulating mitochondrial adaptation and glycolytic reprogramming during diabetic β-cell dysfunction.

Materials and Methods

Animals

Eight-week-old *C57 BLKS/JGpt* db/db male mice (*BKS- Leprem2Cd479/Gpt*, *n*=12), and *C57BL/6J* male mice (20–25 g, *n*=12) were purchased from Gempharmatech co. Ltd (Nanjing, China). All mice were housed under 12h light-dark cycles at 24±1°C with free access to rodent feed and water. in the Experimental Animal Centre of Children's Hospital of Soochow University. All mice experiments were approved through the Animal Ethics Committee of Suzhou University and were performed according to the Guidelines for Ethical Conduct in the Care and Use of Animals. The animal suffering was made to minimize for the best of efforts. The mice were divided into four groups, some db/db mice and *C57BL/6J* male mice treated with NMN (200mg/kg, n=10), others treated with double distilled water(n=10). During the 8 weeks of administration, the body weight was measured weekly, and the blood glucose level was measured every four weeks. After the administration, the mice were treated in accordance with ethical standards, blood and animal tissues were taken for determination of relevant indicators.

Cells

Rat pancreatic islet cell tumor (INS1) cells are a gift from Xiaoxing Yin's group at the

School of Pharmacy, Xuzhou Medical University. Normal INS1 cells are adherent growth cells and are routinely cultured in BASIC RPMI 1640 (sugar concentration 11.1 mmol/L) medium containing 10% fetal bovine serum (FBS), 50μmol/L mercaptoethanol, 0.5% penicillin, and 5% CO2, Cultivate in a constant temperature incubator at 37°C.

Blood glucose measurement

The glucose measurement kit was purchased from Bioassay Systems, USA. Plasma tissue samples were prepared by orbital blood sampling, centrifuged, and the supernatant was absorbed for use. The blood glucose level of mice was measured according to the instructions.

Immunohistochemistry

Pancreatic tissue fixed in 4% formaldehyde was embedded in paraffin for morphological examination. The 4-μm thick coronal sections were stained with insulin-1 (Servicebio, Wuhan, China), according to the instruction of manufacturer. Finally, Pancreatic beta cells were observed with an Olympus CX31 microscope. Using the image analysis software Image-pro-plus 6.0, the integrated optical density (IOD) was used as an indicator to quantitatively analyze the immunohistochemical staining pictures of each group. The result is quantified by the ratio of the average integrated optical density (IOD) to the statistical area (area) of the picture, and its size indicates the intensity of the immune response of the relevant protein.

CCK-8 method to determine the survival rate of INS1 cells

The main component of CCK-8 reagent is a water-soluble tetrazolium salt WST-8, namely 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-Benzene disulfonate)-2H-tetrazole monosodium salt. It continuously reflects with the dehydrogenase in living cells to make the color of the solution deepen. Normal cultured INS1 cells were inoculated into 96-well plates at a density of 1×104/ml, with 6 multiple Wells in each group. After 12h, the cells were treated with medicine for 48 hours. Add 10 μl of CCK solution to each well, do not create bubbles, and then continue to culture for 1-4 hours. The light absorption value is measured at 450 nm with a microplate reader, which can indirectly reflect the number of living cells.

Mitochondrial morphology detection

Normally cultured INS1 cells were induced with high glucose and NMN was given for 48h. 4% formaldehyde fixation and punching, TOM20 antibody labeled mitochondrial TOM20, immunofluorescence reagent purchased from Thermo Fisher Scientific in the United States to

observe the fluorescence of TOM20, thereby detecting the mitochondrial morphology.

Lactic acid determination

The lactic acid determination kit was purchased from Bioassay Systems, USA. Routinely cultured INS1 cells were induced by high glucose and NMN (50mM) for 48h, the changes in cell lactic acid content were measured according to the instructions. The assay is based on the enzymatic conversion of lactate to pyruvate, coupled with the reduction of NAD^+ to NADH. Subsequently, NADH reduces a tetrazolium salt (2-p-iodophenyl-3-nitrophenyl tetrazolium chloride) in the presence of diaphorase, generating a formazan product that exhibits a strong absorbance peak at 490 nm. The absorbance is directly proportional to the amount of LDH released, which correlates with the degree of cell death.

Mitochondrial ATP levels

The measurement of mitochondrial ATP levels is a critical assay in biomedical research, as ATP (adenosine triphosphate) serves as the primary energy currency of cells, and its production is tightly linked to mitochondrial function. Remove the culture medium and add lysis buffer to each well of a 6-well plate at a ratio of 200 μl per well (equivalent to 1/10 of the total culture medium volume of 2 ml) to lyse the cells. Add 100 μl of ATP detection working solution to each assay well. Then, add 20 μl of the sample or standard to each well, mix quickly using a pipette, and wait for at least 2 seconds before measuring the relative light units (RLU) using a luminometer.

Mitochondrial ROS and Membrane Potential Detection

Mitochondrial reactive oxygen species (ROS) and TMRM are critical indicators of mitochondrial function and cellular health. To detect mitochondrial reactive oxygen species (ROS) and membrane potential in live cells using Mito-SOX and TMRM staining, respectively, and visualize nuclear staining with Hoechst.

Flow cytometry

Cells were seeded into a 12-well plate, with an appropriate volume of culture medium added to each well. Three replicate wells were set up for each group to ensure experimental reproducibility and statistical significance. According to the kit instructions, 10× Binding Buffer was diluted with deionized water to prepare 1× working solution. 5 μL of Annexin V-FITC (a fluorescent-labeled apoptosis probe) and 10 μL of PI (propidium iodide, a dead cell

dye) were added.The mixture was gently vortexed and incubated at room temperature in the dark for 5 minutes. After staining, samples were analyzed by flow cytometry within 1-2 hours to avoid fluorescence signal decay.

