

20230405201350841085309659541504

1 **Balancing adipocyte production and lipid metabolism to treat**  
2 **diabetes-associated obesity with a novel proteoglycan from**  
3 ***Ganoderma lucidum***

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14

15 **Abstract**

16 <sup>23</sup> Obesity is often accompanied by metabolic disorder and insulin resistance, resulting in  
17 type 2 diabetes. Based on <sup>7</sup> previous findings, *FYGL*, a natural hyperbranched  
18 proteoglycan extracted from the *G. lucidum* fruiting body, can decrease blood glucose  
19 and reduce body weight in diabetic mice. In this article, <sup>22</sup> the underlying mechanism of  
20 *FYGL* in ameliorating diabetes-associated obesity was further <sup>78</sup> investigated both *in vivo*  
21 and *in vitro*. *FYGL* upregulated expression of metabolic genes related to fatty acid  
22 biosynthesis, <sup>52</sup> fatty acid  $\beta$ -oxidation and thermogenesis; downregulated the expression  
23 of insulin resistance-related genes; and significantly increased the number of beige  
24 adipocytes in db/db mice. In addition, *FYGL* inhibited preadipocyte differentiation of <sup>8</sup>  
25 3T3-L1 cells by increasing the expression of FABP-4. *FYGL* not only promoted fatty  
26 acid synthesis but also more significantly promoted triglyceride degradation and  
27 metabolism by activating the AMPK signalling pathway, therefore preventing fat  
28 accumulation, balancing adipocyte production and lipid metabolism, and regulating  
29 metabolic disorders and unhealthy obesity. <sup>19</sup> *FYGL* could be used as a promising  
30 pharmacological agent for the treatment of metabolic disorder-related obesity.

31

32 **Keywords:** metabolic disorder; obesity; diabetes; *Ganoderma lucidum*; adipocytes;  
33 lipid metabolism; 3T3-L1; AMPK $\alpha$  signalling pathway

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## Introduction

36  
37 Type 2 diabetes mellitus (T2DM) is a chronic degenerative disease, and 60% of T2DM  
38 patients are obese as a result of metabolic disorder and insulin resistance as well as  
39 impaired energy homeostasis [1-6]. Adipose tissues play an important role in surplus  
40 energy storage and energy metabolism [7]. Adipose tissue comprises white adipose  
41 tissue (WAT) and brown or beige adipose tissue (BAT). WAT mainly functions to store  
42 fat in the form of lipid droplets and secrete adipokines to regulate the metabolism of  
43 tissues such as muscle and liver tissues [8]. BAT mainly functions to dissipate excess  
44 energy through thermogenesis to maintain a stable body weight, and it secretes many  
45 adipokines to affect the physiology of a variety of organ systems and tissues, such as the  
46 liver, heart and muscle [9,10]. Accumulating evidence has suggested that a high ratio  
47 of white to beige adipocytes is associated with insulin resistance [3,5].

48 Adipocytes are differentiated from preadipocytes; therefore, many studies have  
49 focused on inhibiting the differentiation of preadipocytes in addition to lipid  
50 metabolism to treat obesity [11,12]. Mesenchymal stem cells (MSCs) undergo a two-  
51 step process to differentiate into adipocytes: MSCs first differentiate into preadipocytes,  
52 and preadipocytes continue to differentiate into mature adipocytes [13,14]. During  
53 adipogenesis, peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and  
54 CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) are marker proteins for preadipocytes  
55 differentiating into mature adipocytes [15,16]. Subsequently, fatty acids are synthesized  
56 in conjunction with the expression of acetyl-CoA carboxylase (ACC $\alpha$ ) and fatty acid  
57 synthase (FAS). Moreover, mature adipocytes further synthesize triglycerides, which

58 aggregate to form lipid droplets [17]. In addition, the triglycerides in lipid droplets are  
59 degraded in conjunction with <sup>7</sup> the expression of adipose triglyceride lipase (ATGL),  
60 hormone-sensitive lipase (HSL), and lipoprotein lipase (LPL) [18,19], which are  
61 regulated by <sup>35</sup> the AMP-activated protein kinase  $\alpha$  (AMPK  $\alpha$ ) signalling pathway;  
62 thermogenesis in BAT is also regulated by this pathway [20,21]. Based on these results,  
63 finding an effective agent to regulate metabolic disorders and alleviate diabetes-  
64 associated obesity is very important.

