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Effects of a *Ganoderma lucidum* Proteoglycan on Type 2 Diabetic Rats and the Recovery of Rat Pancreatic Islets

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Cite This: *ACS Omega* 2023, 8, 17304−17316

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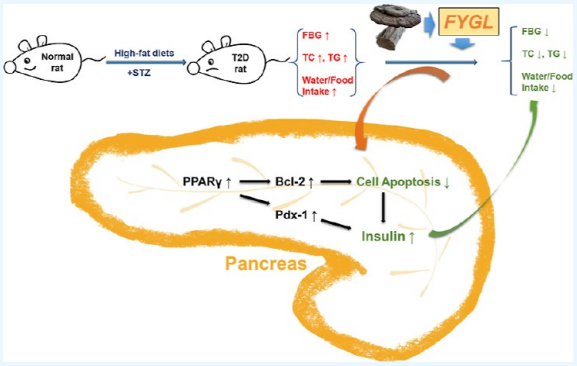
ABSTRACT: Type 2 diabetes (T2D) results from both insulin resistance and pancreatic β-cell dysfunction. A natural proteoglycan extracted from *Ganoderma lucidum*, namely, *FYGL*, has been demonstrated to be capable of ameliorating insulin resistance in previous work. In this work, a T2D rat model induced by streptozocin (STZ) and a high-fat diet was used to investigate the effects of *FYGL* on pancreatic functions, and the transcriptomics of the rat pancreas was used to investigate the biological processes (BP) and signal pathways influenced by *FYGL* on the gene basis. Furthermore, the results of transcriptomics were verified both by histopathological analyses and protein expression. The studies showed that *FYGL* positively regulated T2D-related BP and signaling pathways and recovered the pancreatic function, therefore ameliorating hyperglycemia and hyperlipidemia *in vivo*. Importantly, the recovery of the pancreatic function suggested a crucial strategy to radically treat T2D.



INTRODUCTION

Diabetes is one of the most prevalent and chronic diseases, among which type 2 diabetes (T2D) accounts for about 90% around the world.[1] T2D is thought to result from both insulin deficiency and insulin resistance.[2] There have been many therapies against T2D, such as metformin,[3] pioglitazone,[4] acarbose,[5] glibenclamide,[6] and so on.[7−10] Natural products have many advantages for T2D treatment, such as preferable biosafety, multifunction,[11−13] and multiple active sites.[14] Therefore, natural products are thought to be important for new-drug discovery.[14]

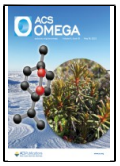
Ganoderma lucidum is a basidiomycete fungus that has been used as a therapy against T2D for a long time in China. Recently, products extracted from *G. lucidum* have been used for curing T2D along with modern technologies. For example, Xu *et al.* showed that insulin sensitivity could be improved by *G. lucidum* polysaccharides by regulating inflammatory cytokines and gut microbiota composition.[15] In our previous studies, a proteoglycan was successfully extracted from the *G. lucidum* fruit body, which was named Fudan-Yueyang *Ganoderma lucidum* (*FYGL*).[16] Nowadays, it is feasible for the large-scale culture of *G. lucidum*, and approximately 1% *FYGL* can be extracted from dried *G. lucidum*.[16] Three fractions can be separated from *FYGL*: the heteropolysaccharide *FYGL-1*, the proteoglycan *FYGL-2*, and the highly branched proteoglycan *FYGL-3*, with molecular weights of 78, 61, and 100 kD, respectively.[17] *FYGL-2* contains 85 ± 2% heteropolysaccharide, and *FYGL-3* contains 82 ± 2% heteropolysaccharide.[17−21] The dominant repeating units of the polysaccharide moieties of *FYGL-1*, *FYGL-2*, and *FYGL-3* and the simulated structure of the protein moiety of *FYGL-2* and *FYGL-3* are shown in Figure 1.[17−21] The percentages of *FYGL-1*, *FYGL-2*, and *FYGL-3* were about 18, 19, and 46%, respectively.[17,18,20,22]

Our previous studies have demonstrated that *FYGL* could inhibit the activity of protein tyrosine phosphatase-1B (PTP1B), improve insulin sensitivity, and then ameliorate T2D in db/db mice.[23] However, some other effects of *FYGL* against T2D were still unclear, such as the effects on the pancreas of T2D rats. In this work, the effects of *FYGL* against T2D were explored in high-fat diets and streptozocin (STZ)-induced T2D Sprague−Dawley rats. Furthermore, the effects of *FYGL* on the pancreas of T2D rats were explored by histopathological and immunohistochemical analyses. Specifically, transcriptomics and Elisa analyses were used to explore the pathways affected by *FYGL* in T2D rats.

RESULTS AND DISCUSSION

Symptoms of T2D Rats Ameliorated by *FYGL*. As shown in Figure 2A, high fasting blood glucose (FBG) was a typical