Statistical analysis

GraphPad Prism 9.5 was used for statistical analysis and graphing. Differences between the groups were assessed using one-way of analysis of variance followed by Dunnett's multiple comparisons test. The data are shown as the mean ± SEMs of at least three biologically independent experiments.

Results

Nicotinamide Mononucleotide (NMN) Protects Pancreatic β-Cells against hyperglycemia-induced mitochondrial dysfunction

Pancreatic β-cells are highly dependent on mitochondrial oxidative metabolism to maintain glucose-stimulated insulin secretion. To determine whether NMN protects β-cells from diabetic stress, INS1 cells were exposed to high glucose (HG, 60 mmol/L) and treated with NMN or other metabolic protective compounds (vitamin C, coenzyme Q9 (Q9), glucagon-like peptide-1 (GLP-1), and metformin). Among the tested compounds, NMN exhibited the strongest protective effect against HG-induced cellular injury, as determined by CCK-8 assays (Figure 1A-B). We next evaluated mitochondrial functional alterations, which represent a major determinant of β-cell failure. HG stimulation markedly increased mitochondrial ROS production, reduced mitochondrial membrane potential, and impaired ATP generation. However, NMN supplementation significantly reversed these mitochondrial defects, restoring mitochondrial energetic capacity and reducing oxidative stress (Figure 1C-F).

These findings indicate that NMN effectively preserves β-cell viability by maintaining mitochondrial functional homeostasis under hyperglycemic stress.

NMN promotes mitochondrial adaptive responses and glycolytic metabolic remodeling

Mitochondrial dysfunction activates cellular stress adaptation pathways aimed at restoring organelle homeostasis. We therefore investigated whether NMN modulates mitochondrial stress responses under hyperglycemic conditions. High glucose exposure failed to sufficiently activate mitochondrial adaptive signaling, whereas NMN treatment selectively increased the

expression of mitochondrial stress-response marker HSP60 without affecting ER stress marker GRP78 (Figure 2A-B), suggesting preferential activation of mitochondrial adaptive pathways. Because mitochondrial stress adaptation is closely associated with metabolic remodeling, we next examined glycolytic activity. HG stimulation reduced lactate production and suppressed glycolytic enzymes, including HIF-1α, PKM2, GAPDH, and LDHA. NMN treatment restored glycolytic activity and increased the expression of these glycolytic regulators, while reducing PDHA1 expression (Figure 2C-D).

Together, these results suggest that NMN promotes mitochondrial adaptation through metabolic reprogramming, favoring glycolytic ATP production under diabetic stress conditions.

HIF-1α is required for NMN-mediated metabolic adaptation and β-cell protection

HIF-1α is a central transcription factor controlling glycolytic adaptation during cellular stress. To determine whether HIF-1α mediates the protective effects of NMN, HIF-1α expression was silenced using siRNA. HIF-1α knockdown markedly abolished the beneficial effects of NMN on HG-induced β-cell injury. Specifically, loss of HIF-1α prevented NMN-mediated reduction of mitochondrial ROS accumulation, restoration of ATP production, and induction of mitochondrial adaptive responses (Figure 3A-G). Furthermore, HIF-1α deficiency blocked NMN-induced upregulation of glycolytic enzymes, including PKM2, GAPDH, and LDHA, while maintaining elevated PDHA1 expression (Figure 3H-I).

These findings demonstrate that HIF-1α is an essential mediator of NMN-induced glycolytic remodeling and mitochondrial protection.

NMN improves glucose metabolism and preserves pancreatic islet integrity in db/db mice

To determine whether the protective effects of NMN extend to an in vivo diabetic setting, db/db mice were treated with NMN for 8 weeks. NMN administration significantly reduced body weight gain and improved fasting blood glucose levels compared with untreated db/db mice (Figure 4A-B). In addition, NMN increased serum insulin concentrations, suggesting improved pancreatic endocrine function (Figure 4C). Histological examination revealed that diabetic mice exhibited disrupted islet architecture and reduced insulin expression. NMN treatment markedly preserved pancreatic islet morphology and enhanced insulin immunoreactivity (Figure 4D-E).

These findings demonstrate that NMN alleviates β-cell dysfunction and improves glucose homeostasis in diabetic mice.

NMN restores HIF-1α-dependent glycolytic adaptation in diabetic pancreatic tissues

To validate whether NMN regulates glycolytic metabolism in vivo, we analyzed metabolic pathway markers in pancreatic tissues from db/db mice. Consistent with in vitro findings, diabetic mice exhibited reduced expression of glycolytic regulators, including HIF-1α, GAPDH, and LDHA, accompanied by increased PDHA1 expression. NMN administration restored glycolytic enzyme expression and normalized PDHA1 levels (Figure 5A-B).

These results indicate that NMN promotes a compensatory glycolytic metabolic program in diabetic pancreatic tissues.

SIRT6 mediates NMN-induced activation of the HIF-1α metabolic pathway

Because NMN functions primarily through NAD^+ metabolism, we investigated potential NAD^+-dependent regulators involved in HIF-1α activation.

Among several candidate sirtuins examined, SIRT6 showed the most prominent NMN-dependent regulation. SIRT6 expression was reduced in diabetic conditions but restored following NMN treatment (Figure 6A). Furthermore, SIRT6 knockdown abolished NMN-mediated restoration of HIF-1α expression and downstream glycolytic enzymes under HG stimulation (Figure 6B).

These data identify SIRT6 as an upstream regulator of HIF-1α-dependent metabolic adaptation induced by NMN.

DISCUSSION

Pancreatic β-cell failure is a defining feature of T2DM progression and represents a major limitation of current therapeutic strategies. Although multiple metabolic stresses contribute to β-cell deterioration, mitochondrial dysfunction has emerged as a central pathogenic mechanism because β-cells rely heavily on mitochondrial oxidative metabolism to couple nutrient sensing with insulin secretion. In this study, we demonstrate that pharmacological restoration of NAD^+ metabolism through NMN supplementation preserves β-cell function under diabetic stress by activating a SIRT6-HIF-1α-dependent mitochondrial adaptive program and promoting glycolytic metabolic remodeling.

