Tertiary Butylhydroquinone Ameliorates Insulin Resistance and Liver Steatosis in Type 2 Diabetes Mellitus

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Research

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Abstract:

Aims: This study aimed to evaluate the effects and mechanisms of tertiary butylhydroquinone (TBHQ) on insulin resistance (IR) and diabetic liver steatosis.

Methods: Male ApoE$^{-/-}$ mice were received streptozocin (STZ) injection and a high-sugar-high-fat diet to form type 2 diabetes mellitus (T2DM). Then, the mice were given TBHQ for six weeks. Body weight, fasting blood-glucose (FBG), postprandial blood glucose (PBG), insulin and oral glucose tolerance test (OGTT) were detected on all the mice. Hematoxylin-eosin staining and western-blot were performed to detect the morphological structure and the target proteins expression in liver tissues. In vitro, HepG2 cells were induced by HClO and insulin to develop IR. Western-blot was used to evaluate the related proteins expression. Hoechst staining was conducted to measure cell apoptosis.

Results: Mice that received STZ injection and a high-sugar-high-fat diet developed T2DM. TBHQ reduced blood glucose level, improved glucose tolerance, alleviated liver steatosis in diabetic mice. Moreover, TBHQ significantly increased AMPKα2, GLUT4 and GSK3β expression, up-regulated PI3K and AKT phosphorylation level in diabetic mice liver. Notably, TBHQ down-regulated HClO and insulin-induced cell IR and inhibited cell apoptosis via AMPKα2/PI3K/AKT pathway.

Conclusion: TBHQ alleviated IR and liver steatosis in T2DM mice and the mechanism may relate to AMPKα2/PI3K/AKT pathway.

Keywords: Type 2 diabetes mellitus, Insulin resistance, Liver steatosis, Tertiary butylhydroquinone, AMPKα2

List of abbreviations

AKT	protein kinase B
AMPK	AMP-activated protein kinase
BCA	bicinchoninic acid
DMEM	dulbeccoo' s modified eagle' s medium
ELISA	enzyme-linked immunosorbent assay
FBG	fasting blood glucose
FBS	fetal bovine serum
GLUT4	glucose transport-4
GSK3β	glycogen synthase kinase-3β
HClO	hypochlorous acid
HE	hematoxylin-eosin
IDF	International Diabetes Federation
IR	insulin resistance
OGTT	oral glucose tolerance test
PAGE	polyacrylamide gel electrophoresis
PBG	postprandial blood glucose
PI3K	phosphatidylinositol 3-kinase
PVDF	polyvinylidenefluoride
SDS	sodium dodecyl sulfate
STZ	streptozocin
siRNA	small interference ribose nucleic acid
T2DM	type 2 diabetes mellitus
TBHQ	Tertiary butylhydroquinone

Key Messages

Tertiary butylhydroquinone can treat type 2 diabetes mellitus.

1.Introduction

According to the 8th edition of the Global Diabetes Map published by the International Diabetes Federation (IDF), there are almost 425 million people suffering from diabetes worldwide, and it is estimated that there will be nearly 700 million people with diabetes by 2045. Among them, patients with type 2 diabetes mellitus (T2DM) account for more than 90%[1]. T2DM is due to insulin resistance (IR)[2,3], characterized by a defect of the peripheral tissues, including liver, adipose tissues, and skeletal muscles, in response to insulin[4]. IR is the core mechanism of T2DM and thus has become the main target of T2DM treatment at this stage[5, 6].

Tertiary butylhydroquinone (TBHQ) is the most commonly used synthetic antioxidant[7] and can enhance the effect of insulin *in vivo*[8, 9]. TBHQ could also remarkably increase the phosphorylation level of protein kinase B (AKT), which could reflect insulin sensitivity[10] in hepatocytes and nerve cells[11]. However, the underlying mechanism of TBHQ increasing AKT phosphorylation thus enhancing insulin effect is unclear.

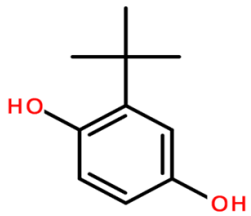
The AMP-activated protein kinase (AMPK) is a heterotrimeric protein composed of a catalytic subunit (α), which have two isoforms (1α and 2α), and two regulatory subunits (β and γ). The 2α subunit isoform, PRKAA2, is mainly found in liver. It is reported that TBHQ has an activation effect on AMPKα2 factor[12], which leads to inhibiting glucose production[13] and regulating of insulin sensitivity[14-17] in liver.

Based on these reports, we speculated that the protective effect of TBHQ on liver function in diabetes may be due, in part, to the ability of TBHQ to up-regulation gene expression of AMPKα2 factor.

2. Materials and methods

2.1. Materials

Tertiary butylhydroquinone (TBHQ), streptozocin (STZ), insulin and HClO were purchased from Sigma. Chemical formula of TBHQ is as follows.



Fetal bovine serum (FBS) and dulbeccoo' s modified eagle' s medium (DMEM) were obtained from Gibco BRL. Bicinchoninic acid (BCA) protein assay kit, hematoxylin-eosin (HE) staining kit and the Hoechst staining kit were purchased from Beyotime Biotechnology Co., Ltd.. p-AKT, AMPKα2, glycogen synthase kinase-3β (GSK3β) and glucose transport-4 (GLUT4) were purchased from Abcam. Phosphatidylinositol 3-kinase (PI3K) and AKT were purchased from Shanghai Sangon Co., Ltd.. p-PI3K was obtained from Cell Signaling Technology. β-actin was purchased from Beyotime Biotechnology Co., Ltd.. PRKAA2 small interference ribose nucleic acid (siRNA) and riboFECT™ CP transfection kit were obtained from Guangzhou RiboBio Co., Ltd..

2.2. Protocols for animal experiments

Male ApoE$^{-/-}$ mice were obtained from Model Animal Research Center GemPharmatech Co., Ltd. of Nanjing University. These animals researches were approved by the Zhengzhou University Veterinary Medicine Animal Care and Use Committee. Mice were maintained at 25 °C under a 12-hour light/12-hour dark cycle. After one week of acclimation period, animals with a body weight of 24±0.5 g were randomly segregated into five groups (n=10 per group):

Group	Treatment
Control	The mice were fed with a conventional diet for eleven weeks.
+TBHQ	The mice were fed with a conventional diet for eleven weeks. During the last six weeks[18, 19], the mice were accepted intragastric administration of 60 mg/kg TBHQ.
T2DM	The mice were fed with a high-sucrose-high-fat diet for two weeks, then the mice were fasted for 10-h and injected STZ (50 mg/kg) which was dissolved in sodium citrate saline buffer (pH=4.5) and injected immediately within a few minutes to avoid degradation through the tail vein[20]. Last, the mice were fed a high-sucrose-high-fat diet for another two weeks to develop diabetes. The diabetic mice were fed with a high-sucrose-high-fat diet for the last six weeks.

+TBHQ	The diabetic mice were fed with a high-sucrose-high-fat diet and accepted intragastric administration of 60 mg/kg TBHQ for the last six weeks.
+rosiglitazone	The diabetic mice were fed with a high-sucrose-high-fat diet and accepted intragastric administration of 7 mg/kg rosiglitazone for the last six weeks[21, 22].

The high-sucrose-high-fat diet (sucrose: lard: sodium cholate: cholesterol: conventional feed=20: 10: 7: 3: 60) was given to all the mice except for the control group and the control + TBHQ group during the entire treatment period[23]. The mice were fed a high-sucrose-high-fat diet and received administrations of TBHQ or rosiglitazone after β-cell damage (Figure. 1A).

2.3. Biochemical study

The body weight and blood glucose of all mice were measured each week during the experimental period. All animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and then sacrificed by cervical dislocation at the end of the eleven weeks. The blood was collected from the mice eye socket vein and centrifuged at 4000 rpm for 15 min at 4 °C to separate serum. Serum level of fasting insulin was determined by their commercially available enzyme-linked immunosorbent assay (ELISA) kits (Beyotime, Shanghai, China). The freshly isolated liver tissues were collected to analyze genes expression. The liver tissues were fixed in 4% paraformaldehyde for HE staining.

2.4. Detection of FBG, PBG and oral Glucose Tolerance Test (OGTT)

For FBG, blood was collected after 12 h of food deprivation from the tail vein of mice. For PBG, blood was collected after the mice ate 2 hours. Blood glucose was measured with an automatic blood glucose meter (Glutest Pro, Sanwa Chemical, Japan).