Received: April 2, 2023
Accepted: April 27, 2023
Published: May 5, 2023



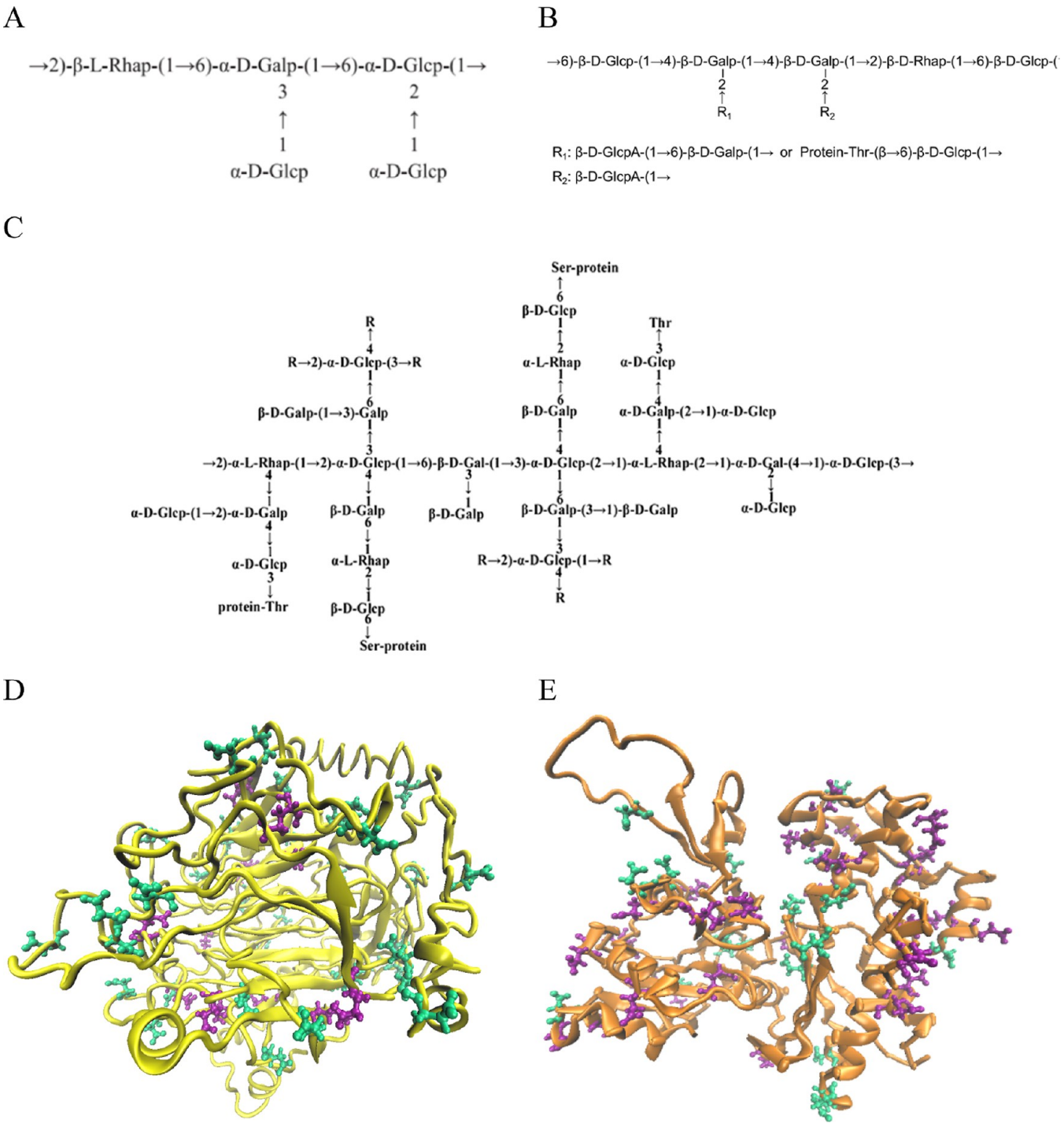


Figure 1. Structures of the polysaccharide components of (A) *FYGL-1*, (B) *FYGL-2*, and (C) *FYGL-3* characterized by chemical analysis and NMR spectrum and the simulated structures of the protein moiety of (D) *FYGL-2* and (E) *FYGL-3*.[17−21] p: pyranose; f: furanose; Thr: threonine; Ser: serine. The acidic amino acids Asp and Glu are marked in green and purple, respectively.

symptom for the T2D rats. FGB of T2D rats decreased dramatically with the effect of the positive control drug, metformin, in the first week, but it rose gradually during the following three weeks, indicating a decreasing effect of metformin. With the treatment of *FYGL*, the FBG of the T2D rats was significantly decreased, and a higher dose of *FYGL* showed a better effect. Furthermore, the hypoglycemic effects of *FYGL* were sustained over 4 weeks. Meanwhile, as shown in Figure 2B,C, the water and food intake of T2D rats was decreased significantly under the effect of *FYGL*, with the high dose of *FYGL* being the most effective. However, as shown in Figure 2D, the effect of metformin or *FYGL* on the body weight (BW) of T2D rats was not observed in this experiment.

Normally, T2D rats are intolerant to high blood glucose resulting from disordered glucose metabolism. The oral glucose tolerance test (OGTT) was used to reflect the ability of blood glucose regulation in T2D rats.[24] As shown in Figure 2E, normal rats showed inconspicuous glycemic variability after being orally given glucose. But the blood glucose in the model group increased rapidly within 30 min and kept at a higher level within

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ACS Omega 2023, 8, 17304−17316

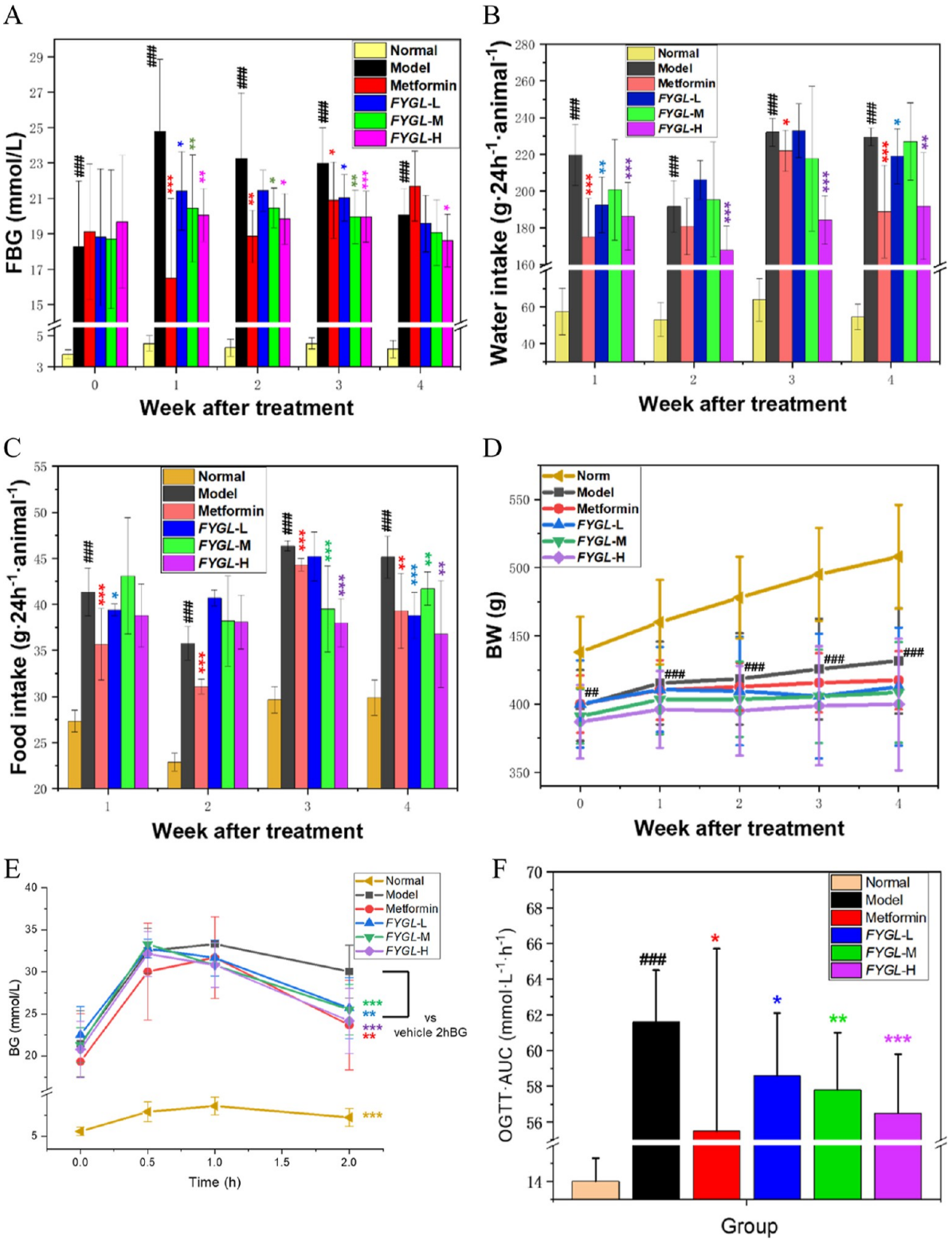

Figure 2. (A) FBG, (B) water intake, (C) food intake, and (D) BW of T2D rats and the influence of *FYGL*, where normal: normal group, model: model group, metformin: 200 mg/kg metformin group as the positive drug group, *FYGL*-L/M/H: *FYGL* groups with dosages of 225(low)/450(middle)/900(high) mg/kg, similarly hereinafter. (E) Effects of *FYGL* on the OGTT and (F) the area under the curves (AUC) of OGTT of T2D rats. $^{\#\#\#}p < 0.001$ *vs* the normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* the model group ($n = 14$).

2 h after the rats were orally given glucose. Under the effect of *FYGL*, blood glucose decreased significantly half an hour after the rats were given glucose. Figure 2F also showed a higher oral glucose tolerance ability for the T2D rats affected by metformin or *FYGL*. These results showed that the oral glucose tolerance ability of T2D rats was obviously recovered by *FYGL*.