65 Some antiobesity drugs, such as orlistat and liraglutide, have been applied  
66 clinically in recent years [22,23]. Orlistat controls body weight by inhibiting pancreatic  
67 lipases but has side effects, such as faecal incontinence and flatulence. [24]. Liraglutide  
68 controls body weight by suppressing gastric emptying and food intake, increasing  
69 satiety, and limiting nutrient absorption by increasing pancreatic <sup>65</sup>  $\beta$  cell proliferation,  
70 regenerating  $\beta$  cells, and alleviating insulin resistance but also has side effects such as  
71 nausea, vomiting, and diarrhoea [25]. Metformin, a first-line therapeutic agent for  
72 diabetes, is an AMPK activator capable of increasing insulin sensitivity and decreasing  
73 body weight, but it also has side effects such as abdominal distension, diarrhoea and  
74 gastrointestinal intolerance [26]. In recent years, some natural medicinal plants <sup>1</sup> have  
75 been used in the treatment of obesity and metabolic diseases because of their safety  
76 [27]. *Hibiscus rosa-sinensis* flowers were reported to be capable of decreasing obesity  
77 by reducing adipogenesis and activating AMPK to promote fatty acid oxidation [12].  
78 *Momordica charantia* extracts can activate the AMPK signalling pathway, reduce  
79 adipogenic <sup>13</sup> gene expression and peroxisome proliferator-activated receptor (PPAR)

80 signalling in adipose tissue, and increase lipid oxidation in adipose tissue, thereby  
81 reducing obesity and insulin resistance [28,29]. In addition, cannabidiol can promote  
82 adipocyte browning for the treatment of metabolic diseases [30].

83 Previously, Teng et al. extracted a proteoglycan called *FYGL* (Fudan-Yueyang *G.*  
84 *lucidum*) from the fruiting body of *Ganoderma lucidum*, a traditional Chinese medicinal  
85 herb used for immunoregulation, anti-inflammation, anti-diabetes and anti-  
86 cancer[31,32]. The dominant sequence of *FYGL* is shown in Figure 1 [33,34]. *FYGL* is  
87 a hyperbranched proteoglycan with a molecular weight of  $2.6 \times 10^5$  Da and a saccharide:  
88 protein ratio of 77:17 [33,34]. *FYGL* has been proven capable of decreasing fasting  
89 blood glucose through inhibition of the activity of protein tyrosine phosphatase 1 B  
90 (PTP1B), an insulin resistance receptor, both *in vitro* [35] and *in vivo* [36,37], as well  
91 as reducing body weight in ob/ob mice [38]. However, the underlying mechanism by  
92 which *FYGL* controls body weight is unknown.

93 In this work, the mechanism of *FYGL* antidiabetic associated with obesity was  
94 investigated both *in vivo* and *in vitro*. In *in vivo* studies, adipose tissue from db/db  
95 diabetic mice was used to analyse the expression of genes related to fatty acid  
96 biosynthesis and metabolism, thermogenesis, and insulin sensitivity, which are  
97 beneficial for BAT functions. In *in vitro* studies, the 3T3-L1 cell line was used to  
98 investigate the underlying mechanism by which *FYGL* alleviates obesity. 3T3-L1 cells  
99 are preadipocytes and normally differentiate into mature adipocytes [39]. The effects  
100 of *FYGL* on preadipocyte differentiation and mature adipocyte lipid metabolism were  
101 investigated by multiple approaches, including analysis of protein expression in

102 preadipocytes and the signalling pathways of lipid metabolism in mature adipocytes.

## 103 **Materials and Methods**

### 104 **Materials**

105 <sup>2</sup> Fruiting bodies of *G. lucidum* grown in northeastern China were purchased from  
106 Leiyunshang Pharmaceutical Co. Ltd (Shanghai, China). The preparation of *FYGL* was  
107 described in previous work [36]. <sup>15</sup> Dulbecco's modified Eagle's medium (DMEM), foetal  
108 bovine serum (FBS), and penicillin/streptomycin antibiotics were purchased from  
109 <sup>7</sup> Gibco Co. Ltd (USA). 3T3-L1 cells were obtained from Procell Life Science &  
110 <sup>1</sup> Technology Co. Ltd (Wuhan, China). Fluorescein isothiocyanate (FITC), 4',6-  
111 diamidino-2-phenylindole (DAPI), rhodamine-labelled phalloidin and super ECL  
112 detection reagent were <sup>11</sup> provided by Yeasen Co. Ltd (Shanghai, China). A cell counting  
113 <sup>kit-8 (CCK-8)</sup>, a modified oil red O staining kit, a bicinchoninic acid (BCA) kit,  
114 newborn calf serum (NCS), RIPA lysis buffer, dexamethasone, 3-Isobutyl-1-  
115 methylxanthine (IBMX), paraformaldehyde, Triton X-100, <sup>2</sup> anti-rabbit IgG (H + L), and  
116 a horseradish peroxidase (HRP)-labelled secondary antibody were purchased from  
117 <sup>53</sup> Beyotime Co. Ltd (Shanghai, China). Dimethyl sulfoxide (DMSO) was provided by  
118 <sup>Sigma–Aldrich</sup> (Taufkirchen, Germany). Triglyceride (TG) <sup>12</sup> assay kits were obtained  
119 from Jiancheng Bioengineering Institute (Nanjing, China). The RNAprep pure cell kit  
120 was acquired from TIANGEN Biotech Co. Ltd (Beijing, China). The HiScript III All-  
121 <sup>16</sup> in-one RT SuperMix kit (#R333) and Taq Pro Universal SYBR qPCR Master Mix kit  
122 <sup>(#Q712)</sup> <sup>10</sup> were purchased from Vazyme Biotech Co. Ltd (Nanjing, China). Primary  
123 <sup>8</sup> antibodies against peroxisome proliferator-activated receptor gamma (PPAR, A11183),