At the end of the experiment, OGTT was performed. For OGTT, rats were orally loaded with glucose (1.0 g/kg) after 12 h of fasting. Blood glucose levels were measured as described above at the indicated time after glucose administration. The operation procedure could see literatures of Zhang WX et.al and Bin-Jumh MN et.al[23, 24].

2.5. HE staining

The freshly isolated Liver tissues were immersed in 4% aqueous buffered paraformaldehyde at the room temperature for overnight fixation and then dehydrated,

embedded in paraffin and sliced into 4 μm sections. The paraffin-embedded tissue sections were routinely stained with HE staining to conduct histologic analysis[25].

2.6. Western blot analysis

Total protein was isolated from the Liver tissues and cultured HepG2 cells with RIPA buffer containing 1% PMSF (Beyotime) for 30-60 min on ice. The lysates were centrifuged at 12,000 rpm for 15 min at 4 °C and the protein concentrations were determined by BCA Protein Assay Kit (Beyotime, China). A fixed amount of protein (20–60 μg) was separated from each sample by using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE). These proteins were transferred onto polyvinylidenefluoride (PVDF) membranes (Millipore, USA). Then the membranes were blocked with 5% non-fat milk or BSA at room temperature for 1 h, and incubated with primary antibodies at 4 °C over night, finally the membrane was washed thoroughly and incubated with a peroxidase-conjugated mouse anti-mouse or anti-rabbit antibody (1:5000; ABclonal) at room temperature for 1 h. The chemiluminescence signals were detected using ECL (millipore). Densitometric analysis was conducted using Image J 1.43 software (National Institutes of Health, New York City, NY, USA). The specific operation steps could see articles of Zhang W et.al and Liu H et.al[26, 27]. Primary antibodies against GLUT4 (1:1000, ab654, Abcam, USA), GSK3β (1:1000, ab32391, Abcam, USA), p-AKT (1:1000, ab38449, Abcam, USA), p-PI3K (1:1000, 17366, CST, USA), AKT (1:500, D199241, Sangon, Shanghai, China), PI3K (1:500, D162051, Sangon, Shanghai, China), AMPK α2 (1:1000, ab97275, Abcam, USA) and β-actin (1:1000, AF0003, Beyotime, Shanghai, China) were used.

2.7. Cell culture and treatment

HepG2 cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum in a 5% CO_2 atmosphere at 37 °C. To formation the insulin resistant model in HepG2 cells, cells were exposed to HClO (200 mM) for 40 min and then insulin for 25 min. PRKAA2 siRNA (5′-GTTTAGATGTTGTTGGAAAdTdT-3′) and Negative control siRNA (5′-GACUACUG-GUCGUUGAACUdTdT-3′) which were purchased from Ribobio (Ribobio Co. LTD, Guangzhou, China) was transfected by using ribo FECT^{TM}CP transfection kit (Ribobio Co. LTD, Guangzhou, China). Two series of experiments were designed. The first series of experiments investigated the role of hypoxia on PASMCs and the involvement of AMPKα2. The cells were divided into 8 groups as

follows: 1) Control group, 2) TBHQ group, 3) HClO group, 4) Insulin group, 5) HClO+TBHQ group, 6) HClO+insulin group, 7) TBHQ+insulin group, and 8) HClO+TBHQ+insulin group. The expression of GLUT4, GSK3β, p-AKT, p-PI3K, AKT, PI3K, AMPK α2, β-actin and cell apoptosis were analyzed. The second series of experiments were designed to explore the role of AMPK α2 in diabetic liver steatosis and the involvement of the AMPK α2/PI3K/AKT pathway. The cells were divided into 12 groups as follows: 1) Control group, 2) PRKAA2 siRNA group, 3) TBHQ group, 4) TBHQ+ PRKAA2 siRNA group, 5) TBHQ+Insulin group, 6) TBHQ+ PRKAA2 siRNA+insulin group, 7) HClO group, 8) PRKAA2 siRNA+HClO group, 9) HClO+insulin group, 10) PRKAA2 siRNA+HClO+insulin group, 11) HClO+TBHQ+insulin group, 12) PRKAA2 siRNA+HClO+TBHQ+insulin group. The expression of GLUT4, GSK3β, p-AKT, p-PI3K, AKT, PI3K, AMPK α2 and β-actin were analyzed.

2.8. Hoechst staining

The cells (approximately 3000 cells/well) were seeded into 96-well plates with DMEM containing 10% FBS for 24 h before the HClO and insulin treatment. Then, the cells were treated with TBHQ for 30 min. Hoechst staining kit (Beyotime, Shanghai, China) was used to detect the cell apoptosis according to the manufacturer's instructions.

2.9. Quantitative RT-PCR analysis

Quantitative RT-PCR analysis was conducted using the SYBR Premix Ex Taq (TaKaRa). Briefly, total RNA was extracted from HepG2 cells using a RNAiso plus kit (TaKaRa) according to the supplier's instructions. Quantitative RT-PCR was conducted to assess AMPK α2 expression using an ABI step one plus sequence Detection System (Applied Biosystems). GAPDH was used for normalization, and the relative expression levels for each target gene were calculated using the $2^{-\Delta\Delta Ct}$ approach as previously reported[17]. The primers used to amplify each target gene were as follows: human PRKAA2: forward: 5′- GGAGAACATCAATTAACAGGCC -3′, reverse: 5′- CCAACAACATCTAAACTGCGAA -3′; human GAPDH forward: 5′- CAAATTCCATGGCACCGTCA-3′, reverse: 5′- GGTCATGAGTCCTTCCACGA-3′was used as an internal control.

2.10. Statistical analysis

Statistical analysis was performed using SPSS software. All quantitative results data were expressed as mean ± SD. All results were performed by one-way ANOVA

with Newman-Student-Keuls test for multiple comparisons, and both sides $P<0.05$ were considered significant.

3. Results

3.1. TBHQ obviously alleviated the abnormal of glucose metabolism in diabetic mice

A high-sucrose-high-fat diet treatment and STZ injection induced type 2 diabetes mellitus (T2DM). Specifically, the mice developed T2DM with significant increases in FBG and PBG concentrations in serum (Figure 1C and 1D), a rise in oral glucose tolerance test (OGTT) levels (Figure 1E), and an decrease in body weight (Figure 1B). However, after six weeks of rosiglitazone or TBHQ treatment, the FBG, PBG, OGTT levels were significantly decreased and the body weight was obviously increased. Moreover, TBHQ produced an equivalent or more evident effect on hypoglycemic activity in diabetic mice compared with rosiglitazone treatment. Taken together, these data indicate that the persistent application of TBHQ relieves glucose metabolic abnormalities and the body weight increase in T2DM mice.

3.2. TBHQ effectively alleviated liver steatosis of diabetic mice and increased cell survival in HepG2 cells

HE staining (Figure 2A) revealed that in normal mice, no abnormality of the hepatocyte architecture and morphology were observed. The hepatocytes were arranged in cords and radiated around the central vein. In the liver of T2DM mice, severe hepatocyte necrosis and macrovesicular steatosis were appeared. However, after rosiglitazone or TBHQ treatment, the degeneration of the hepatocyte was markedly alleviated, the macrovesicular steatosis and cell death were partially recovered. These results demonstrate that the persistent application of TBHQ exhibited a beneficial effect on diabetes-mediated pathological changes of the mice liver.

Hoechst staining was used to explore the effect of TBHQ on HepG2 cells apoptosis. As shown in Figure 2B, HClO and insulin treatment promotes cells apoptosis. The number of cells with apoptotic morphology appearing condensed or fragmented nuclei was counted. However, after TBHQ treatment the number of apoptosis HepG2 cells markly downregulated. TBHQ displayed varying degrees of apoptosis (64.65% for HClO group, 71.56% for HClO+insulin group, 23.05% for HClO+TBHQ+insulin group, 20.08% for HClO+TBHQ group). These results indicated that TBHQ subsequently increase the survival ability of HepG2 cells.