Effects of *FYGL* on Differentially Expressed Genes of the T2D Rat Pancreas. The differentially expressed genes

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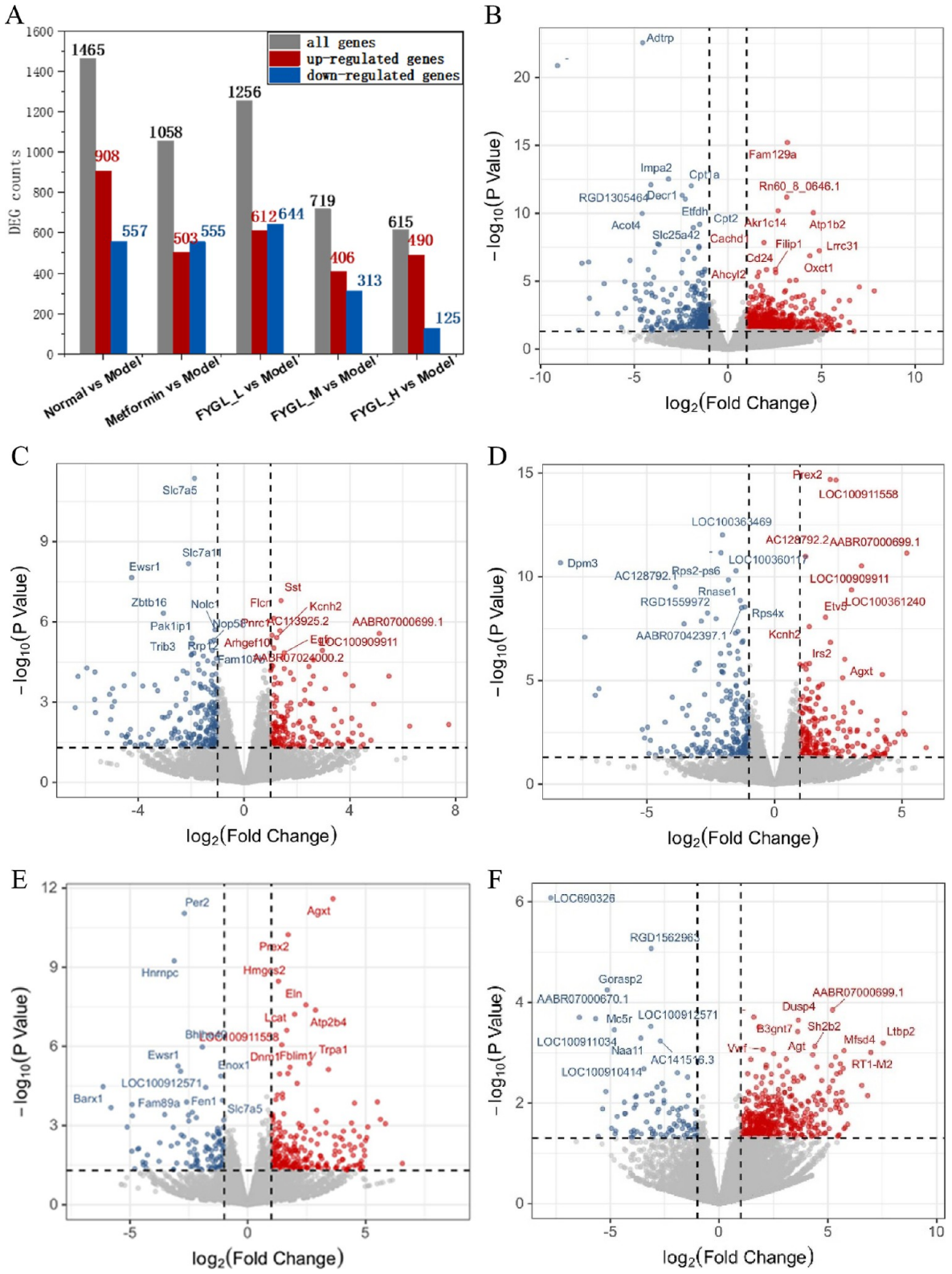

Figure 3. (A) DEG number statistics of each group compared with the model group. DEGs statistics between the model group and (B) normal, (C) metformin, (D) low-dose *FYGL* (*FYGL*-L), (E) middle-dose *FYGL* (*FYGL*-M), and (F) high-dose *FYGL* (*FYGL*-H) groups, respectively, displayed by the volcano plot. Red dots represented up-regulated genes, blue dots represented down-regulated genes, and gray points were not-significant genes. DESeq2 p-value < 0.05, $|\log_2\text{FoldChange}| > 1$.[25,26]

(DEGs) between the model group and other groups were counted by the methods described in the work of Love *et al.*,[25] where genes with a DESeq2 p-value < 0.05 and |log2FoldChange| > 1 were chosen as DEGs.[25−27] As shown in Figure 3, there were 615 DEGs for the *FYGL*-H (high doses of *FYGL*) group compared with the model group, in which 79.6% DEGs were upregulated. In the normal group, more DEGs were upregulated as well. More detailed effects of *FYGL* on the

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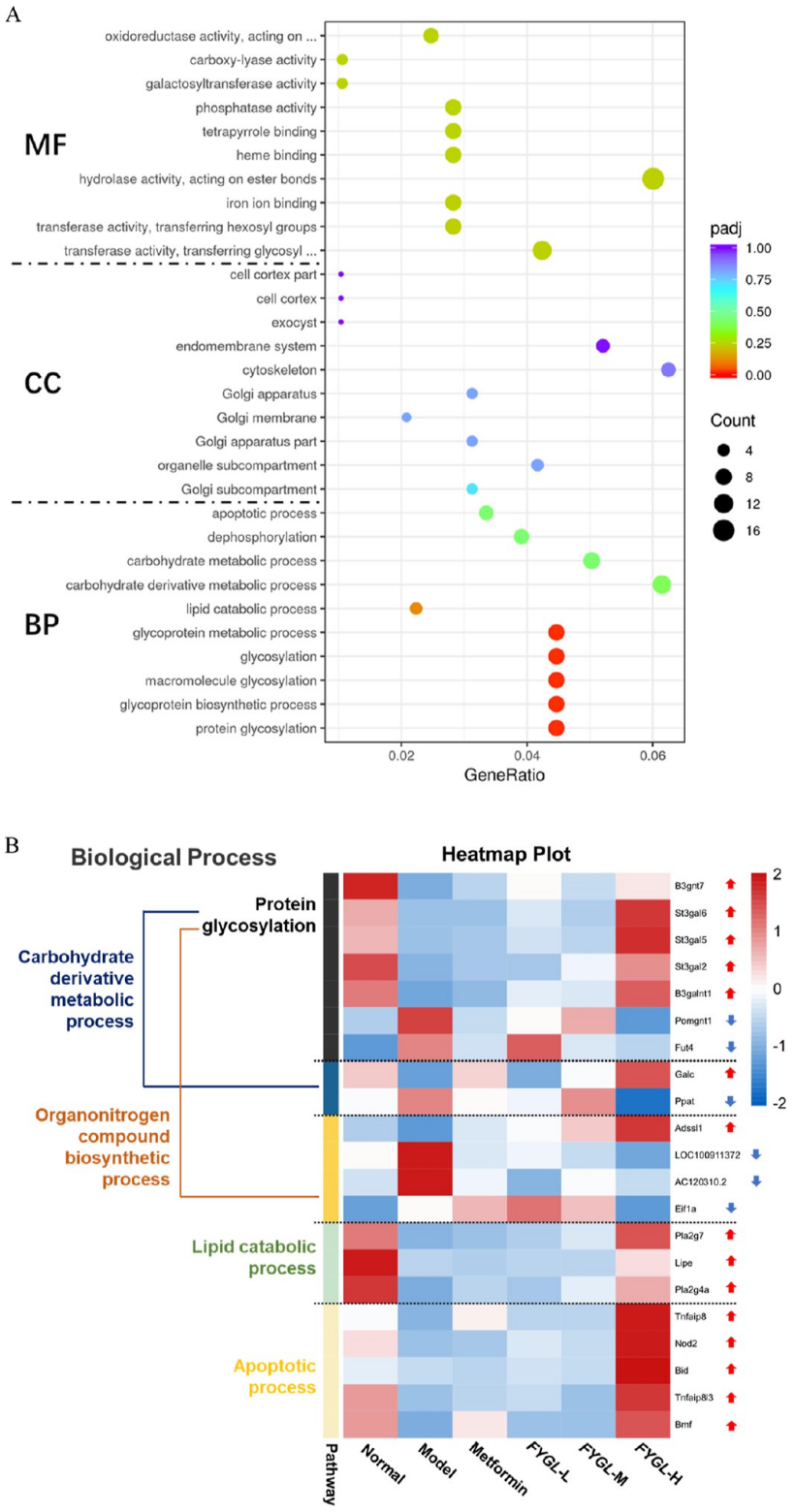