124 lipoprotein lipase (LPL, A16252), and  $\beta$ -actin (AC026)<sup>11</sup> were purchased from ABclonal  
125 Technology Co. Ltd (Wuhan, China). Primary antibodies against<sup>8</sup> CCAAT/enhancer-  
126 binding protein  $\alpha$  (C/EBP $\alpha$ , ab40764), fatty acid synthase (FAS, ab128870), fatty acid  
127 binding protein 4 (FABP-4, ab92501), adipose triglyceride lipase (ATGL, ab109251),  
128 AMPK $\alpha$ 1 (ab32047), AMPK $\alpha$ 1 (phospho T183) + AMPK $\alpha$ 2 (phospho T172) (p-  
129 AMPK $\alpha$ , ab133448)<sup>69</sup> were purchased from Abcam (Cambridge, MA, USA). Primary  
130 antibodies against<sup>46</sup> hormone-sensitive lipase (HSL, #4107) were purchased from Cell  
131 Signaling Technology (CST, Beverly, MA, USA).

### 132 **Animal trial**

133 All male BKS-DB (*db/db*) mice (4 weeks old)<sup>11</sup> and wild-type BKS-DB (*db/m*) mice<sup>1</sup>  
134 were purchased from GemPharmatech Co. Ltd, Nanjing, China. Mice were housed in  
135 the specific pathogen-free (SPF) Animal Experimental Center of the School of  
136 Pharmacy, Fudan University,<sup>48</sup> at a constant temperature ( $22 \pm 2$  °C) on a 12 h/12 h  
137 light/dark cycle and were provided standard food and water.<sup>20</sup> All animal trials were  
138 conducted following protocols approved by the Fudan University Institutional Animal  
139 Care and Use Committee. Subsequent experimental procedures were performed<sup>4</sup>  
140 according to the method described in previous works [40,41].<sup>1</sup> Mice were randomly  
141 divided into six groups ( $n = 12$  mice per group): (1) normal group (wild-type BKS mice<sup>1</sup>  
142 treated with saline); (2) control group (*db/db* mice treated with saline); (3) positive  
143 control group (*db/db* mice<sup>1</sup> treated with 225 mg/kg metformin); (4) low-dose group  
144 (*db/db* mice treated with 225 mg/kg FYGL); (5) middle-dose group (*db/db* mice treated  
145 with 450 mg/kg FYGL); and (6) high-dose group (*db/db* mice treated with 900 mg/kg



146 **FYGL**<sup>1</sup>. After 7 weeks of drug treatment, all the mice were sacrificed.

#### 147 **Histopathological analysis of beige adipose tissue**

148 Beige adipose tissue (BAT) was extracted from the scapulae of db/db mice and were  
149 fixed, sectioned, and mounted. The sections<sup>55</sup> were stained with haematoxylin and eosin  
150 (H&E) and observed by microscopy (NanoZoomer 2.0-HT, Japan). Adipocyte numbers  
151 are shown as ratios of the adipocyte number to the area of the selected region (a  
152 randomly selected circle with an area of 0.1 mm<sup>2</sup>) in the images.

#### 153 **RNA sequencing (RNA-seq) analysis of BAT**

154 Total RNA was extracted from beige adipose tissue.<sup>3</sup> RNA purity was checked using a  
155 NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA integrity was  
156 assessed using the RNA Nano 6000 Assay Kit for the Bioanalyzer 2100 system (Agilent  
157 Technologies, CA, USA). Sequencing libraries were generated using the NEBNext®  
158 Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the  
159 manufacturer's recommendations, and index codes were added to attribute sequences  
160 to each sample. Clustering of the index-coded samples was performed on a cBot Cluster  
161 Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to  
162 the manufacturer's instructions. After cluster generation, the library preparations were  
163 sequenced on the Illumina NovaSeq platform, and 150 bp paired-end reads were  
164 generated. Every group was analysed with three biological replicates. Differential  
165 expression analyses between two conditions or groups (two biological replicates per  
166 condition) were performed using the DESeq2 R package (1.16.1). Genes with an  
167 adjusted *P* value<sup>9</sup> of < 0.05 determined by DESeq2 were considered differentially

168 expressed. Gene Ontology (GO) enrichment analysis of the differentially expressed  
169 genes was implemented in the clusterProfiler R package, in which gene length bias was  
170 corrected. GO terms with a corrected *P* value of less than 0.05 were considered  
171 significantly enriched with differentially expressed genes. The clusterProfiler R  
172 package was used to test the statistical enrichment of the differentially expressed genes  
173 in KEGG pathways.