3.3. Effects of TBHQ treatment on PI3K/AKT pathway in diabetic mice and insulin resistance (IR) HepG2 cells

The related proteins levels of the glucose metabolism such as GLUT4, GSK3β, p-PI3K and p-AKT are index of diabetes and IR. We next evaluated the related proteins in hepatic cells to confirm the regulatory effect of TBHQ. We found that TBHQ increased the protein expression of the glucose transport marker GLUT4 (Figure. 3A, 3B) and the glycogen synthase marker GSK3β (Figure. 3C, 3D) when compared to the T2DM group and insulin resistance HepG2 cells. Moreover, the phosphorylation level of PI3K and AKT relatively elevated in diabetic mice and insulin resistance HepG2 cells treatment with rosiglitazone or TBHQ. Previous studies have showed that the phosphorylation level of AKT is upregulated by the PI3K phosphorylation[28-30]. Therefore, the AKT phosphorylation level in liver tissues and HepG2 cells were inspected to discuss whether TBHQ increased p-AKT activity by activating the PI3K pathway and then regulating gluconeogenesis. In a word, the data suggest that TBHQ may inhibit glucose uptake and increase glycogen synthesis via the PI3K/AKT pathway.

3.4. TBHQ might activate AMPKα2 factor in diabetic mice and HepG2 cells with IR

As a crucial cellular energy regulator, AMPKα2 plays a considerable role in glucose utilization[14]. Thus we detected AMPKα2 in our animal and cell experiments and found that the expression of AMPKα2 has significantly decreased in both diabetic mice liver and IR cells (Figure. 4A-B). However, after the treatment of TBHQ, AMPKα2 was significantly increased. Thus, TBHQ could correct the abnormal of glucose metabolism and increase the expression of AMPKα2.

3.5. TBHQ activated AMPKα2 factor to increase insulin sensitivity in HClO and insulin-treated HepG2 cells

In order to illuminate whether TBHQ could improve the glucose metabolism directly through AMPKα2, we knocked down AMPKα2 gene with PRKAA2 siRNA in HepG2 cells and the expression levels of p-AKT, p-PI3K, GLUT4 and GSK3β were detected, respectively. In our results, we found that in HClO and insulin-treated HepG2 cells the expression of p-AKT, p-PI3K, GLUT4 and GSK3β were significantly decreased. After the treatment of TBHQ, the expression of these genes increased, whereas, blocking of AMPKα2 markedly retarded TBHQ-induced upregulation of p-AKT, p-PI3K, GLUT4 and GSK3β (Figure. 5A-D). Taken together,

these results suggest that TBHQ could improve the expression of GLUT4, GSK3β, p-PI3K and p-AKT in IR HepG2 cells via AMPKα2 activition.

4. Discussion

Insulin resistance (IR)[2,3] could lead to type 2 diabetes mellitus (T2DM), which is characterized by a defect of the liver in response to insulin[4]. TBHQ, as a food antioxidant, can increase the effect of insulin, thus reducing IR[7-9]. However, whether TBHQ can alleviate IR thus controlling liver steatosis in T2DM and the mechanisms behind the effects have not been clearly defined. Our data presented here was the first to show the effectiveness of TBHQ on improving liver steatosis in T2DM, and the mechanism of the anti-IR effect of TBHQ related to AMPKα2/PI3K/AKT pathway. The data suggested that the persistent application of TBHQ could effectively reduce the levels of blood glucose, increase the body weight, alleviate liver steatosis and activate AMPKα2. Also a parallel experiment in cells was conducted. In vitro studies suggested that TBHQ could increase the survival of HClO and insulin induced IR HepG2 cells and the mechanism related to AMPKα2/PI3K/AKT pathway. In brief, TBHQ may potentially be used as a medicinal compound for the treatment of T2DM via activating AMPKα2/PI3K/AKT pathway.

TBHQ, which has antioxidant and anti-inflammatory effects, can protect pancreatic islet cells from damage and increase the insulin sensitivity *in vivo* to improve T2DM. Liver tissue, as a main detoxifying organ, often damaged by metabolic overload, lipid accumulation and viruses factors. Moreover, liver tissue will occur metabolic disturbance in T2DM. TBHQ can inhibit liver cell apoptosis, which will protect the liver from acute and chronic toxin-mediated injury[12, 31, 32]. According to these evidences, we hypothesized that TBHQ can alleviate liver injury in T2DM, and our subsequently experiment support this point (Figure. 2).

AMPKα2 is a key component in the regulation of insulin sensitivity[33, 34], and the lack of AMPKα2 can inhibit the resistibility of insulin in glucose uptake, which leading to abnormal glucose tolerance *in vivo*[35-37]. TBHQ can activate AMPKα2 and promote the autophagy of hepatocytes, thus exerting the effect of anti-fatty acid[17]. In our experiments, we detected the expression of AMPKα2 both *in vivo* and *in vitro* studies. Consistently, we found that AMPKα2 expression was decreased in both diabetic mice liver and insulin resistance cells. However, after TBHQ treatment, the expression of AMPKα2 was significantly increased. Previous reports have suggested that insulin can tremendous decrease blood glucose by improving phosphorylates

AKT and PI3K[28, 29, 35], which is association with GSK3β and GLUT4[36-37]. AMPKα2 can induce AKT activation by phosphorylating two major residues: Ser473 and Thr308[38, 39]. Thus we speculated that TBHQ alleviates IR and liver steatosis via activating AMPKα2/PI3K/AKT pathway. In order to further explore the underlying mechanism, we used AMPKα2 siRNA in HepG2 cells and found that the TBHQ could enhance the phosphorylation levels of AKT and PI3K, thus significantly improving the role of GLUT4 and GSK3β in the liver of diabetic mice and IR HepG2 cells. After AMPKα2 knockdown, PI3K/AKT pathway was significantly influenced. Our results suggested that TBHQ alleviated PI3K/AKT pathway directly via AMPKα2 activation in insulin resistance cells.

Clinical analysis has suggested that rosiglitazone should be used with caution in patients with cardiac insufficiency, severe cardiovascular disease and hypertension[40]. It also may increase the occurrence of cardiovascular complications in the treatment of T2DM. Therefore, developing high-efficiency and safe drugs that can prevent diabetes is very necessary. TBHQ is mainly used as food additive. TBHQ is safety and low toxicity, and its chemical structure is modified to enhance the antioxidant effect. More importantly, in our study we found that TBHQ can improve IR and liver steatosis except for the effect of antioxidant. Thus TBHQ is expected to be the ideal medicine for treating T2DM.

As we all know, diabetes can cause damage of the liver, skeletal muscle and fat. The shortcoming of this study is that we have only explored the effect of TBHQ on relieving liver injury, we have not probed the role of TBHQ in inhibiting the injury of skeletal muscle and fat. In the next study, we will use C2C12 and 3T3-L1 cells to research the effect of TBHQ in these diseased organs. As different tissues have different sensitivities to TBHQ, we will use multiple doses of TBHQ to explore the optimal dose of TBHQ in diabetes. Only in this way can we make TBHQ be an ideal medicine and be used as soon as possible for treating T2DM patients.

In summary, this study demonstrates that TBHQ alleviates T2DM via AMPKα2 activation, and then AMPKα2 mediates IR via AKT/PI3K signaling pathway (Figure. 6). Therefore, TBHQ can be a potential medicine and AMPKα2 is a pharmacological target for the therapy of T2DM.

Conclusion

This study demonstrated that TBHQ can ameliorate insulin resistance and liver steatosis via AMPKα2/AKT/PI3K signaling pathway in diabetic rats. Our results provide evidence for the clinical use of TBHQ to treat T2DM. The mechanisms underlying the anti-diabetic effects of TBHQ need further elucidation.

Authors' contributions

YYL, LP and SP conceived and designed the experiments. ZTT, ZCN and LTH performed the experiments. HN and WSX analyzed the data. Wrote the paper: XJ and WGR. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

Availability of data and materials

The data and materials generated or analyzed during this study are available from the corresponding author on reasonable request.

Consent for publish

The manuscript is approved by all authors for publication.

Ethics approval and consent to participate

The experimental protocol was established according to the ethical guidelines and was approved by the Experimental Animal Ethics Committee of Xinxiang medical University

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Figure legends

Figure 1. Effects of Tert-butylhydroquinone (TBHQ) treatment on body weight, fasting blood glucose (FBG), postprandial blood glucose (PBG), and oral glucose tolerance test (OGTT) in type 2 diabetes mellitus (T2DM) mice. A. the protocol of the animal experiment; B. FBG; C. PBG; D. body weight; E. OGTT. $^{**}P<0.01$ *vs*. Control, $^{\#\#}P<0.01$ *vs*. T2DM. Data are represented as mean ± SD (n=10).