Figure 4. GO enrichment analysis of the T2D rat pancreas transcriptome. (A) Top ten MF, CC, and BP according to the DEGs between the model and the *FYGL*-H group. The lower padj value indicated a higher geostatistical significance of differences. The count number referred to the extent of DEGs related to the MF/CC/BP. (B) DEG heatmap of preferentially enriched BP related to T2D and the effects of high doses of *FYGL*. DEGs with a red color were strongly expressed, and DEGs with a blue color were weakly expressed.

transcriptomics of the T2D rat pancreas were further analyzed by Gene Ontology (GO)[28] enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG)[29] pathway enrichment analysis.

T2D-Related Biological Processes Positively Regulated by *FYGL*. GO enrichment analysis was used to classify the gene function at the molecular and cellular levels. GO enrichment analysis can be divided into three sub-ontologies:

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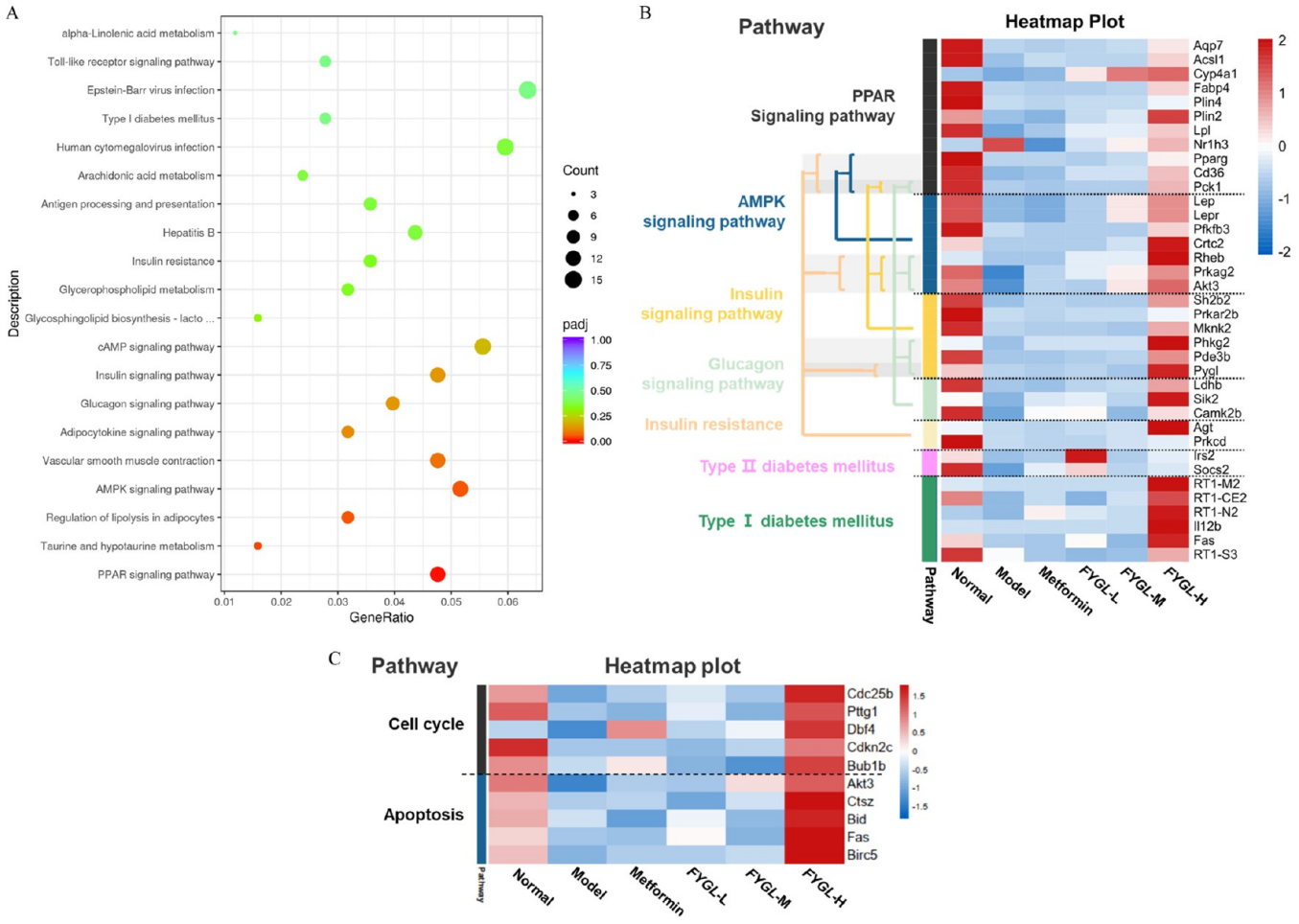

Figure 5. KEGG pathway enrichment analysis of the T2D rat pancreas transcriptome. (A) Top 20 pathways affected by a high dose of *FYGL*. The lower padj value indicated the higher geostatistical significance of differences. The count number referred to the extent of DEGs related to the pathway. (B) DEG heatmap of some preferentially enriched pathways related to T2D and the effects of *FYGL*. (C)DEG heatmap of the cell cycle and apoptosis pathways related to T2D and the effects of *FYGL*. DEGs in red color were strongly expressed, and DEGs in blue color were weakly expressed.

biological process (BP), molecular function (MF), and cellular component (CC). In this work, DEGs involved in specific BP were mainly analyzed. As shown in Figure 4A, BP related to carbon and nitrogen metabolism were enriched according to the DEGs in the *FYGL*-H group, such as glycoprotein metabolic, lipid catabolic, and carbohydrate and carbohydrate derivative metabolic. Many studies have indicated that T2D is always accompanied by metabolic disorders and notable obesity.[30] Energy metabolisms such as carbon and nitrogen metabolism are largely regulated by insulin, and these metabolic processes are disordered in T2D rats due to insulin secretion deficiency and insulin resistance.[31] Storlien *et al.* and Kraegen *et al.* showed that excess lipid accumulation, such as triglyceride (TG) accumulation, of diet-fed rats usually occurs in T2D.[32,33]

As shown in Figure 4B, DEGs of some preferentially enriched BP were closely associated with T2D. For example, for DEGs related to the carbohydrate derivative metabolic process, six of them (B3gnt7, St3gal6, St3gal5, St3gal2, B3galnt1, and Galc) were downregulated, and three of them (Pomgnt1, Fut4, and Ppat) were upregulated in T2D rats compared with normal rats. However, the six downregulated genes and the three upregulated genes in T2D rats were recovered by metformin or *FYGL*, in which the effect of high-dose *FYGL* was the most obvious. This phenomenon indicated that the carbohydrate derivative metabolic process was disordered in T2D rats and then recovered by *FYGL*. Similarly, protein glycosylation, organonitrogen compound biosynthetic, lipid catabolic, and apoptotic processes were also disordered in T2D rats, and these T2D-related BP were also recovered by *FYGL*.