Previous studies have established that mitochondrial damage caused by chronic hyperglycemia contributes to β-cell exhaustion through excessive ROS accumulation, impaired ATP production, and defective insulin secretion. Consistent with these observations, our study showed that high glucose exposure induced substantial mitochondrial dysfunction in β-cells, characterized by increased mitochondrial oxidative stress, decreased membrane potential, and reduced ATP production. Importantly, NMN treatment effectively reversed these abnormalities, indicating that enhancing mitochondrial resilience may represent a promising strategy for maintaining β-cell viability under diabetic conditions.

Rather than simply suppressing oxidative stress, cells rely on adaptive mitochondrial signaling networks to restore metabolic homeostasis during stress. The mitochondrial unfolded protein response (UPRmt) represents an important mitochondrial-to-nuclear communication pathway that promotes proteostasis and metabolic adaptation. However, whether insufficient activation of mitochondrial adaptive responses contributes to β-cell failure remains poorly understood. Our findings reveal that high glucose stress alone was insufficient to induce robust mitochondrial adaptive responses, whereas NMN supplementation selectively enhanced mitochondrial stress-associated signaling, suggesting that impaired mitochondrial adaptation may represent an important component of diabetic β-cell dysfunction.

A key finding of this study is the identification of HIF-1α as an essential mediator of NMN-induced metabolic adaptation. HIF-1α is traditionally recognized as a regulator of cellular responses to hypoxia, but accumulating evidence indicates that HIF-1α also coordinates metabolic flexibility by enhancing glycolytic flux and maintaining ATP production under conditions of mitochondrial stress. In our study, NMN restored HIF-1α expression and increased glycolytic enzyme expression, whereas HIF-1α depletion abolished the protective effects of NMN on mitochondrial function and cell survival. These findings indicate that HIF-1α-dependent metabolic remodeling is required for NMN-mediated β-cell protection.

The activation of glycolysis observed in our study may appear counterintuitive because β-cells are highly dependent on mitochondrial oxidative metabolism for glucose-stimulated insulin secretion. However, under pathological conditions associated with mitochondrial impairment, enhanced glycolytic capacity may serve as an adaptive mechanism to maintain ATP availability and prevent energy collapse. Thus, NMN-induced glycolytic remodeling may

represent a compensatory response that allows β-cells to preserve cellular energy balance during diabetic stress rather than a permanent metabolic shift away from mitochondrial metabolism.

NAD^+ metabolism has emerged as a critical regulator of mitochondrial function and cellular stress resistance. NMN, as an NAD^+ precursor, improves mitochondrial activity in multiple disease models; however, the downstream molecular mechanisms remain incompletely understood. Our findings identify SIRT6 as a key mediator linking NMN metabolism to HIF-1α activation. SIRT6 expression was reduced under diabetic conditions but restored following NMN treatment, while SIRT6 depletion prevented HIF-1α restoration and metabolic adaptation. These results suggest that the SIRT6-HIF-1α axis represents an important signaling pathway through which NMN enhances β-cell stress resistance.

Consistent with cellular findings, NMN administration improved glucose homeostasis and preserved pancreatic islet integrity in db/db mice. These in vivo results support the translational potential of targeting NAD^+ metabolism to delay β-cell decline during T2DM progression. However, several limitations remain. First, although our data demonstrate that NMN activates mitochondrial adaptive responses, further studies using additional mitochondrial stress-response markers and genetic models are required to fully define the contribution of specific UPRmt components. Second, whether SIRT6 directly regulates HIF-1α stability, transcriptional activity, or post-translational modification requires further investigation. Finally, validation in human pancreatic islets will be necessary to determine the clinical relevance of this pathway.

In conclusion, this study reveals a previously unrecognized role of the SIRT6-HIF-1α signaling pathway in NMN-mediated β-cell protection. By enhancing mitochondrial adaptive capacity and promoting glycolytic metabolic flexibility, NMN preserves β-cell function under diabetic stress. These findings provide new mechanistic insights into NAD^+ metabolism-based strategies for preventing β-cell failure in T2DM.

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Author contributions

YC, QZ and GQ designed the study. YC, QZ and GQ performed most of the experiments. QW and CM performed some of the biochemical and cellular experiments. YC analyzed the data. YC drafted the manuscript. CG, RW and MW revised the manuscript. All the authors read and approved the manuscript.

Competing interests

The authors declare no competing interests.

Data and materials availability

All the data are available in the main text.

Ethics declarations

All experimental methods were performed in accordance with the relevant guidelines and regulations. Animal experiments were conducted according to the regulations established by the Animal Ethics Committee of Soochow University, and protocols approved by the ethical committee of Children's Hospital of Soochow University. The use of cell lines in this study complied with the relevant ethical guidelines for cell research.