Figure 2. Effects of TBHQ on liver steatosis of diabetic mice and on HepG2 cells survival. A. HE staining of the liver tissue, glycogen was staining as red and nucleus was staining as blue (×200). B. Hoechst staining of HepG2 cells (×200), the red arrow indicated the apoptotic cell.

Figure 3. The expression of the related protein level of glucose metabolism in the liver of the mice and the HepG2 cells. A-B. the expression of GLUT4 in the liver of the mice and the HepG2 cells; C-D. the expression of GSK3β in the liver of the mice and the HepG2 cells; E-F. the phosphorylation level of PI3K in the liver of the mice and the HepG2 cells; G-H. the phosphorylation level of AKT in the liver of the mice and the HepG2 cells. $^{*}P<0.05$ *vs*. Control, $^{\$}P<0.05$ *vs*. T2DM, $^{\&}P<0.05$ *vs*. HClO+insulin. Results are represented as mean ± SD (n=3).

Figure 4. Effect of TBHQ on the expression of AMPKα2. A. the expression of AMPKα2 in the liver of the mice; B. the expression of AMPKα2 in HepG2 cells. $^{*}P<0.05$ *vs*. Control, $^{\#}P<0.05$ *vs*. T2DM, $^{\&}P<0.05$ *vs*. HClO+insulin. Results are represented as mean ± SD (n=3).

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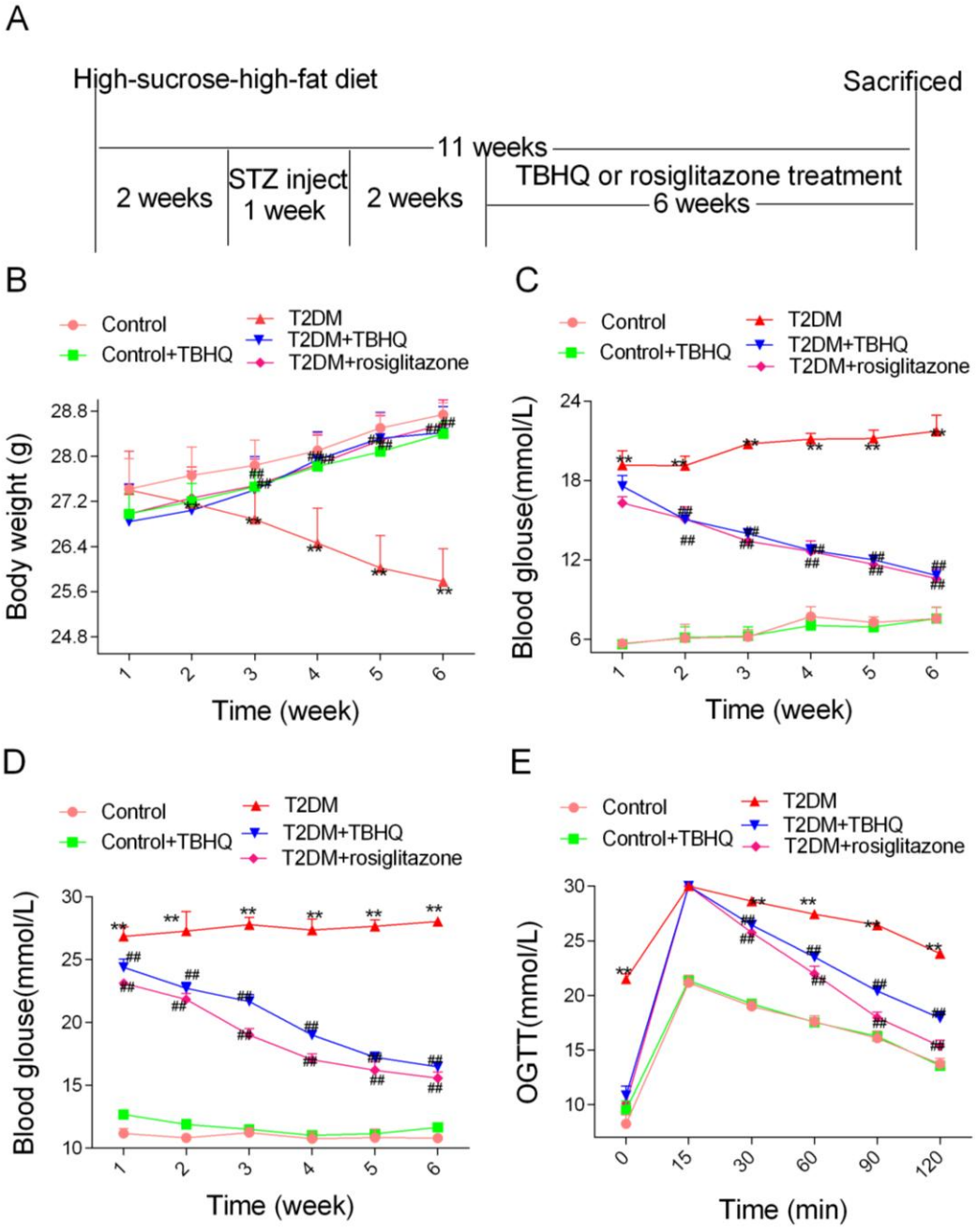
Figure 1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STZ inject
1 week
2 weeks
TBHQ or rosiglitazone treatment
6 weeks
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Control
Control+TBHQ
T2DM
T2DM+TBHQ
T2DM+rosiglitazone
Body weight (g)
Time (week)
C
Blood glouse(mmol/L)
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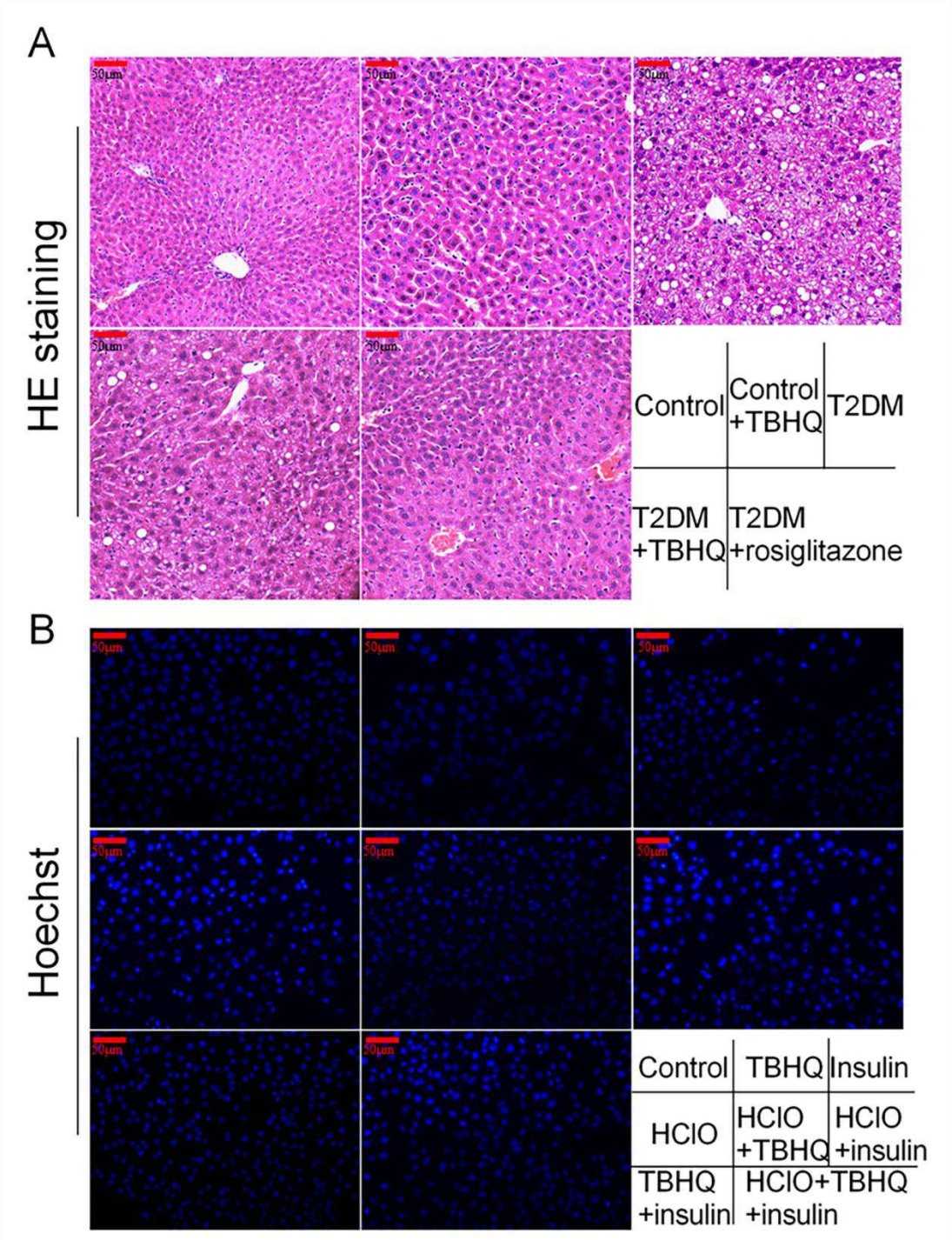
E
OGTT(mmol/L)
Time (min)