Pathways Positively Regulated by *FYGL* on KEGG Analysis. KEGG enrichment analysis was used to understand the related functions or pathways of the DEGs.[34] As shown in Figure 5A, many diabetes-related pathways were preferentially affected by *FYGL*, such as PPAR (peroxisome proliferator-activated receptor),[35] AMPK (AMP-activated protein kinase),[36] glucagon/insulin signaling, insulin resistance, and type I diabetes mellitus pathways. The PPAR pathways, including PPARα, PPARβ/δ, and PPARγ pathways, have been thought to be potent modulators of metabolic homeostasis and are also highly correlated with diabetes. For example, PPARγ deficiency in the pancreas could result in hyperglycemia with insulin secretion deficiency.[35] Gupta *et al.* showed that about 40% Pdx-1 (pancreatic duodenal homeobox 1) expression in mouse β-cells was PPARγ-dependent, and the downregulation of Pdx-1 in the pancreas could indirectly affect β-cell function and survival.[37]

As shown in Figure 5B, 10 DEGs (Aqp7, Acsl1, Cyp4a1, Fabp4, Plin4, Plin2, Lpl, Pparg, Cd36, and Pck1) were downregulated, and a DEG (Nr1h3) was upregulated in the PPAR signaling pathway in the model group compared with that in the normal group. With the effect of *FYGL*, the abnormal

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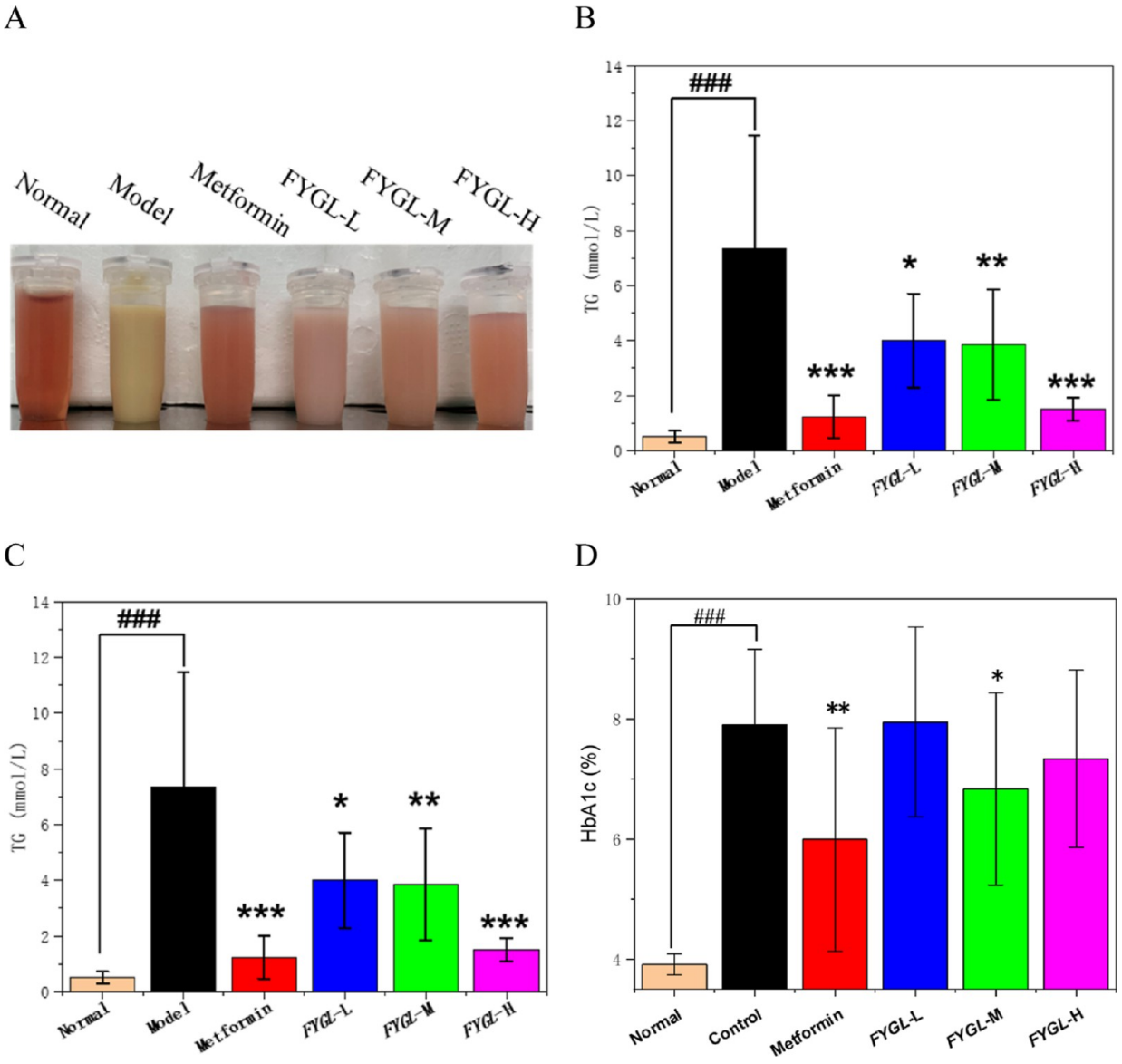

Figure 6. (A) Plasma of rats in different groups. (B) Concentration of TG in rat plasma. (C) Concentration of TC in rat plasma. (D) Percentage of glycosylated hemoglobin (HbA1c)/Hb in the whole blood of rats. $^{###}p < 0.001$ *vs* the normal model group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* the model group ($n = 14$).

expression of these DEGs in T2D rats was revised, indicating that PPAR pathways in the rat pancreas were positively regulated by *FYGL*. Similarly, other pathways in the T2D rat pancreas directly or indirectly related to diabetes were also positively regulated by *FYGL*, such as AMPK, glucagon/insulin signaling, insulin resistance, and type I and II diabetes mellitus pathways. The AMPK pathway is thought to be a master regulator of lipid and glucose metabolism.[31,36] These phenomena indicated that the pancreatic function of T2D could be regulated by *FYGL* in many ways.

GO enrichment analysis of the T2D rat pancreas transcriptome indicated that the apoptotic process of the T2D rat pancreas was ameliorated by a high dose of *FYGL*. In addition, the cell cycle and apoptosis pathways were ameliorated as well, according to the KEGG enrichment analysis, as shown in Figure 5C. Both GO and KEGG enrichment analyses indicated that the apoptotic process in the pancreas of T2D rats could be positively regulated by *FYGL*.

Decrease of Lipids and Glycosylated Hemoglobin (HbA1c) in Blood by *FYGL*. GO and KEGG enrichment analyses of transcriptomics indicated that the processes of lipid metabolism and protein glycosylation in T2D rats were positively regulated by *FYGL*. As shown in Figure 6A, the plasma of the rats in the model group was viscous and lactescent, resulting from the disordered lipid metabolism in T2D rats. The milky and lactescent blood increased the risk of cardiovascular metabolism and peripheral atherosclerosis disease.[38] Interestingly, the lactescent plasma of T2D rats tended to be clear with the influence of metformin or *FYGL*, indicating that lipid metabolism was ameliorated by metformin or *FYGL*. What is more, the higher dose of *FYGL* showed the better effect. TGs and cholesterol were chosen as the typical lipids in plasma, and quantitative analysis shown in Figure 6B,C indicated that the concentrations of TGs and total cholesterol (TC) in the plasma of T2D rats were significantly decreased by *FYGL*. These phenomena confirmed the predictions of GO and KEGG enrichment analyses.