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Figures

Figure 1

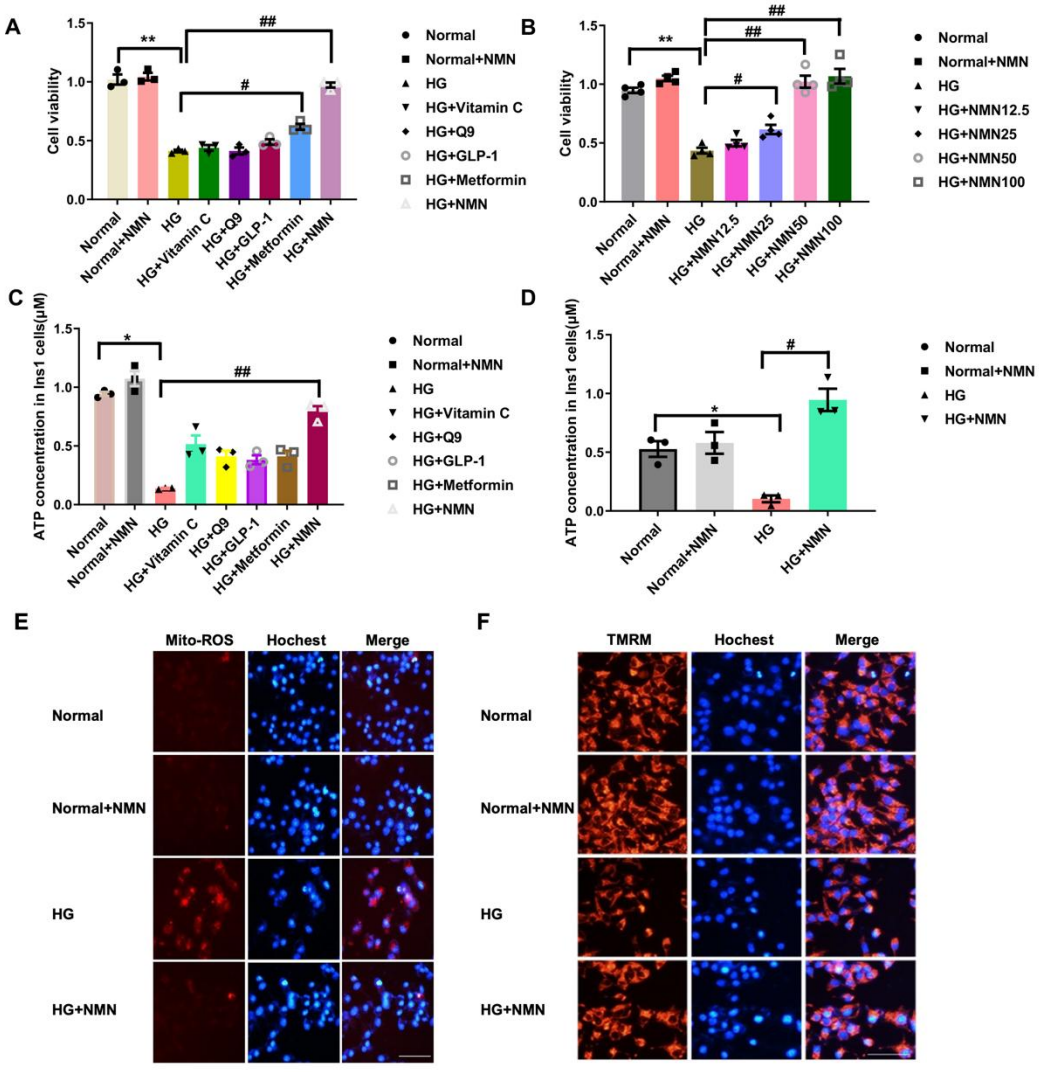


Figure 1. NMN protects INS1 β-cells against high glucose-induced mitochondrial impairment. (A) Cell viability analysis of INS1 cells treated with high glucose (HG) and indicated metabolic protective compounds. NMN exhibited the strongest protective effect against HG-induced cellular injury.

(B) Dose-dependent effects of NMN on INS1 cell viability under HG stimulation.

(C–F) Assessment of mitochondrial functional alterations following HG exposure with or without NMN treatment. NMN supplementation restored mitochondrial ATP production (C and D), reduced mitochondrial ROS accumulation (E), and preserved mitochondrial membrane potential (F).

Data are presented as mean ± SEM from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. # $p<0.05$, ## $p< 0.01$, * $p<0.05$, ** $p<0.01$.