Figure 2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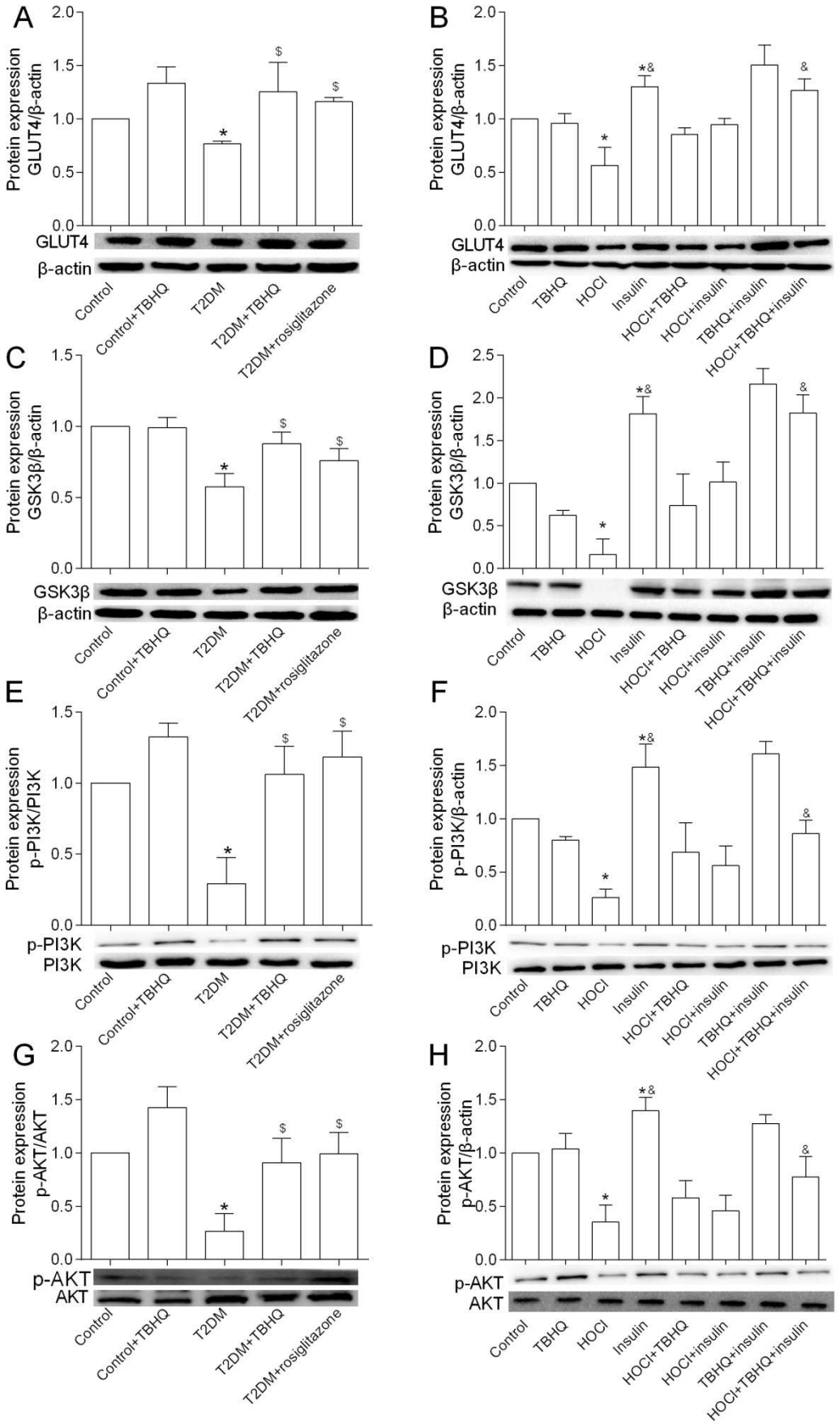
HClO
HClO +TBHQ
HClO +insulin
TBHQ +insulin
HClO+TBHQ +insulin

Figure 3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Protein expression GSK3β/β-actin
GSK3β
β-actin
D
Protein expression GSK3β/β-actin
GSK3β
β-actin
E
Protein expression p-PI3K/PI3K
p-PI3K
PI3K
F
Protein expression p-PI3K/β-actin
p-PI3K
PI3K
G
Protein expression p-AKT/AKT
p-AKT
AKT
H
Protein expression p-AKT/β-actin
p-AKT
AKT
Control
Control+TBHQ
T2DM
T2DM+TBHQ
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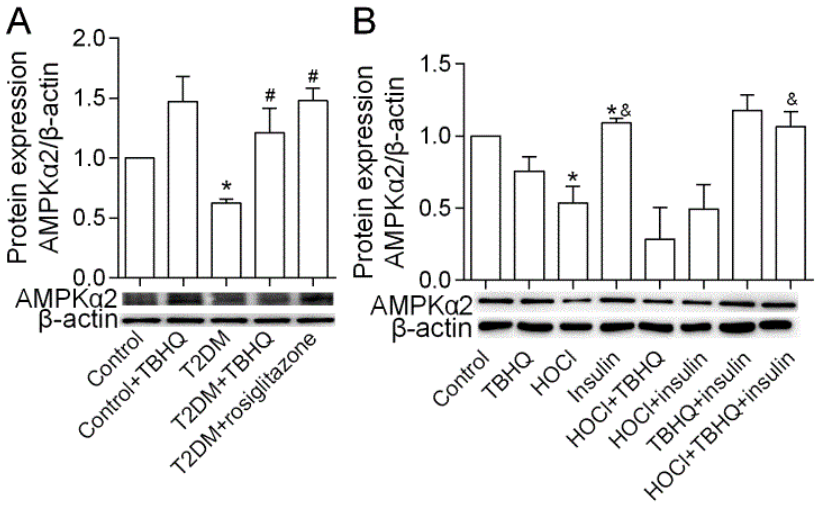
HOCl+TBHQ+insulin