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The percentage of HbA1c/hemoglobin (Hb) in the whole blood of rats can reflect the average level of blood glucose within a period of time. As shown in Figure 6D, the percentage of HbA1c/Hb was decreased by middle and high doses of *FYGL* in four weeks, and a significant decrease was observed in the *FYGL*-M group. It could be explained by the insufficient experimental duration that the percentage of HbA1c/Hb in the high-dose group did not show a significant difference from that in the model group.

Recovery of Damaged Islets in T2D Rats Affected by *FYGL*. The islet histomorphology of rats is shown in Figure 7. In the model group, islets were shrunken and degenerative, and the morphological symmetry of islets was broken. Meanwhile, the density of pancreatic β-cells decreased in the model group compared with the normal group. With the effects of *FYGL*, the area of islets and the density of β-cells were recovered significantly, indicating that islets of T2D rats could be recovered by *FYGL*, which was consistent with the GO and KEGG enrichment analyses of transcriptomics. Zhou and Melton showed that β-cells could be recovered by β-cell self-replication.[39]

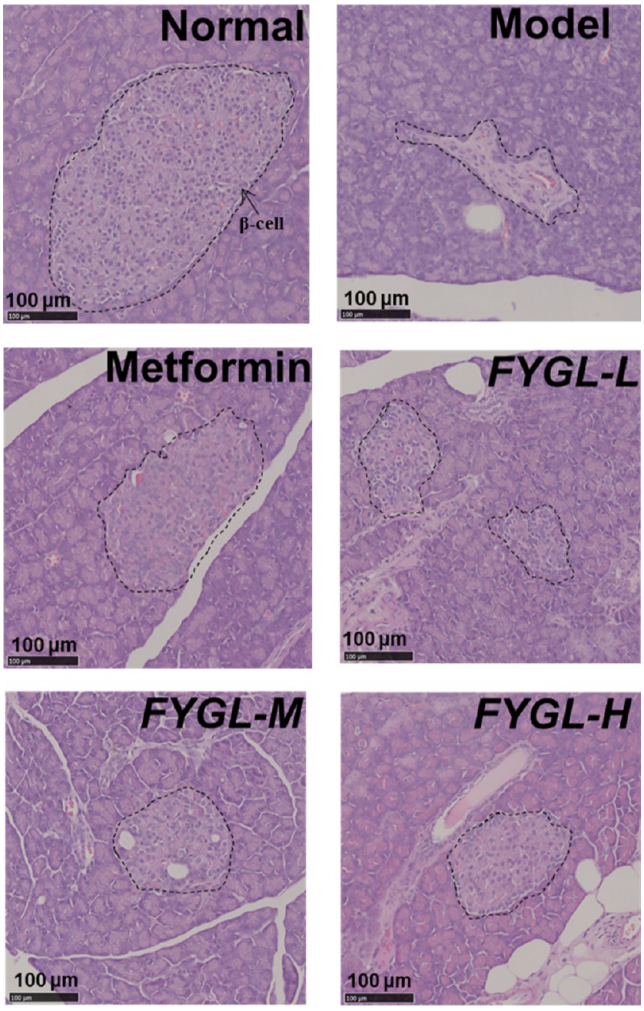

Figure 7. Histopathology of pancreatic islets stained by hematoxylin&eosin (H&E) staining.

Insulin immunohistochemistry was used to observe the function of pancreatic β-cells. As shown in Figure 8A, insulin secretion sharply decreased along with the degeneration of pancreatic islets in the model group, and the relative insulin level in islets was quantified in Figure 8B, which confirmed the dysfunction of insulin synthesis in T2D rats. Under the influence of *FYGL*, the islets and the insulin level were significantly recovered by *FYGL* in a dose-dependent manner. The recovery of insulin secretion in T2D rats resulted from the recovery of the β-cell amount and functions, and therefore, the blood glucose balance was positively regulated by *FYGL*, as shown in Figure 8C. These results were consistent with the KEGG pathway enrichment analysis of pancreatic transcriptomics. Importantly, the recovery of islets by *FYGL* was meaningful for both type I and II diabetes.

Activation of PPARγ Signaling in the T2D Rat Pancreas by *FYGL*. KEGG pathway enrichment analysis showed that PPAR signaling in the rat pancreas was positively regulated by *FYGL* and that the expression of PPARγ (named "Pparg" in Figure 5) could be up-regulated by *FYGL*. According to the Elisa analysis, as shown in Figure 9A, the relative concentration of PPARγ in the pancreas of T2D rats was higher with the influence of *FYGL*, which was consistent with the conclusion of the transcriptomics analysis in Figure 5.

Bcl-2 (B-cell lymphoma-2), known as an apoptosis suppressor protein (an antiapoptotic protein), could be upregulated by PPARγ to inhibit cell apoptosis.[40] As shown in Figure 9B, the expression of Bcl-2 was significantly decreased in T2D rats and was significantly upregulated with the influence of *FYGL*. which explained the recovery of pancreatic islets in T2D rats influenced by *FYGL* in Figure 7.

Pdx-1 is an important insulin activator and is essential for the function of pancreatic β-cells.[41] The expression of Pdx-1 is also positively related to PPARγ. As shown in Figure 9C, Pdx-1 in T2D rat pancreas was significantly upregulated by *FYGL*, which could result in the recovery of the β-cell functions for synthesizing and secreting insulin. The influences of *FYGL* on T2D rats are summarized in Figure 10.

CONCLUSIONS

FYGL significantly ameliorated typical pathological characteristics of T2D, such as hyperglycemia, hyperlipidemia, polydipsia, polyphagia, abnormal OGTT, and high HbA1c *in vivo*. The GO enrichment analysis showed that *FYGL* preferentially enriched and positively regulated the genes related to the processes including glucose, glucose derivatives and lipid metabolisms, and ameliorated cell apoptosis in the rat pancreas. The KEGG enrichment analysis showed that *FYGL* regulated multiple T2D-related signaling pathways, such as PPAR, AMPK, glucagon/insulin, insulin resistance, and type I and II diabetes mellitus. Also, the expression of PPARγ in the T2D rat pancreas was recovered with the effects of *FYGL*, resulting in the upregulation of Pdx-1 and Bcl-2 proteins, which were beneficial for insulin secretion and islet cell activity. Importantly, *FYGL* increased the amounts of β-cells and recovered the functions of pancreatic islets, which suggested a crucial strategy to radically treat T2D.