Figure 2

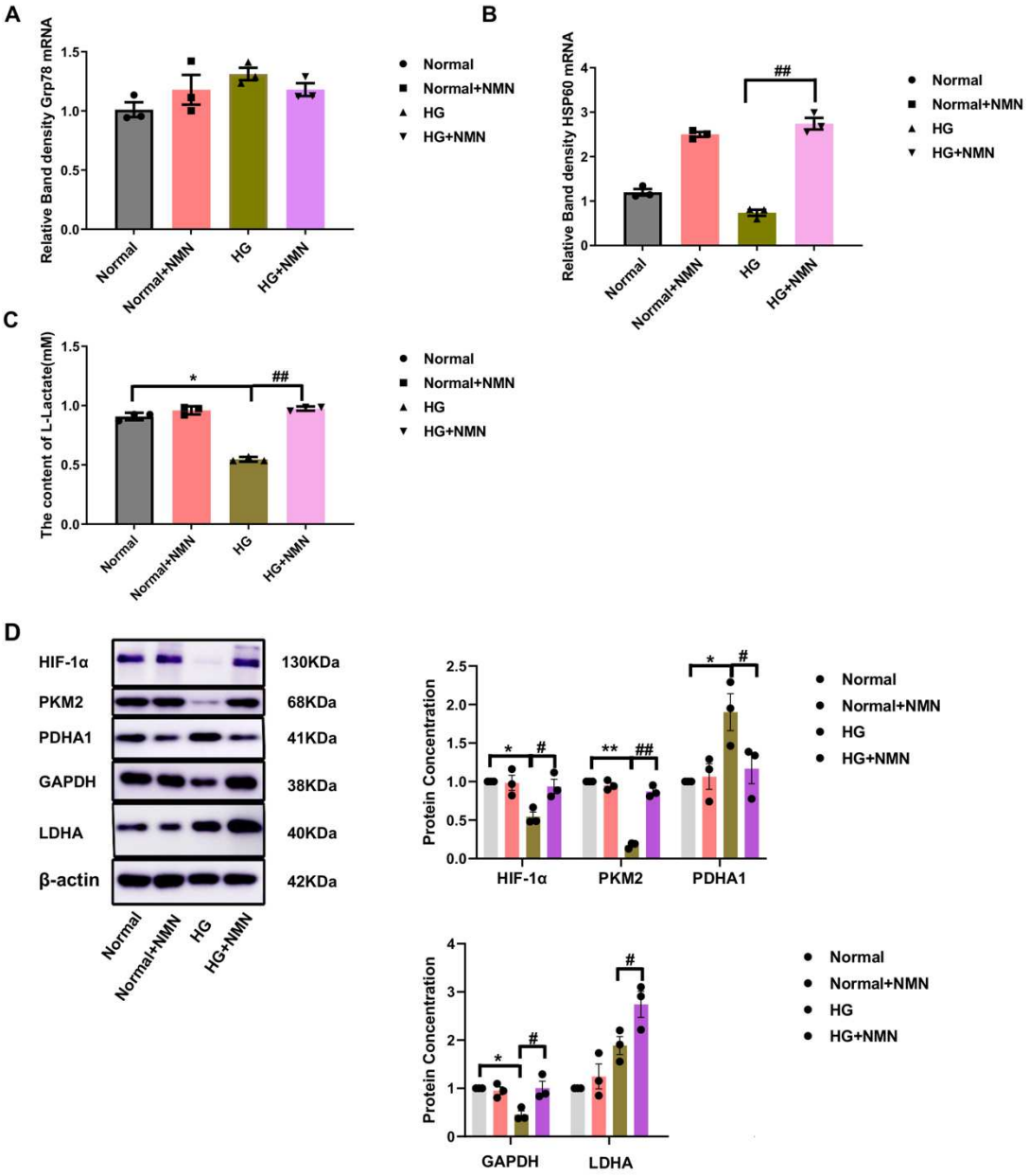


Figure 2. NMN promotes mitochondrial stress adaptation and glycolytic metabolic reprogramming in β-cells.

(A–B) Expression analysis of mitochondrial adaptive response marker HSP60 and endoplasmic reticulum stress marker GRP78 in INS1 cells following HG and NMN treatment.

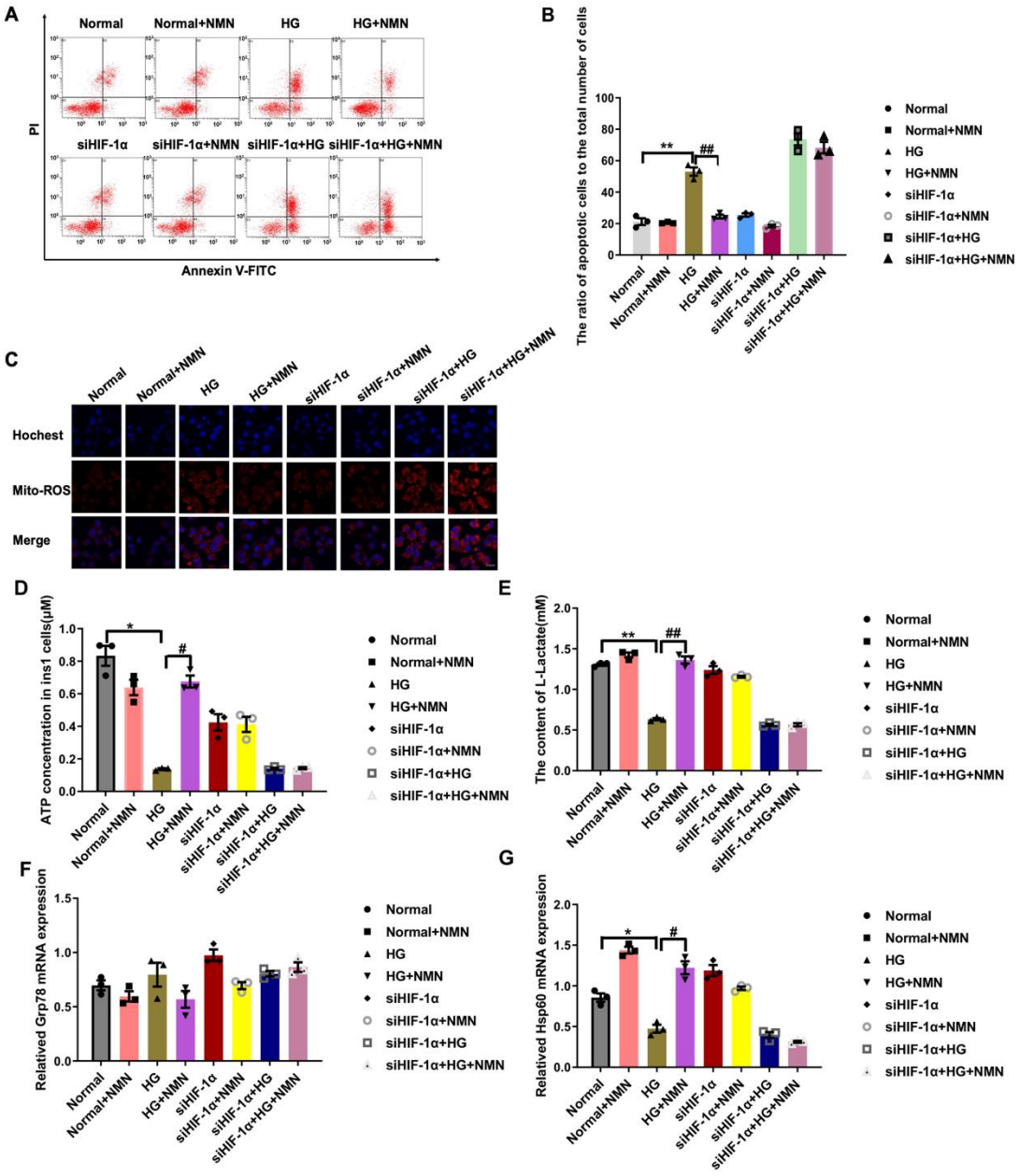
(C) Lactate production analysis indicating enhanced glycolytic activity following NMN supplementation.