#### 174 **Cell culture and treatment**

175 <sup>40</sup> 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% NCS and  
176 1% penicillin–streptomycin (basal medium I, BMI). When the cells were confluent  
177 <sup>59</sup> (Day 0), adipocyte differentiation was induced by treatment with a cocktail of <sup>27</sup> 5 µg/mL  
178 insulin, 1 µM dexamethasone, and 0.5 mM isobutyl methylxanthine in DMEM  
179 supplemented with 10% FBS and 1% penicillin–streptomycin (differentiation medium  
180 I, DMI). After 48 h <sup>24</sup> (Day 2), the medium was changed to DMEM containing 10% FBS,  
181 1% penicillin–streptomycin, and 5 µg/mL insulin for 48 h (differentiation medium II,  
182 <sup>5</sup> DMII). On Day 4, insulin was removed from the medium, and the cells were maintained  
183 <sup>3</sup> in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin (basal  
184 medium II, BMII), and the medium was changed every two days thereafter [42]. During  
185 <sup>6</sup> differentiation, cells were treated with different concentrations of *FYGL* (0, 50, 100,  
186 200, 400, and 800 µg/mL). Undifferentiated cells cultured in BMI were used as the  
187 blank control group, and differentiated cells cultured in BMI without *FYGL* were used  
188 as the model groups.

#### 189 **Uptake of *FYGL* in 3T3-L1 cells**

190 Three milligrams of FITC fluorescence agent was dissolved in 0.3 mL of DMSO to  
191 prepare a FITC solution with a concentration of 10 mg/mL, and then the solution was  
192 diluted to 1 mg/mL with sodium buffer (SB). *FYGL* (10 mg) was dissolved in 10 mL  
193 SB to form a 1 mg/mL *FYGL* solution, which was mixed with the diluted FITC solution  
194 at a volume ratio of 10:1. The mixture was stirred at low temperature (ice bath) to allow  
195 the formation of fluorescent FITC-*FYGL* complexes. After the coupling reaction was  
196 allowed to proceed overnight, the solution was dialyzed with a 1 kDa dialysis bag to  
197 filter free FITC and then cryodesiccated.

198 3T3-L1 cells were seeded on microscope cover glasses in a 24-well plate at a  
199 density of  $1 \times 10^4$  cells per well and were incubated with FITC-*FYGL* complexes (200  
200  $\mu\text{g/mL}$ ) for 4 h. Nuclei and F-actin (filamentous actin) in 3T3-L1 cells were stained by  
201 DAPI and phalloidin-TRITC (phalloidin-tetramethyl rhodamine), respectively. Cell  
202 images were acquired with a C2<sup>+</sup> laser scanning confocal microscope (Nikon, Japan).  
203 Moreover, 3T3-L1 cells were treated with the indicated concentrations of FITC-*FYGL*  
204 (0, 50, 100, 200, 400, 800  $\mu\text{g/mL}$ ) for 4 h, and then the fluorescence intensity was  
205 determined by flow cytometry (Gallios, Beckman Coulter) to visualize the uptake of  
206 *FYGL* in the cells.

#### 207 **Measurement of cell viability**

208 Cell viability was measured by a cell counting kit-8 (CCK-8) assay. In brief, 3T3-L1  
209 cells were plated into 96-well plates at a density of  $5 \times 10^3$  cells per well and incubated  
210 to near confluence. Some cells were incubated in DMI with different concentrations of  
211 *FYGL* (0, 100, 200, 400, and 800  $\mu\text{g/mL}$ ) for 24 h. After treatment for 24 h, the medium

212 was discarded, and fresh DMI containing CCK-8 solution was added to the 96-well  
213 plates. Approximately 1 h later, a multimode microplate reader (Cytation3, BioTek,  
214 U.S.A.) was used to measure the optical density (OD) at 450 nm.

#### 215 **5 Triglyceride quantification**

216 Triglyceride (TG) concentrations were determined using a commercial kit (Jiancheng  
217 Bioengineering Institute, China). Briefly, differentiated 3T3-L1 cells were washed  
218 twice with phosphate-buffered saline (PBS) and harvested by scraping from the culture  
219 plate in PBS containing 1% Triton X-100 on Day 6. Cell homogenates were obtained  
220 by sonication, and TG concentrations were determined using a commercial kit  
221 according to the manufacturer's instructions. Protein concentrations were measured  
222 using the bicinchoninic acid (BCA) protein assay kit (Beyotime, China) and used for  
223 quantification of proteins in samples.