MATERIALS AND METHODS

Materials. *FYGL* was prepared as described previously.[16] Sprague−Dawley rats (Animal Certification Number: 20170005034927) at 4 weeks old were purchased from the SLRC Shanghai Animal Center, China. The blood glucometer and blood glucose test strip were purchased from Bayer Vital GmbH, Leverkusen, Germany. Citric acid monohydrate, citric acid trisodium salt dihydrate, and STZ were purchased from

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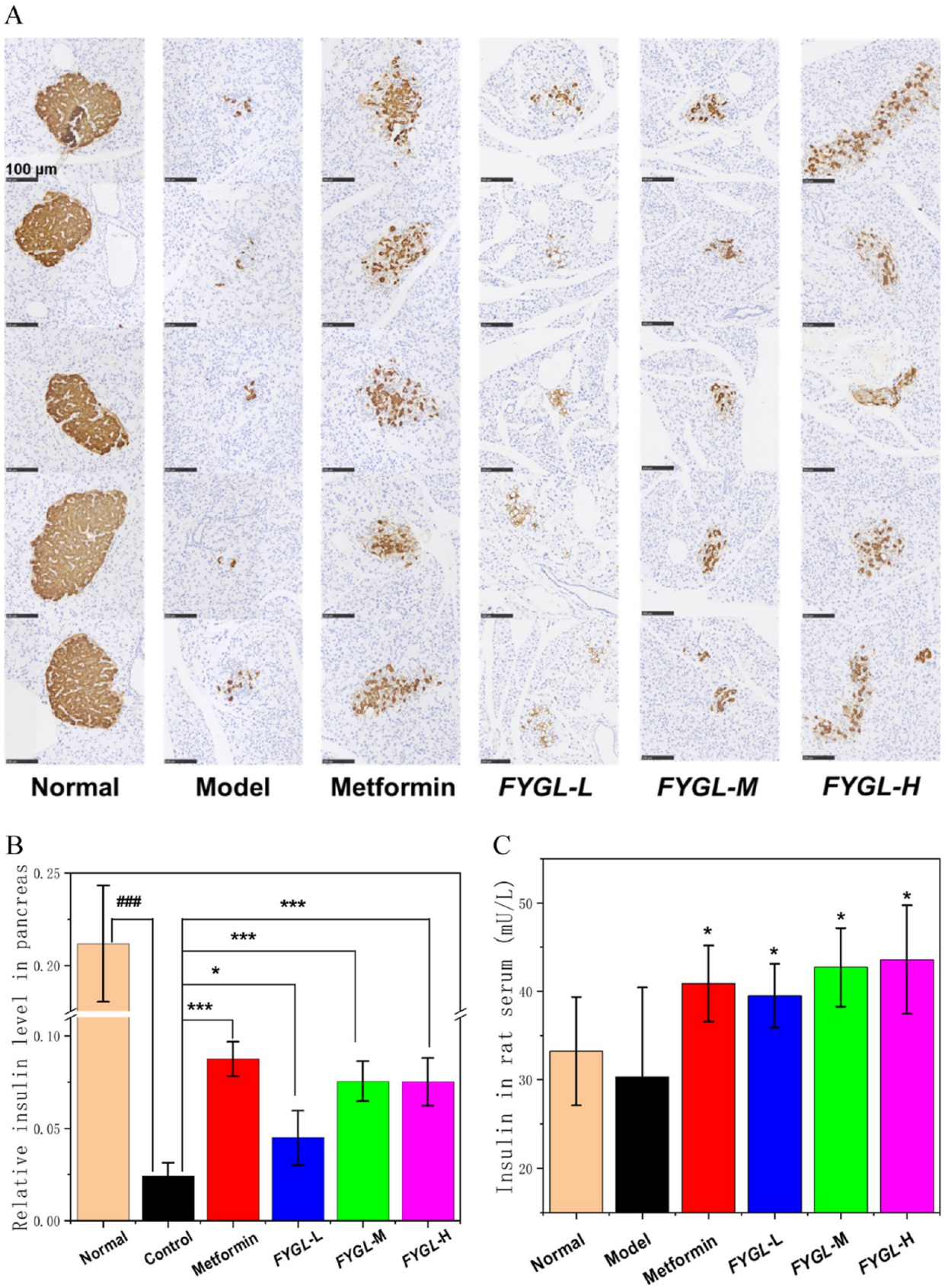

Figure 8. (A) Insulin immunohistochemistry in rat pancreatic islets. (B) Relative insulin level in islets. (C) Concentration of insulin in rat serum. $^{###}p < 0.001$ *vs* the normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* the model group ($n = 4$).

Yeasen Biotechnology Co., Ltd., Shanghai, China. An anti-insulin antibody (art. no. ab282459) and a second antibody were purchased from Abcam, USA. Urethane and other reagents were purchased from Macklin Biochemical Co., Ltd., Shanghai,

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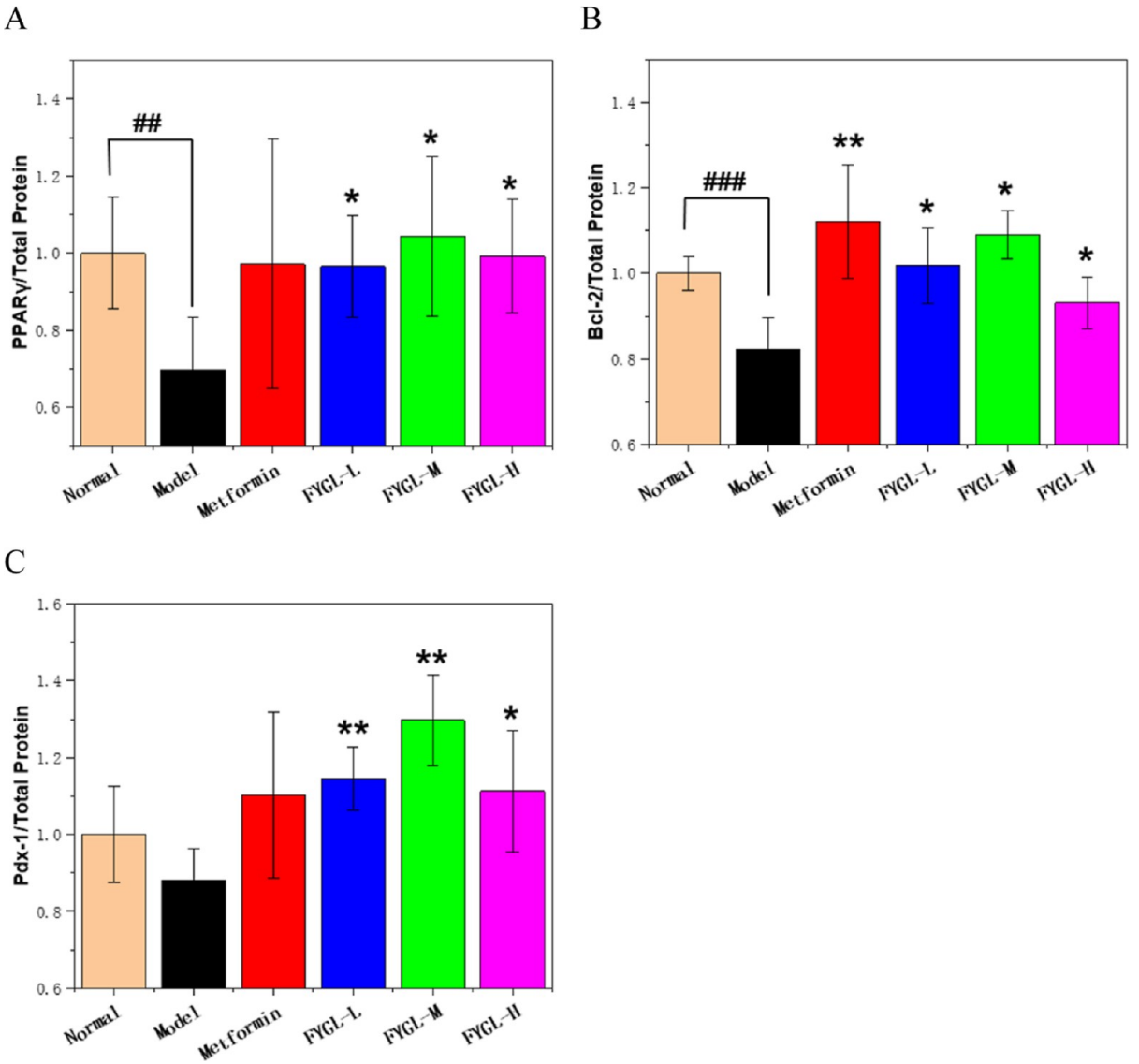

Figure 9. Relative expressions of (A) PPARγ, (B) Bcl-2, and (C) Pdx-1 in the rat pancreas, referred to the normal group. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs* the normal group; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* the model group ($n = 4$).