(D) Western blot analysis of glycolytic regulators (HIF-1α, PKM2, GAPDH, LDHA) and

mitochondrial oxidative metabolism marker PDHA1.

Data are presented as mean ± SEM from independent experiments. #p<0.05,##p<0.01,*p<0.05,**p<0.01.

Figure 3


A
Normal
Normal+NMN
HG
HG+NMN
siHIF-1α
siHIF-1α+NMN
siHIF-1α+HG
siHIF-1α+HG+NMN
PI
Annexin V-FITC
B
The ratio of apoptotic cells to the total number of cells
C
Hochest
Mito-ROS
Merge
D
ATP concentration in ins1 cells(μM)
E
The content of L-Lactate(mM)
F
Relatived Grp78 mRNA expression
G
Relatived Hsp60 mRNA expression

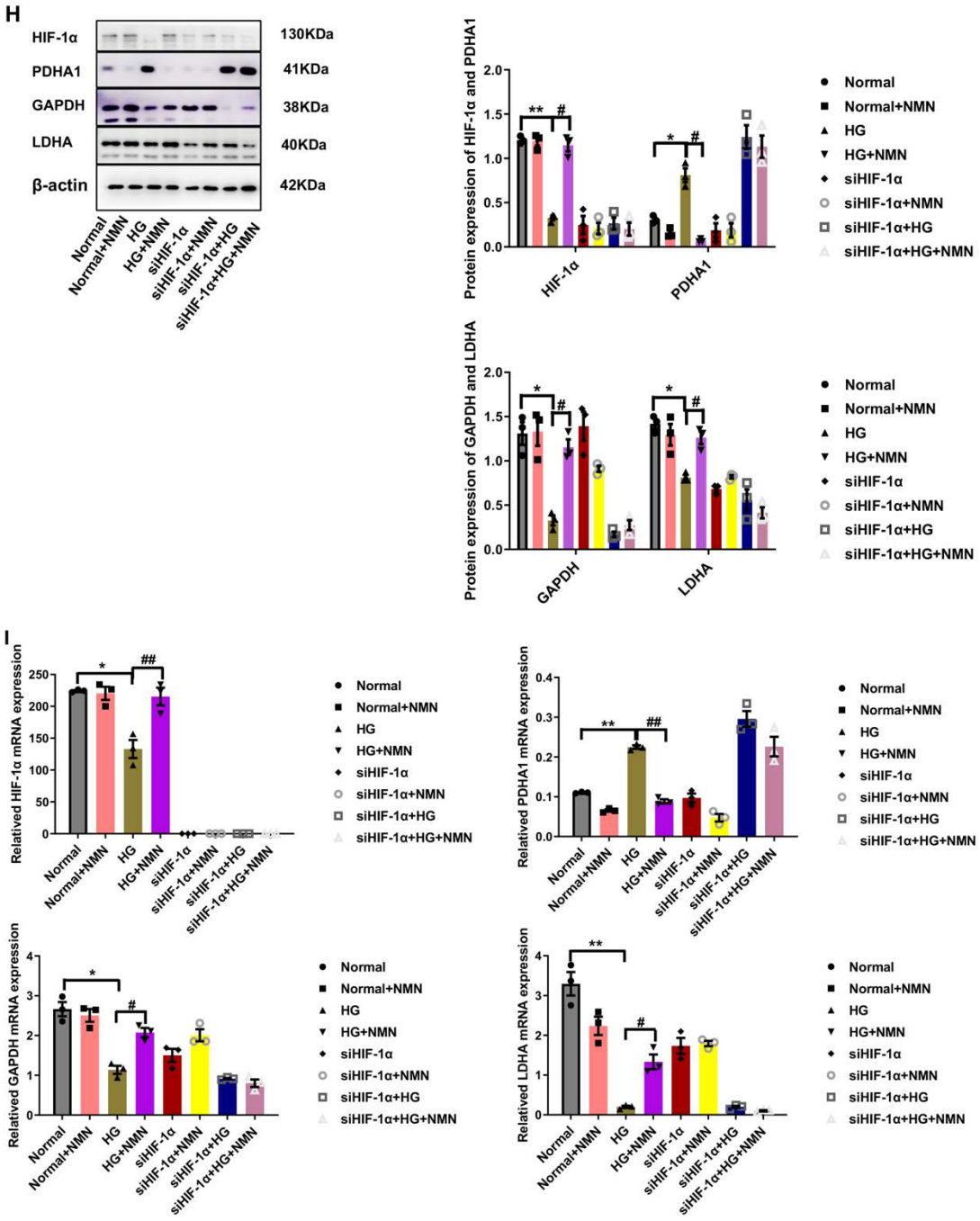


Figure 3: Figure 3. HIF-1α mediates NMN-induced metabolic remodeling and protection against hyperglycemic injury.

(A–B) Flow cytometric analysis of apoptosis in control and HIF-1α knockdown INS1 cells treated with HG and NMN.

(C–G) Effects of HIF-1α depletion on NMN-mediated regulation of mitochondrial ROS production, ATP generation, mitochondrial membrane potential, and mitochondrial adaptive responses.

(H–I) Western blot and quantitative PCR analyses of glycolytic pathway-associated genes following

HIF-1α knockdown.

Data are presented as mean ± SEM from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. #p< 0.05, ##p< 0.01, *p< 0.05, **p< 0.01.