#### 224 **6 Oil red O staining and quantification**

225 Lipid accumulation in cells was measured by oil red O staining. Differentiated 3T3-L1  
226 cells were subjected to oil red O staining with modified oil red O staining kits  
227 (Beyotime, China). Briefly, the cells were washed with phosphate-buffered saline (PBS,  
228 pH 7.4) and then fixed with 10% (v/v) paraformaldehyde at room temperature for 10  
229 min. Then, the fixation solution was removed, and the cells were washed twice with  
230 PBS. The cells were immersed in washing solution for 20 secs. After the washing  
231 solution was discarded, modified oil red O was added and incubated with the cells at  
232 room temperature for 20 min. Then, the staining solution was removed, and the cells  
233 were washed with washing solution once and PBS twice. Finally, cells stained with oil

234 red O were examined via a polarizing microscope (DM2500P, Leica, Germany). In<sup>4</sup>  
235 addition to this gross evaluation, the dye was dissolved in 60% isopropanol solution,  
236 and the absorbance was measured at 510 nm.<sup>1</sup>

#### 237 RNA extraction and RT-qPCR analysis

238 Total RNA was isolated from differentiated 3T3-L1 cells using RNAprep pure cell kits  
239 (TIANGEN, China)<sup>38</sup> according to the manufacturer's instructions. Conversion of total  
240 RNA to single-stranded cDNA was performed using<sup>51</sup> HiScript III All-in-one RT  
241 SuperMix Kits (Vazyme, China). The series of primers shown in Table 1 for  
242 amplification of  $\beta$ -actin (as an internal reference), C/EBP $\alpha$ , FABP4, ATGL, and LPL  
243 were<sup>77</sup> synthesized by Sangon Co. The primers were mixed with the cDNA templates,  
244 and qPCR was then performed<sup>1</sup> with a Taq Pro Universal SYBR qPCR Master Mix kit  
245 (Vazyme, China)<sup>22</sup> on a qPCR instrument (Bio-Rad, Germany) to amplify the DNA of  
246 C/EBP $\alpha$ , FABP4, ATGL, and LPL. The melt curves of the cDNA were analysed to  
247 determine the specificity of amplification, and<sup>68</sup> quantification of relative mRNA levels  
248 was performed using the  $2^{-\Delta\Delta Ct}$  method with normalization to  $\beta$ -actin mRNA.

#### 249 Protein extraction and immunoblot analysis

250 Immunoblot<sup>4</sup> analysis was performed according to the method described in a previous  
251 report with a minor modification [43]. Differentiated 3T3-L1 cells were lysed in RIPA<sup>12</sup>  
252 lysis buffer and centrifuged (12000  $\times$  g, 10 min, 4 °C). Proteins in the lysates were  
253 separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes.  
254 Then, the<sup>13</sup> membranes were blocked in TBST/5% nonfat dry milk powder; incubated  
255 overnight at 4 °C with antibodies against FABP4, PPAR $\gamma$ , CEBP $\alpha$ , AMPK $\alpha$ , p-AMPK $\alpha$ ,

256 ATGL, HSL, LPL, and  $\beta$ -actin; and incubated with a goat anti-rabbit secondary  
257 antibody at room temperature for 1 h. Finally, enhanced chemiluminescence solution  
258 (ECL) was used to detect the proteins on the membranes. The luminescence signals  
259 were recorded with a Chemiscope3300 mini (Clinx Science Instruments, China). Data  
260 were collected from three independent experiments.

#### 261 **Statistical analysis**

262 All data were analysed by SPSS 20.0 (SPSS, Inc., U.S. and are expressed as the mean  
263  $\pm$  S.D. values. One-way ANOVA followed by the Bonferroni correction was performed  
264 to analyse the statistical significance of differences among the groups. A value of  $P <$   
265 0.05 was considered statistically significant.

#### 266 **Results and Discussion**

##### 267 **Effect of FYGL on BAT histopathology *in vivo***

268 Teng previously proved that FYGL can decrease triglycerides and total cholesterol in  
269 SD rats with STZ-induced diabetes [33], which is closely related to lipid biosynthesis  
270 and metabolism. In the present work, BAT in db/db mice was subjected to  
271 histopathological analysis. Figure 2A shows that the size of beige adipocytes was larger  
272 and the numbers were lower in the control group than in the normal group, whereas  
273 treatment with metformin and FYGL reduced the size of adipocytes. Semiquantitative  
274 analysis of H&E staining in Figure 2B showed that FYGL significantly increased the  
275 number of adipocytes per unit area of BAT in a dose-dependent manner and even  
276 outperformed metformin.