Figure 4



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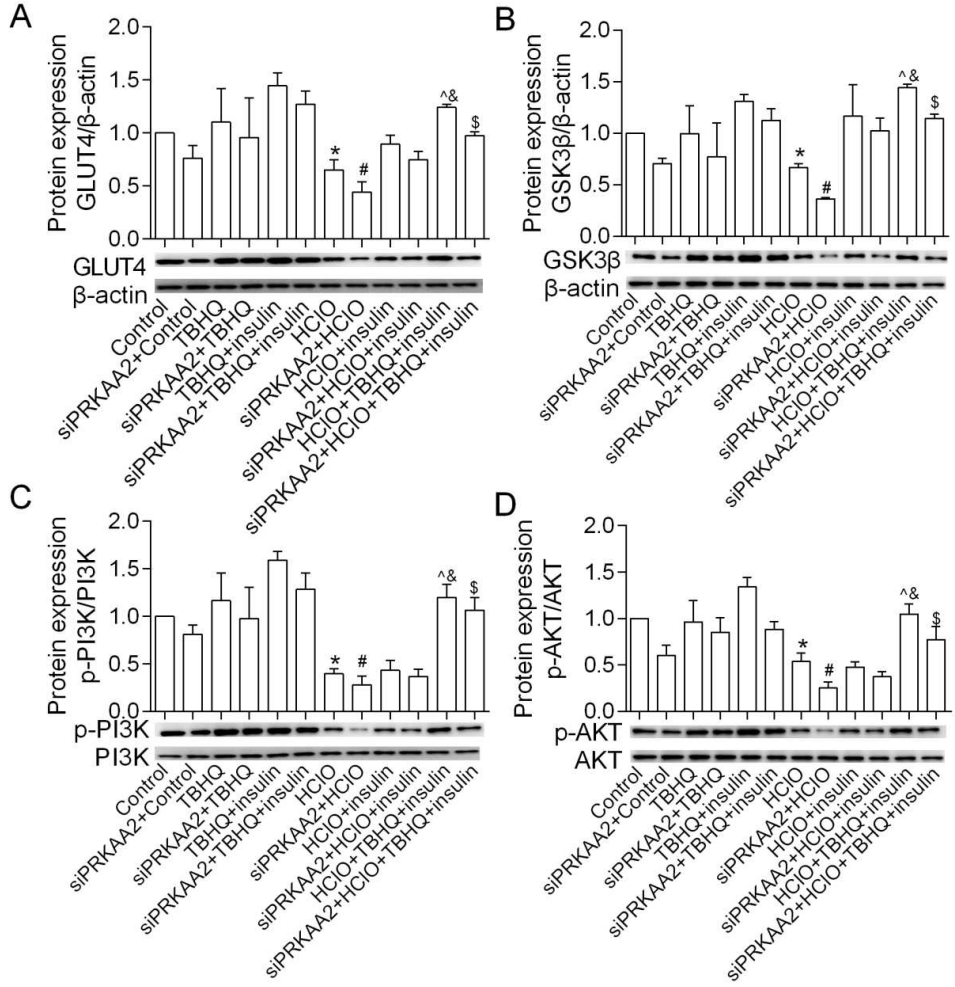
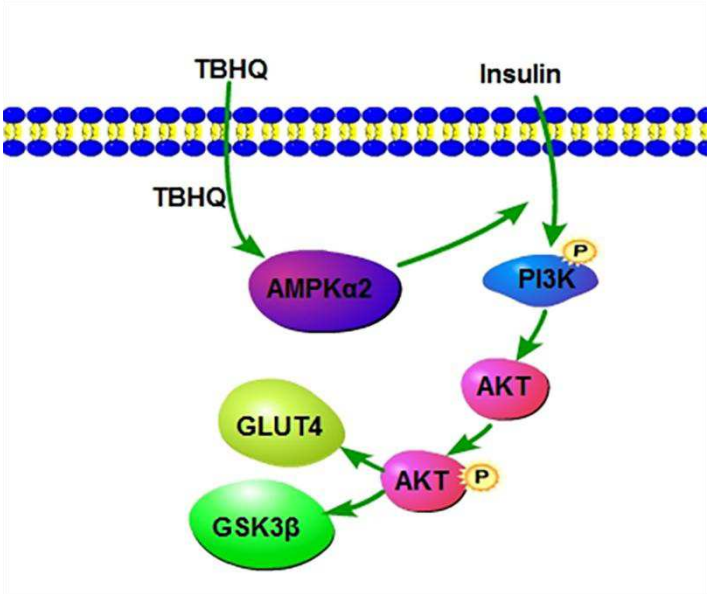
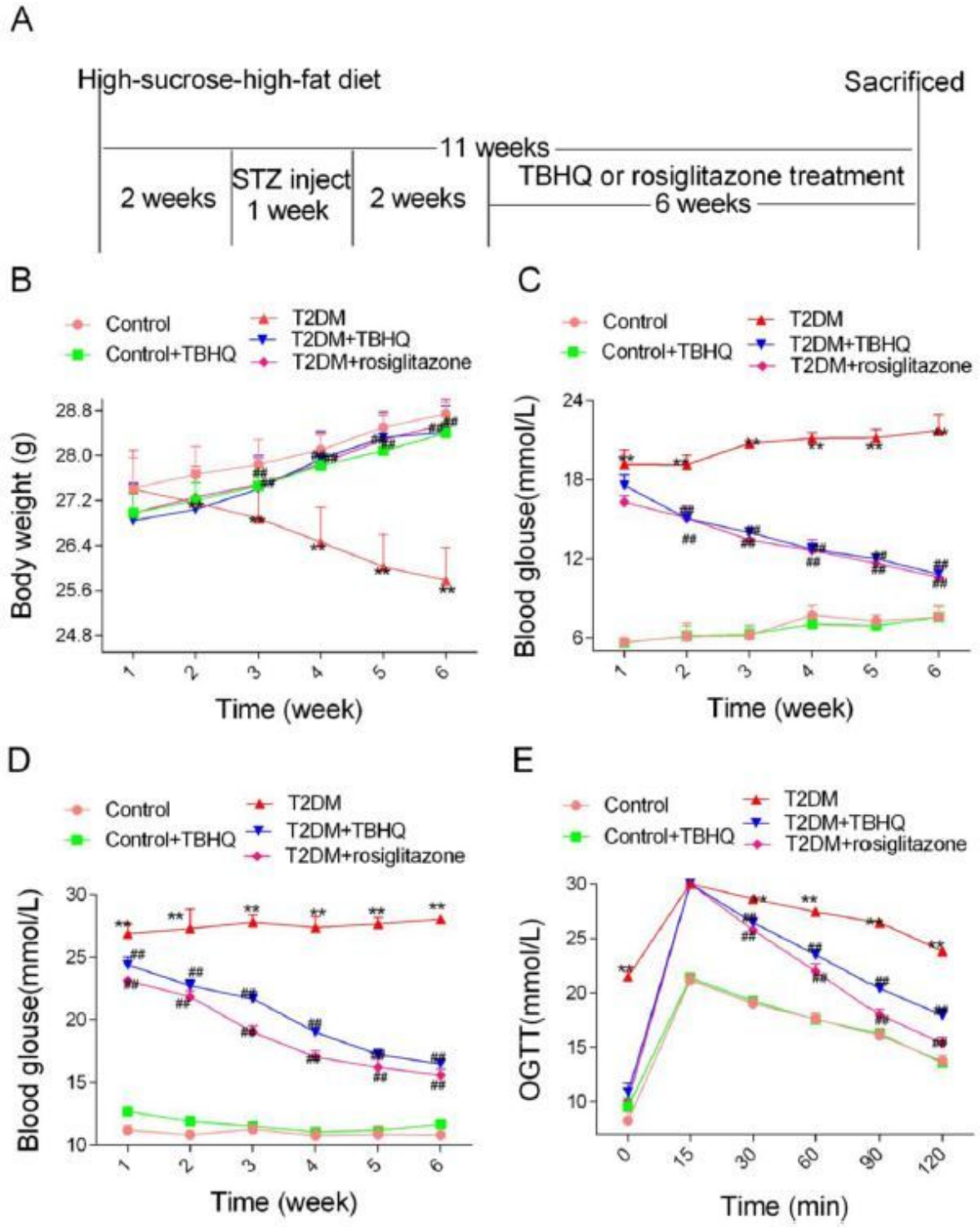