Figure 4

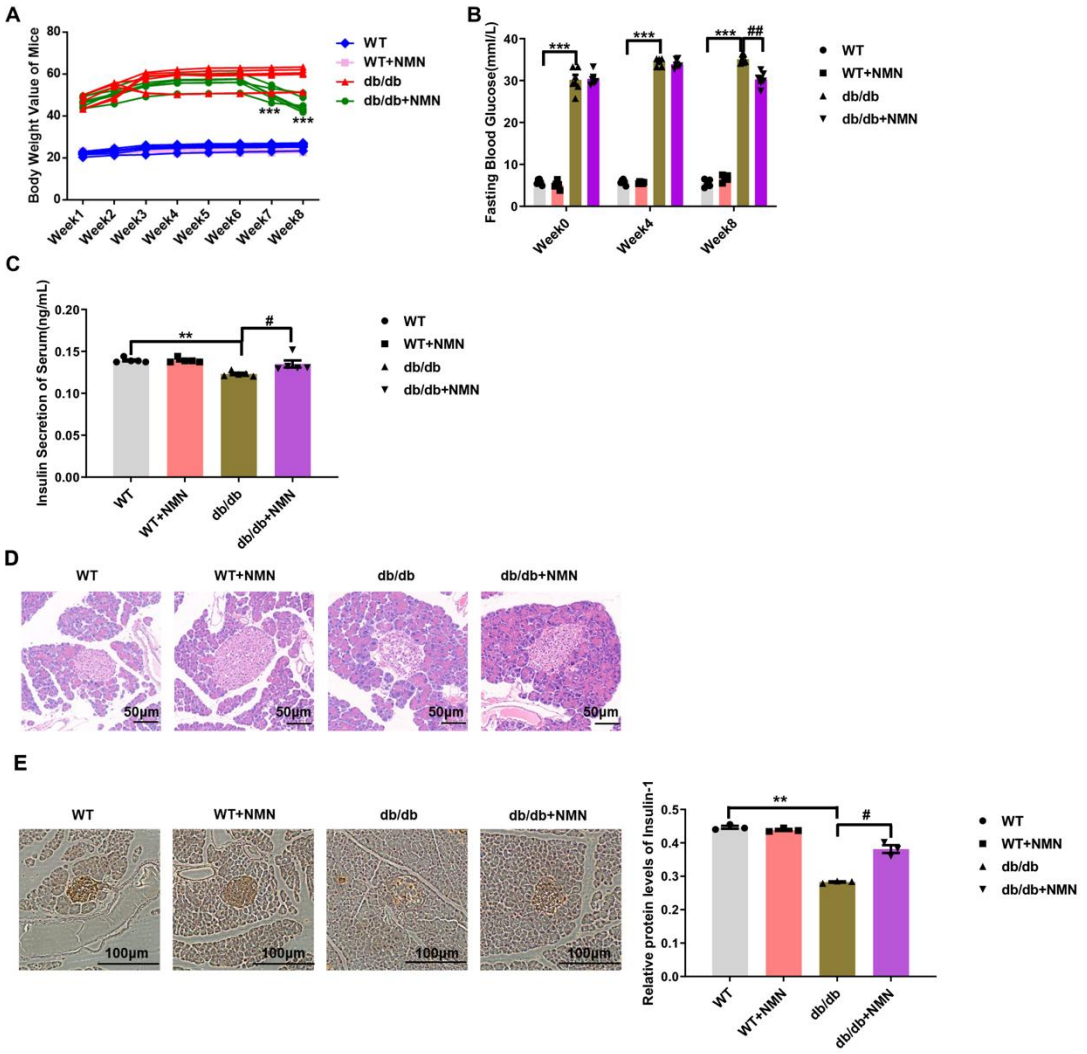


Figure 4. NMN ameliorates diabetic metabolic dysfunction and preserves pancreatic β-cell integrity in db/db mice.

(A) Changes in body weight during 8 weeks of NMN administration.

(B) Fasting blood glucose levels measured during treatment.

(C) Serum insulin levels in control, db/db, and NMN-treated db/db mice.

(D) Representative hematoxylin and eosin staining images of pancreatic islets.

(E) Immunohistochemical analysis of insulin expression in pancreatic tissues.

Data are presented as mean ± SEM from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. #p< 0.05, ##p< 0.01, **p< 0.01, ***p< 0.001.