277 Cypess et al. proved that the amount and activity of BAT are inversely correlated

278 with body mass index [44]; the smaller the size and the greater the number of beige  
279 adipocytes, the healthier the body [45-47]. Ouellet et al. demonstrated that the activity  
280 of beige adipocytes is positively correlated with the level of glucose uptake in cells,  
281 which modulates the blood glucose content [48]. Therefore, increasing the number or  
282 activation of beige adipocytes could be a potential approach to treat type 2 diabetes-  
283 associated obesity [49]. Consistent with those studies, the results of this study showed  
284 that beige adipocytes were significantly enlarged and increased in number in db/db  
285 mice, while these changes were significantly reversed after *FYGL* treatment.

#### 11 **Effect of *FYGL* on lipid metabolism *in vivo***

35 Type 2 diabetes is strongly associated with genes of lipid metabolism[50]. In this work,  
287 BAT transcriptome sequencing was performed to explore the potential molecular  
288 mechanism of lipid metabolism *in vivo*. As shown in Figure 3A, the screening results  
289 of the differentially expressed genes (DEGs) showed that the ratio of upregulated:  
290 downregulated: all significant differentially expressed genes was approximately  
291 0.5:0.5:1 in the metformin and *FYGL* groups compared with the control group, nearly  
292 the same as the ratio in the normal group compared to the control group. Figure 3B  
293 shows the hierarchical clustering heatmap. The large coloured square patterns represent  
294 the upregulated or downregulated genes in the different groups. The change in colour  
295 from blue to red indicates a change in the gene expression from downregulation to  
296 upregulation. The narrow columns on the left show the pathway-related genes. Figure  
297 3B shows that the colour patterns of the DEGs in the control group were different from  
298 those in the normal group for most genes except *Ppp1r3b*, while the colour patterns in  
299

300 the *FYGL* group were similar to those in the normal group. From the pathway indication  
301 in the upper-left corner in Figure 3B, it can be seen that the DEGs were involved in the  
302 pathways of fatty acid synthesis (black), fatty acid oxidation (green), insulin resistance  
303 (yellow), and thermogenesis (purple).

304 As shown in Figure 3B, *FYGL* increased the mRNA levels of *Ppp1r3b*, *Fasn*,  
305 *Acaca*, *CPT2*, and *Acadl* in the BAT of db/db mice compared to those in the control  
306 group. The *Ppp1r3b* gene encodes protein phosphatase 1, which is a critical protein in  
307 glycogen metabolism regulated by insulin [51]. The *Fasn* (encoding FAS [52]) and  
308 *Acaca* (encoding ACC $\alpha$  [53]) genes are involved in fatty acid synthesis [52,53]. *CPT2*  
309 (encoding CPT-II, carnitine palmitoyl transferase II [54]) and *Acadl* (encoding ACADL,  
310 acyl-CoA dehydrogenase long chain [55]) are involved in the  $\beta$ -oxidation of long-chain  
311 fatty acids in mitochondria [54,55]. The imbalance between fatty acid synthesis and  
312 degradation can lead to dyslipidaemia, diabetes and cardiovascular disease [56, 57].  
313 Transcript analysis of those genes in BAT indicated that *FYGL* could upregulate fatty  
314 acid metabolism *in vivo*. Additionally, as shown in Figure 3B, *FYGL* upregulated fatty  
315 acid degradation genes (*CPT2* and *Acadl*) more significantly than fatty acid synthesis  
316 genes (*Fasn* and *Acaca*). In addition, as shown in Figure 3C, *FYGL* increased the levels  
317 of *Cd81* (encoding CD81 [58]) and *Slc25a4* (encoding SLC25A4 [59]) compared to  
318 those in the control group, and the levels of these mRNAs in the *FYGL* group were even  
319 higher than those in the metformin group. CD81 is a marker of beige adipocyte  
320 progenitors. The absence of CD81 leads to diet-induced obesity, insulin resistance, and  
321 adipose tissue inflammation [58]. SLC25A4, a mitochondrial ATP/ADP transporter,



322 regulates BAT thermogenesis through UCP1-independent mechanisms [60]. Beige  
323 adipocytes can produce heat by metabolizing fatty acids. Transcriptome analysis  
324 indicated that *FYGL* could increase the expression of thermogenesis genes (*Cd81* and  
325 *Slc25a4*) in BAT, as indicated by the transition from blue to red in Figure 3C.  
326 Furthermore, *FYGL* and metformin increased the mRNA levels of *Akt2* (encoding  
327 AKT2 [61]) and *Slc2a4* (encoding GLUT-4, glucose transporter-4 [62]), as shown in  
328 Figure 3B. Deficiency of AKT2 and GLUT-4 leads to type 2 diabetes and insulin  
329 resistance [61,63].