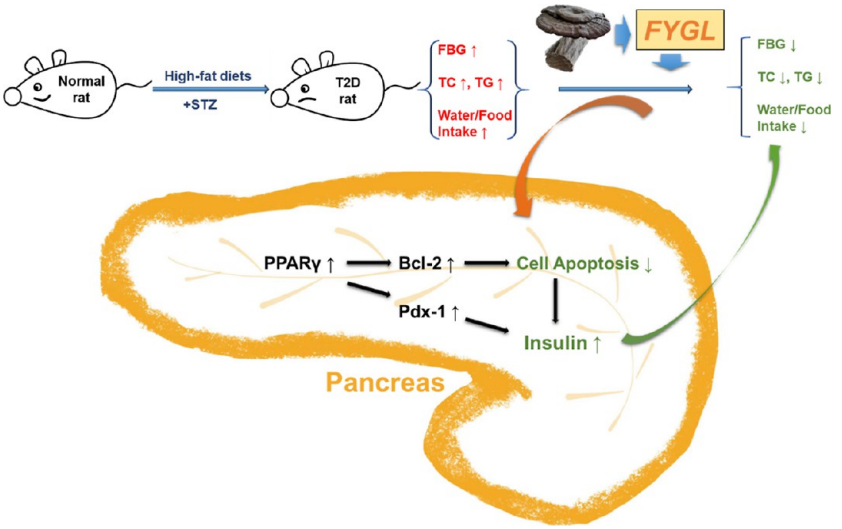

Figure 10. Summary scheme of *FYGL* effects on T2D rats.

https://doi.org/10.1021/acsomega.3c02200
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China. The Elisa kit of insulin was purchased from Crystal Chem, USA, the Elisa kits of PPARγ (art. no. R107668-48T) and Bcl-2 (art. no. PR106976-48T) were purchased from Shanghai sig Biotechnology Co., Ltd., and the Elisa kit of Pdx-1 (art. no. SY-R03915) was purchased from Shanghai Win-win Biotechnology Co., Ltd., China. The BCA (bicinchoninic acid) protein assay kit and RIPA (radio immunoprecipitation assay) lysis buffer were purchased from Beyotime Biotechnology, China.

Animal Experiments. All animal experiments were approved by the Ethics of Animal Experiments Committee of Medical College, Fudan University. All the animals were acclimated to standard laboratory conditions (ventilated room, 20−26 °C, 30−70% humidity, 12 h light/dark cycle) and had free access to standard water and food. After adaptive feeding for 1 week, the rats were randomly divided into normal groups and model groups, and the model rats were treated with a high-fat diet consisting of 10% fat, 2% cholesterol, and 1% bile salt. 4 weeks later, the model rats were treated with STZ (40 mg STZ per kilogram rats, in 0.1 M citric acid/sodium citrate buffer, pH = 4.3), and another 1 week later, the blood glucose of rats was tested by the blood glucose test strip and rats with FBG below 11 mmol/L were treated again with STZ, as described before.[42] Model rats with FBG >11.0 mmol/L were randomly divided into model, metformin, high doses of *FYGL* (*FYGL*-H), middle doses of *FYGL* (*FYGL*-M), and low doses of *FYGL* (*FYGL*-L) groups (n = 14), which were treated with physiological saline, 200 mg/kg metformin, 900 mg/kg, 450 mg/kg, and 225 mg/kg *FYGL* ($LD_{50} \approx 6$ g/kg)[16] by gastric irrigation daily for 4 weeks. FBG, BW, and food and water intake were tested weekly.

Oral Glucose Tolerance. Rats received a gastric gavage of 2.0 g glucose per kilogram after fasting for 12 h. Blood glucose was tested before and after gastric gavage of glucose for rats.

Transcriptomics Analysis. Transcriptomics analysis referred to the methods described in the work of Moreira-Teixeira *et al.*[43] The total RNA of the pancreas was extracted by RiboPure kits (Ambion). The complementary DNA (cDNA) library was prepared by total-reduced RNA by TruSeq Stranded mRNA HT Library Preparation kits (Illumina), and it was initially assessed with HT DNA HiSens Reagent kits (PerkinElmer) using a LabChip GX bioanalyzer (Caliper Life Sciences/PerkinElmer). The DESeq2 p-value based on the negative binomial distribution was used to analyze differential gene expression.[25] GO[28] and KEGG[29] pathway enrichment analyses were, respectively, used to analyze the BP and the signaling pathways affected by *FYGL*.[44]

Blood and Histological Examinations. Rats were anesthetized with 100 mg/kg urethane after fasting for 8 h. Whole blood, serum, and plasma were obtained from the abdominal aorta. Concentrations of TG and TC in plasma were tested by TG and TC testing kits, respectively, in the way of "glycerophosphate oxidase−peroxidase anti peroxidase, GPO−PAP". The proportion of HbA1c to Hb in whole blood was tested by the SIEMENS DCA-Vantage testing system. Pancreatic tissues were embedded in paraffin after being fixed in 10% formaldehyde solution and then cut into 4 μm thick sections. The pathological sections were stained with H&E staining and examined by a light microscope. Similarly, the pathological sections of the pancreas were treated with an anti-insulin antibody (1:1000) overnight at 4 °C and then incubated with secondary antibodies (1:200) for 2 h at 4 °C.[45] Immunohistochemistry of insulin in the pancreas was analyzed with a NanoZoomer 2.0-HT slide scanner by Hamamatsu Photonics.

Protein Relative Expression Measured by Elisa. The concentration of insulin in rat blood and PPARγ, Pdx-1, and Bcl-2 in the rat pancreas were measured by Elisa kits. About 100 mg of rat pancreatic tissue was added into 1 mL RIPA lysis buffer and grinded at −15 °C, and the solid impurity was eliminated by centrifugalizing. The blood sample was diluted 20 times, and the pancreatic extract sample was diluted 5 times before measuring. The concentration of total proteins was measured by the BCA protein assay kit, and the relative expression of a protein was calculated by the equation of 1

$$[X]_{\text{Relative}} = [X] \div [A] \quad (1)$$

where $[X]_{\text{Relative}}$ is the relative expression of the protein X, $[X]$ is the concentration of the protein X, and $[A]$ is the concentration of total proteins.

Statistical Analysis. The experimental data are expressed as the mean ± standard error of the mean. The statistical significance between two groups was evaluated by the software of Microsoft Excel 2016 based on Tukey's test. p-value <0.05 (*) was considered statistically significant, p-value <0.01 (**) was considered statistically very significant, and p-value < 0.001 (***) was considered statistically extremely significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02200.

Extraction of FYGL from Ganoderma lucidum (G. lucidum) (PDF)

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https://doi.org/10.1021/acsomega.3c02200
ACS Omega 2023, 8, 17304−17316

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (nos. 21374022 and 81374032), the National Health Commission of the People's Republic of China (no. 2017ZX09301006), the Science and Technology Commission of Shanghai Municipality (no. 17401902700), and the Shanghai Collaborative Innovation Center of Industrial Transformation of Hospital TCM Preparation.

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https://doi.org/10.1021/acsomega.3c02200
ACS Omega 2023, 8, 17304−17316