Figure 6



Figures



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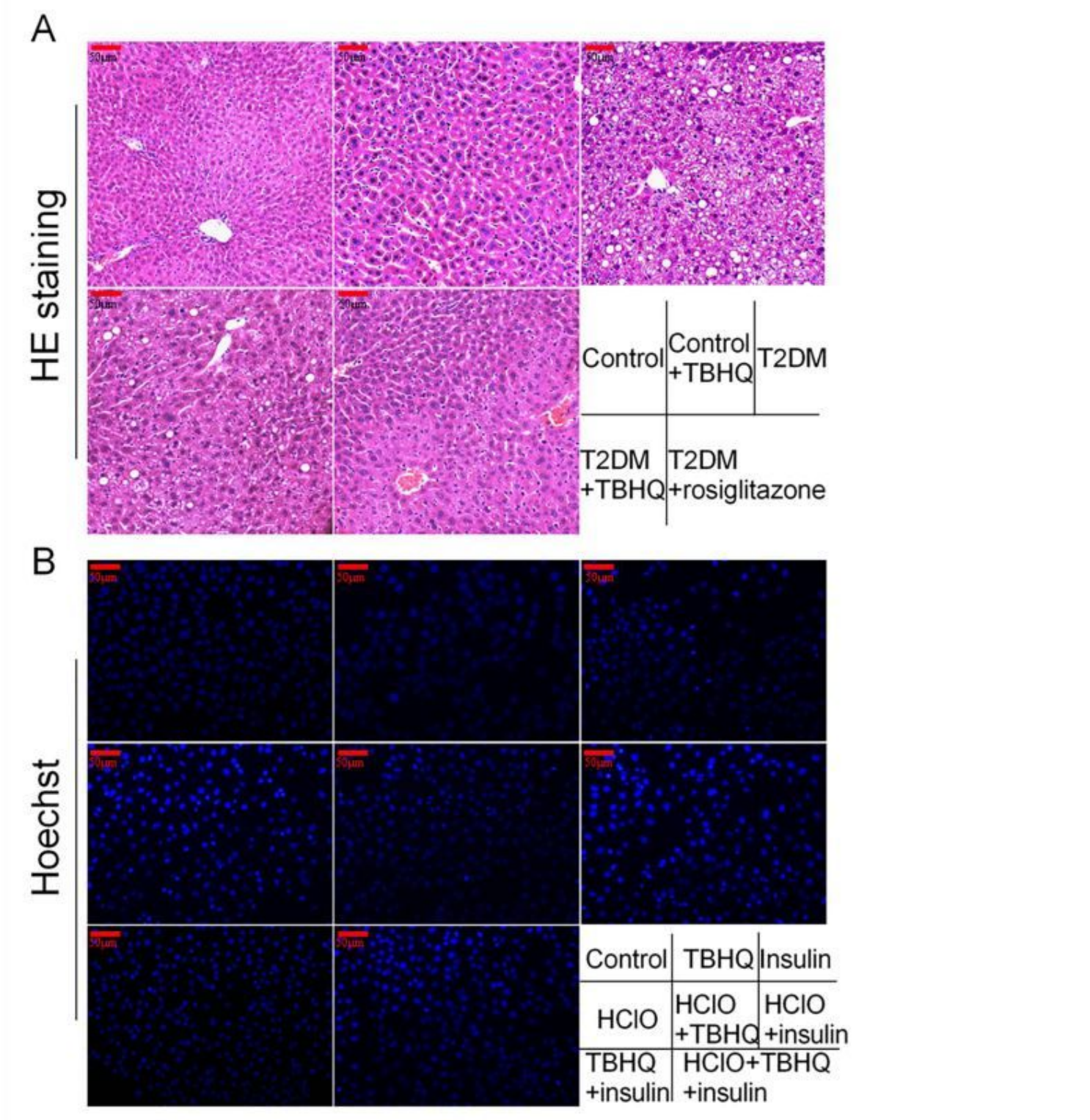

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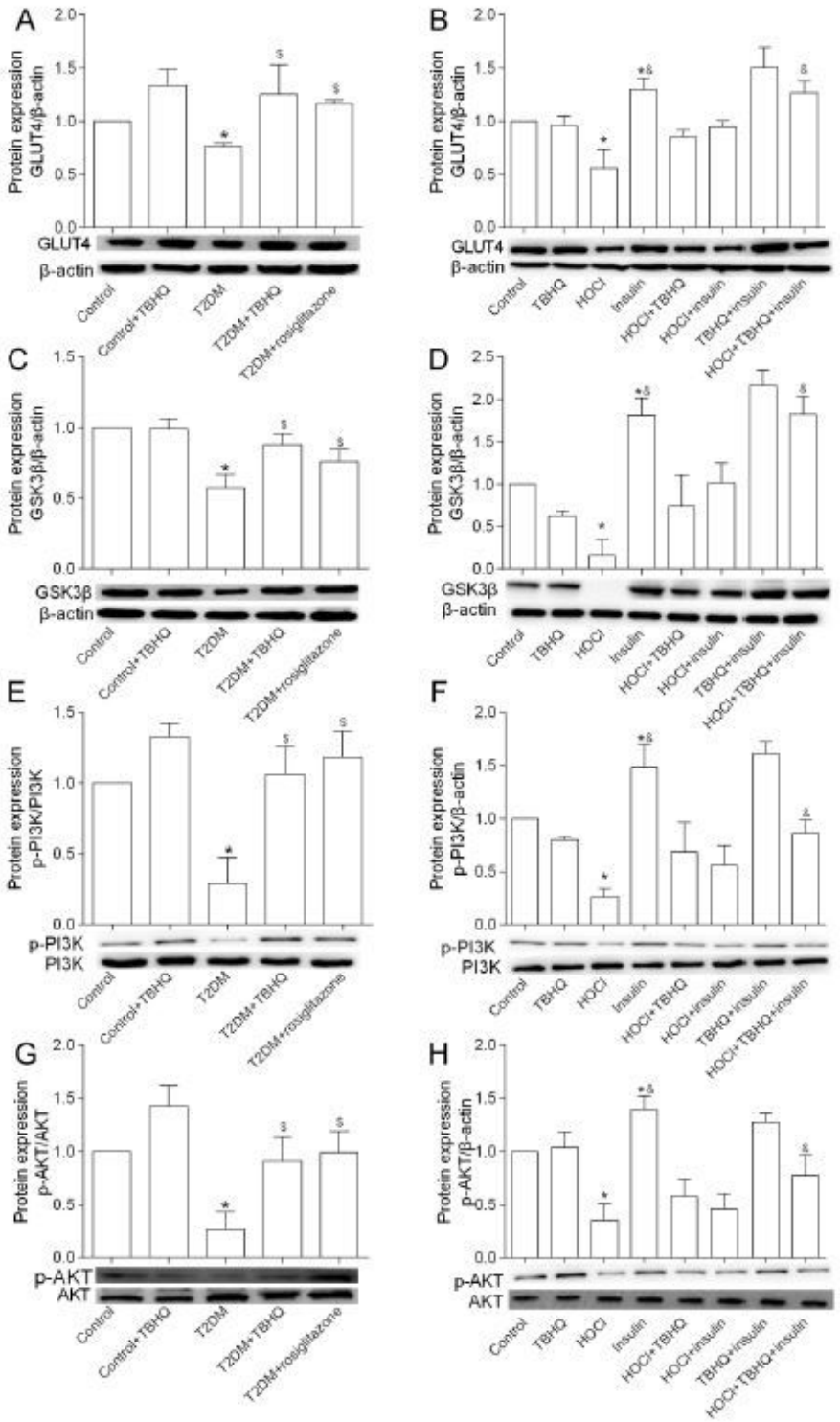

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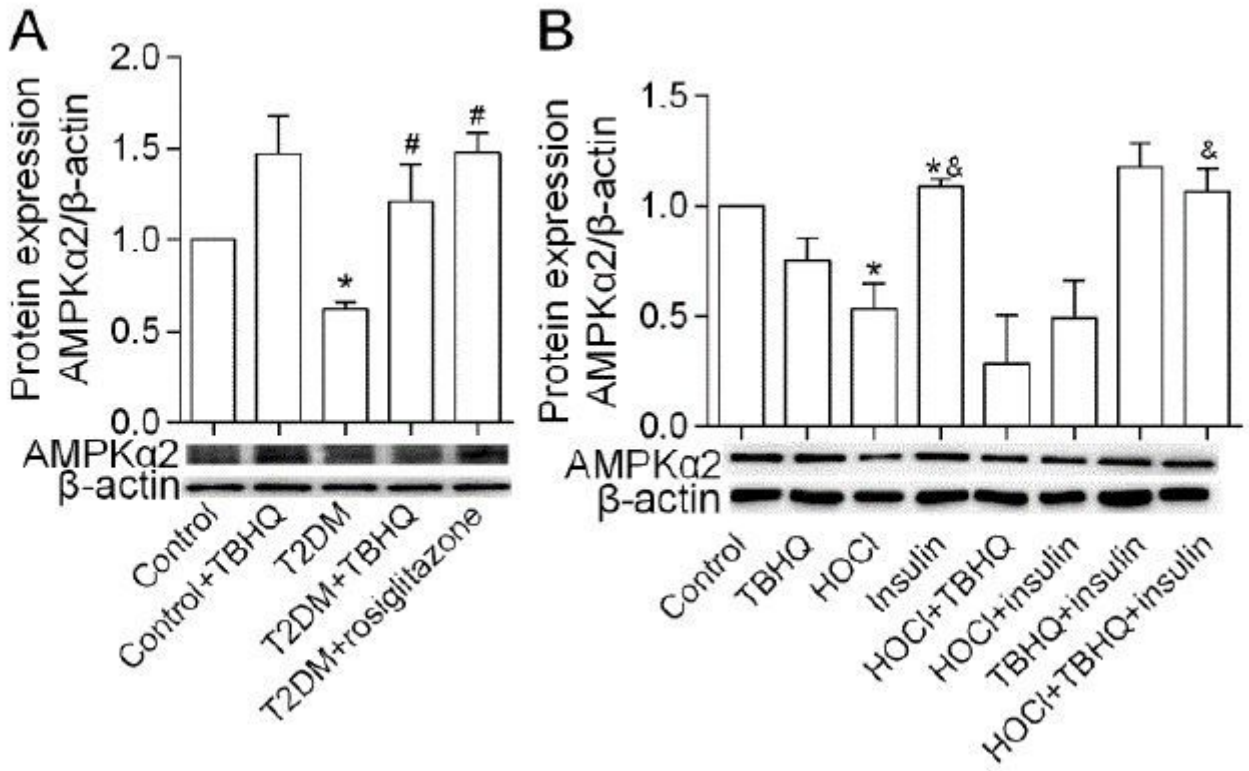