330 The GO (Gene Ontology) database is a comprehensive database describing gene  
331 functions and includes the biological process (BP), cellular component (CC), and  
332 molecular function (MF) ontological categories. Figure 4A shows the bubble plot of the  
333 biological processes in the GO enrichment analysis (*FYGL* vs. control), where the  
334 redder the dot is, the more significant the enrichment of the biological process. Figure  
335 4A shows that DEGs were mainly enriched in terms related to the biological processes  
336 of cellular respiration, fatty acid metabolism, tricarboxylic acid metabolism, fatty acid  
337 oxidation, etc., and that *FYGL* restored BAT functions in db/db mice through those  
338 biological processes. Figure 4B is a directed acyclic graph (DAG, *FYGL* vs. control) of  
339 the GO biological process enrichment analysis results and indicates the relationship of  
340 functions from upregulated to downregulated biological processes. Figure 4C shows  
341 that the biological processes were eventually refined to include only fatty acid  
342 metabolism and cellular respiration. *FYGL* upregulated the fatty acid metabolism  
343 process and promoted thermogenesis in brown adipocytes.

344 KEGG enrichment analysis aims to identify connections between differentially  
345 expressed genes and signalling pathways. Figure 4C shows the bubble plot of the  
346 signalling pathways in the KEGG enrichment analysis (*FYGL* vs. control), where the  
347 redder the dot is, the more significant the enrichment of the signalling pathway. Figure  
348 4B shows that DEGs were predominantly enriched in signalling pathways related to  
349 oxidative phosphorylation, thermogenesis, the citrate cycle (TCA cycle), fatty acid  
350 metabolism, and fatty acid biosynthesis. The data in Figure 4B suggest that *FYGL*  
351 promotes the functions of BAT through those signalling pathways.

352 These findings indicated that *FYGL* could balance fatty acid biosynthesis and  
353 metabolism to effectively dissipate energy, therefore reducing insulin resistance and  
354 increasing insulin sensitivity *in vivo*.

#### 355 Cellular uptake of *FYGL*

356 To reveal the underlying mechanisms of *FYGL* in mediating biological functions,  
357 investigations at the cellular level are necessary. Figure 5A shows the uptake of *FYGL*  
358 (200 µg/mL and 400 µg/mL) in 3T3-L1 cells, as measured by confocal laser scanning  
359 microscopy, where green fluorescence was found in the cells cultured with FITC-*FYGL*,  
360 indicating that *FYGL* could be taken up well into 3T3-L1 cells. Moreover, the results  
361 of flow cytometric analysis of *FYGL* uptake in 3T3-L1 cells are shown in Figure 5B  
362 and Figure 5C; the peak of the curve shifted to the right as the FITC-*FYGL*  
363 concentration increased, and the uptake of *FYGL* in cells occurred in a dose-dependent  
364 manner.

#### 365 Effect of *FYGL* on cell viability

366 To examine the cytotoxicity of *FYGL* in 3T3-L1 adipocytes, cell viability was measured  
367 using the CCK-8 assay. Adipocytes were treated with various concentrations of *FYGL*  
368 (0-800 µg/mL). The CCK-8 assay results shown in Figure 5D demonstrate that *FYGL*  
369 had no obvious cytotoxicity at concentrations up to 800 µg/mL.

#### 370 **Effect of *FYGL* on the accumulation of intracellular triglycerides and lipids**

371 Lipid accumulation in adipocytes is a hallmark of adipogenesis. Mature differentiated  
372 cells accumulate triglycerides, which then converge to form lipid droplets (LDs). *FYGL*  
373 significantly decreased the triglyceride content, as shown in Figure 6A. Moreover, cell  
374 differentiation and lipid accumulation can be identified by oil red O staining and  
375 triglyceride assays. Figure 6B shows that the number of lipid droplets (red staining)  
376 was markedly increased in cells cultured in differentiation medium (DM) but was  
377 significantly decreased when the cells were cultured with *FYGL* (200-400 µg/mL), and  
378 Figure 6C quantitatively shows the effect of *FYGL* on lipid droplet accumulation.  
379 Excessive accumulation of lipid droplets in adipocytes leads to obesity and insulin  
380 resistance [17]. *FYGL* inhibited triglyceride accumulation and lipid droplets in  
381 differentiated adipocytes. The mechanism of inhibition was further investigated as  
382 follows.