Figure 5

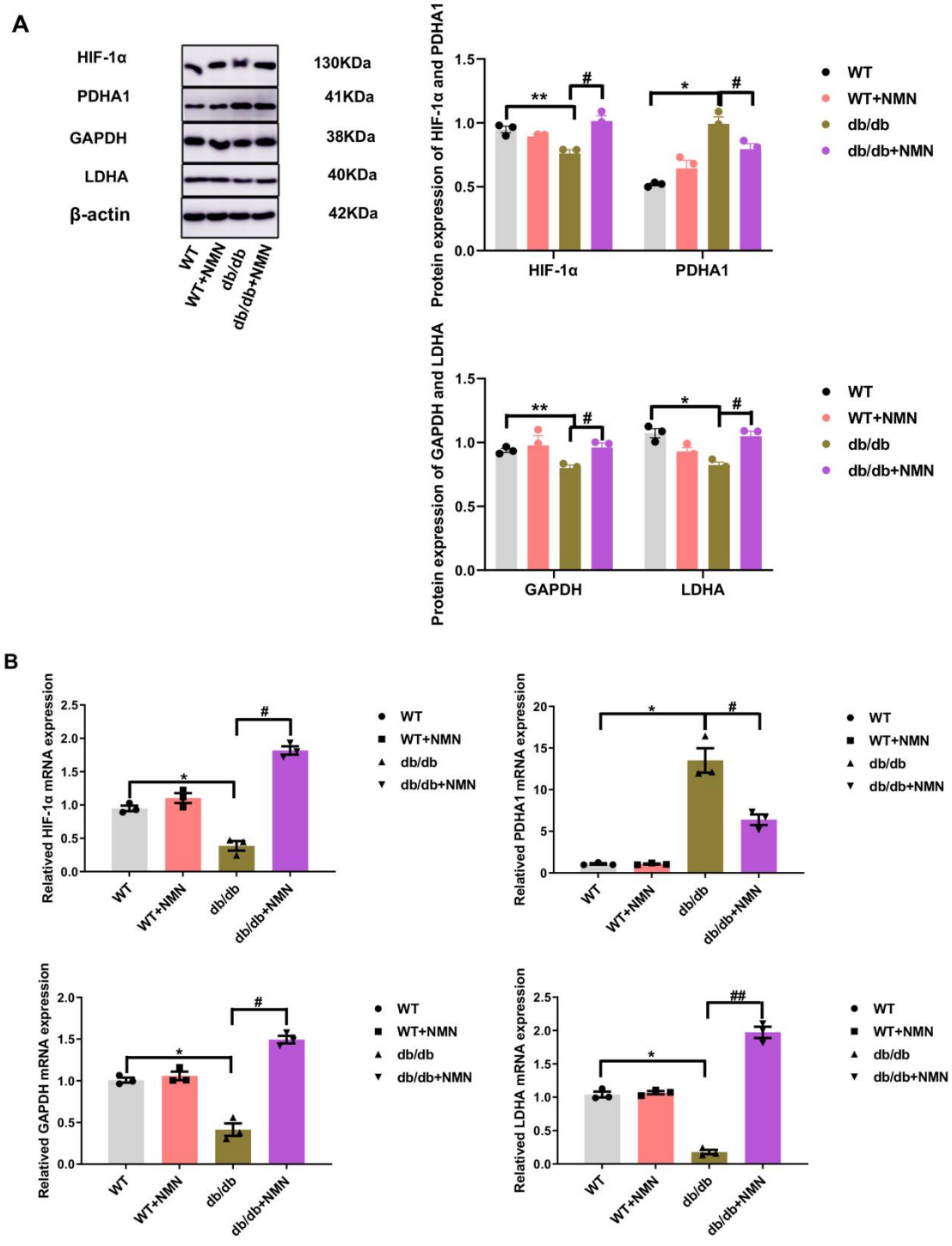


Figure 5. NMN restores glycolytic metabolic adaptation in pancreatic tissues of db/db mice.

(A) Western blot analysis of HIF-1α, PDHA1, GAPDH, and LDHA expression in pancreatic tissues.

(B) Quantitative PCR analysis of corresponding metabolic genes.

Data are presented as mean ± SEM from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. #p<

0.05, ##p< 0.01, **p< 0.01, ***p< 0.001.

Figure 6

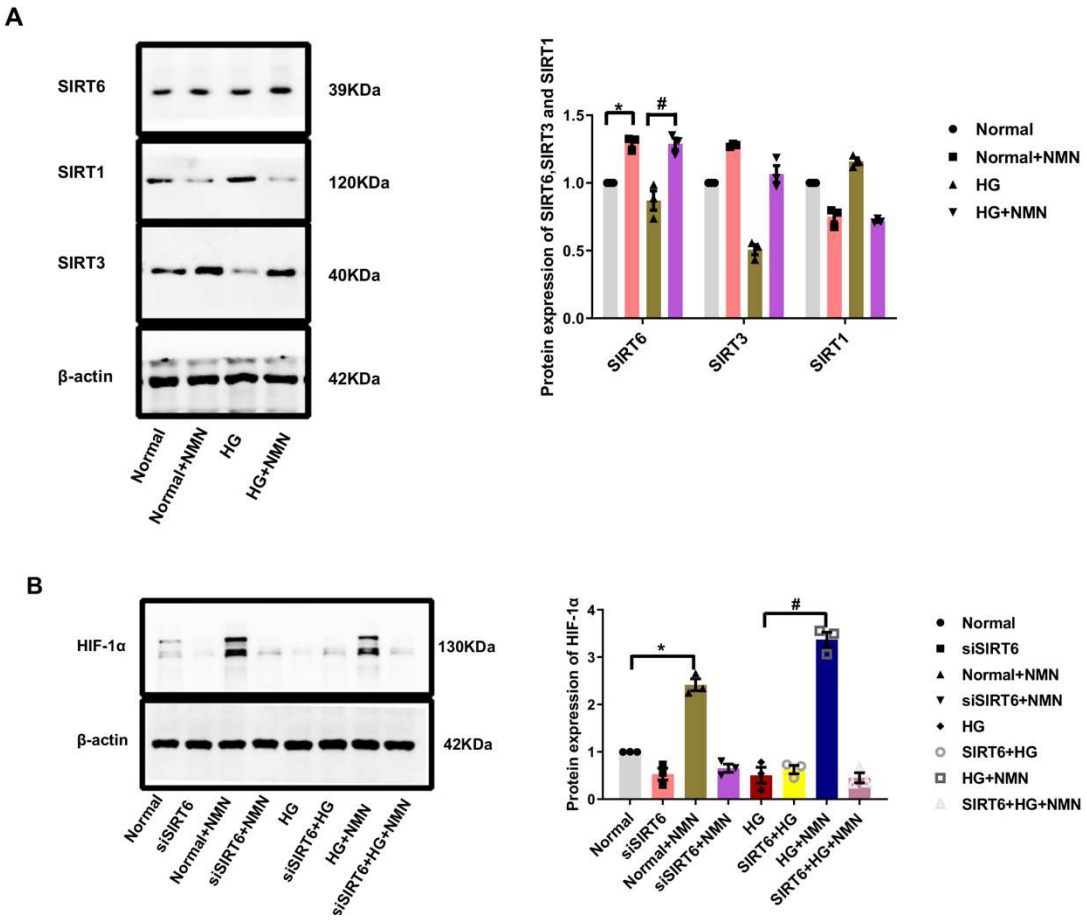


Figure 6. SIRT6 is an upstream regulator of NMN-mediated HIF-1α metabolic signaling.

(A) Expression analysis of NAD^+-dependent sirtuin family members following NMN treatment in diabetic conditions. NMN selectively restored SIRT6 expression.

(B) Effects of SIRT6 knockdown on NMN-induced HIF-1α activation and downstream glycolytic pathway regulation.

Data are presented as mean ± SEM from at least three independent experiments. Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. #p< 0.05, *p< 0.05.