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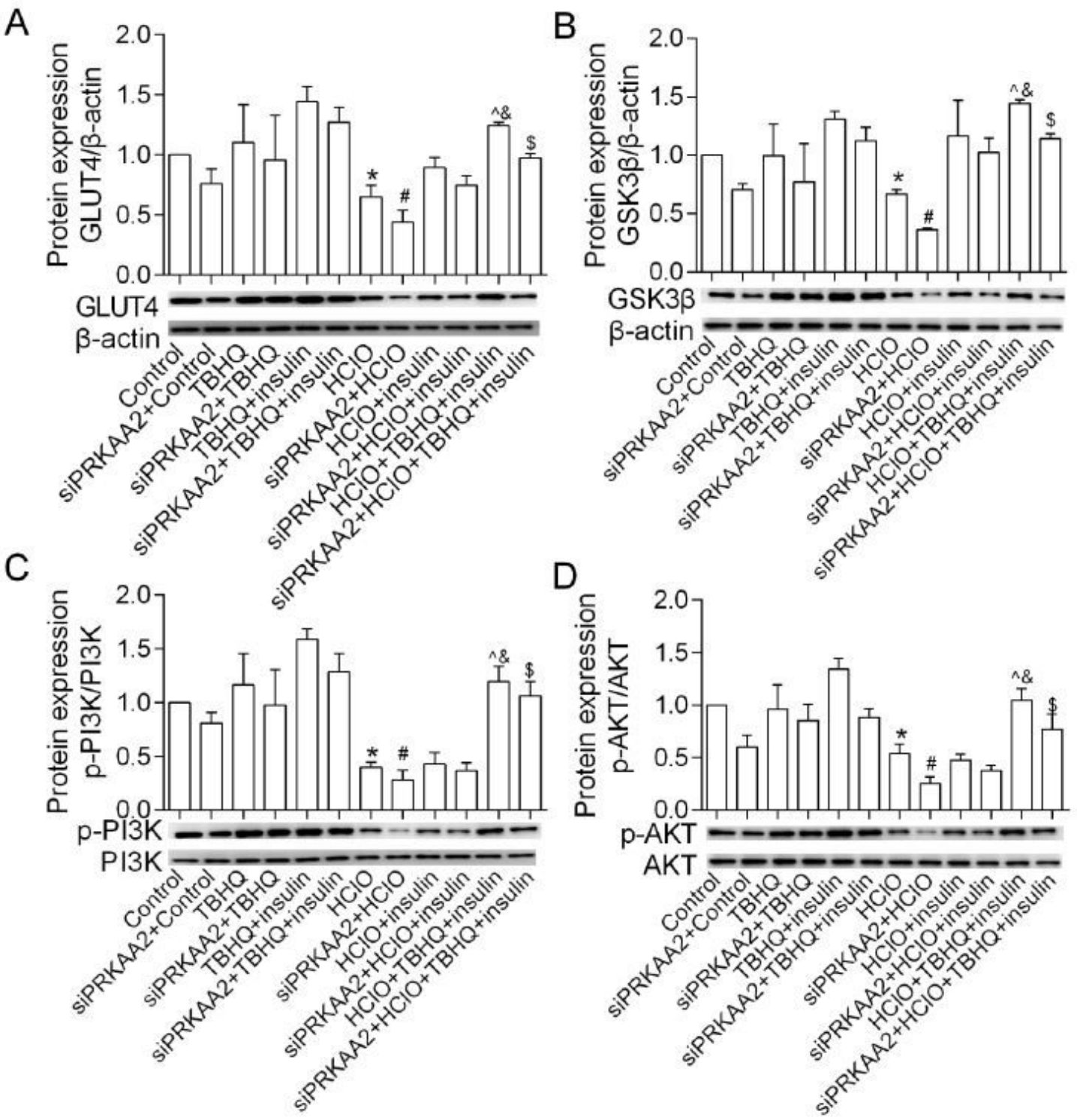

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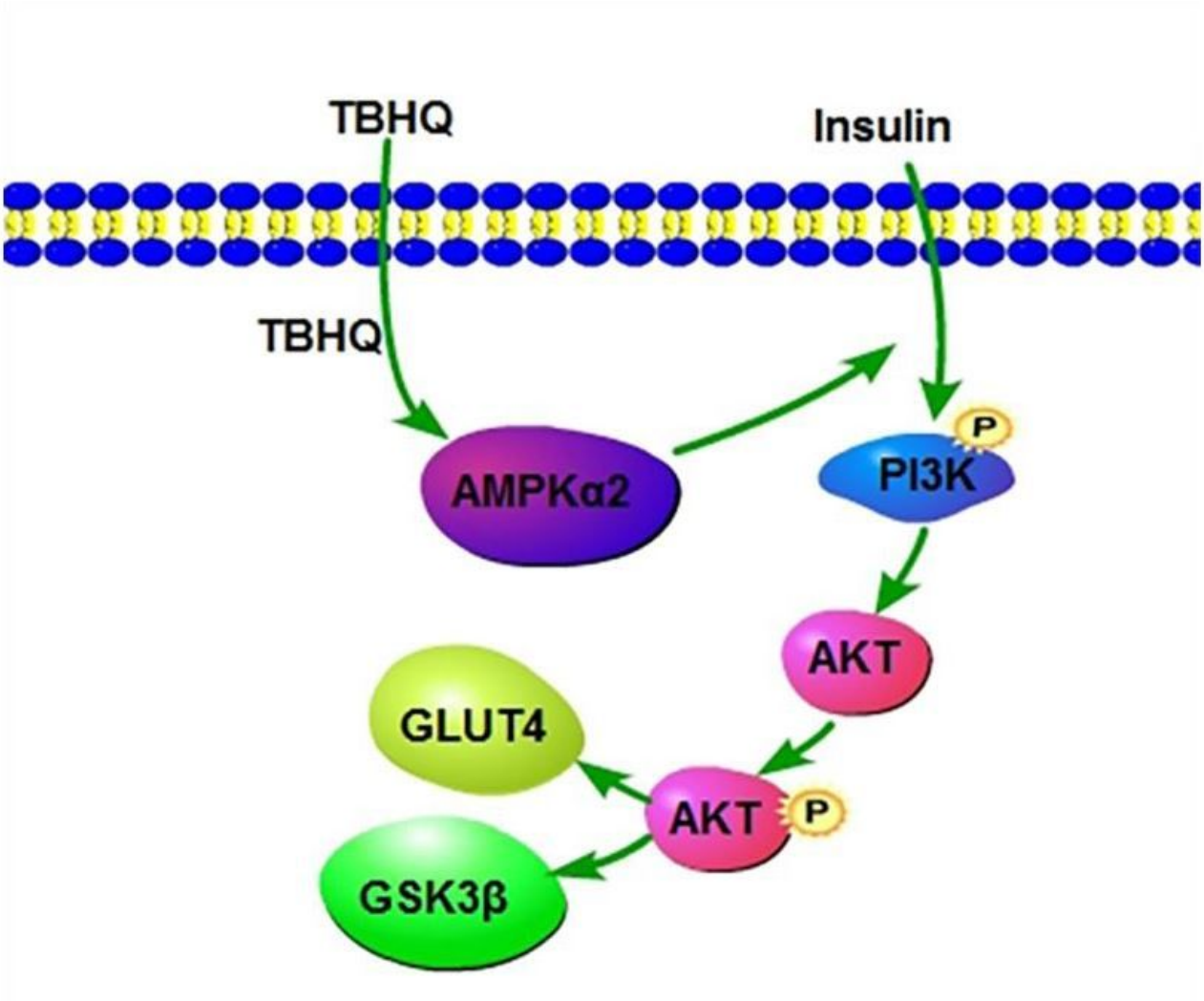

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