#### 383 **Effect of *FYGL* on the expression of adipogenic and lipolytic genes and proteins**

384 Several reports have shown that peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ )  
385 and CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ) are marker proteins of adipocyte  
386 differentiation and adipogenesis [64-66]. Tali et al. found that fatty acid binding  
387 protein-4 (FABP-4)-null preadipocytes can enhance PPAR $\gamma$  expression and activity,

388 while the overexpression of FABP-4 inhibits PPAR $\gamma$  expression and adipogenesis [67].  
389 Furuhashi et al. further found that FABP-4-null mice exhibit decreased lipolysis in  
390 adipocytes and pancreatic  $\beta$  cells and reduced insulin secretion [68]. To reveal the  
391 mechanisms by which *FYGL* inhibits the accumulation of intracellular triglycerides and  
392 lipids, <sup>32</sup> the effect of *FYGL* on the expression of adipogenic and lipolytic genes and  
393 proteins was investigated. Interestingly, *FYGL* <sup>5</sup> significantly increased the transcript  
394 <sup>8</sup> level of FABP-4 (Figure 7A) in 3T3-L1 preadipocytes and considerably increased the  
395 transcript level of C/EBP $\alpha$  in adipocytes cultured in differentiation medium (Figure 7B).  
396 Moreover, *FYGL* increased <sup>32</sup> the mRNA level of lipolytic genes, such as ATGL (Figure  
397 7C) and LPL (Figure 7D).

398 Furthermore, <sup>19</sup> Western blotting was used to analyse the protein expression of FABP-  
399 4, PPAR $\gamma$ , and C/EBP $\alpha$ , as shown in Figure 8A. *FYGL* greatly increased FABP-4  
400 expression, as shown in Figure 8B, and markedly decreased <sup>8</sup> PPAR $\gamma$  and C/EBP $\alpha$   
401 expression, as shown in Figure 8C and 8D. This work proved that *FYGL* <sup>17</sup> could inhibit  
402 the differentiation of 3T3-L1 preadipocytes and promote lipolysis in adipocytes,  
403 therefore reducing lipid droplet accumulation.

#### 404 **Effect of *FYGL* on lipid metabolism and the AMPK $\alpha$ signalling pathway**

405 <sup>56</sup> Studies have shown that fatty acid synthase (FAS) plays an important role in lipogenic  
406 pathways, which are involved in fatty acid biosynthesis [69]. In addition, the AMPK $\alpha$   
407 signalling pathway also plays a critical role in lipolysis [20,70,71]. Activating the  
408 AMPK signalling pathway can increase the activity of the lipases ATGL, HSL, and  
409 LPL, thus promoting the utilization of lipid storage [20,70-72]. ATGL and HSL catalyse

410 triglyceride degradation, releasing the fatty acids in lipid droplets of adipocytes [73],  
411 while adipocytes secrete LPL to degrade triglycerides in VLDL in vessels [74]. As<sup>3</sup>  
412 shown in Figure 9A and 9B, *FYGL* increased the protein expression of FAS.  
413 Additionally, *FYGL* increased the phosphorylation of AMPK $\alpha$  (Figure 9C&D) and  
414 consequently increased the protein expression of lipolysis markers, such as ATGL  
415 (Figure 9E), HSL (Figure 9F), and LPL (Figure 9G).

416 <sup>70</sup>The results of this study indicated that *FYGL* promoted the degradation of lipid  
417 droplets in mature adipocytes by activating the AMPK $\alpha$  signalling pathway. In addition,  
418 *FYGL* increased the protein levels of ATGL (Figure 9E) and HSL (Figure 9F) by 2-fold  
419 compared with that of FAS (Figure 9B) and <sup>60</sup>by 1.5-fold compared with those in the  
420 control group at concentrations higher than 200  $\mu$ g/mL. Therefore, *FYGL* upregulated  
421 lipolysis more significantly than fatty acid biosynthesis, consistent with the animal  
422 experiment results. Taken together, <sup>76</sup>the results of the study on the cellular level showed  
423 that *FYGL* could inhibit lipid accumulation by both suppressing <sup>72</sup>the differentiation of  
424 preadipocytes and promoting the degradation of lipid droplets in mature adipocytes to  
425 alleviate metabolically unhealthy obesity.

## 426 Conclusion

427 <sup>82</sup>In conclusion, this study showed that *FYGL* could increase the number of beige  
428 adipocytes and restore adipocyte morphology, thereby alleviating metabolic disorders  
429 in db/db mice. The mechanism by which *FYGL* alleviates metabolic disorders involves  
430 the balance between fatty acid biosynthesis and metabolism to effectively dissipate  
431 energy in beige adipocytes. In addition, *FYGL* inhibited the differentiation of

432 preadipocytes by increasing FABP-4 gene expression and decreasing PPAR $\gamma$  and  
433 C/EBP $\alpha$  gene levels. Moreover, *FYGL* promoted adipocyte browning by upregulating  
434 *Cd81* gene expression. Furthermore, *FYGL* increased the levels of the lipolysis-related  
435 proteins ATGL, HSL and LPL by activating the AMPK $\alpha$  signalling pathway, therefore  
436 accelerating lipid metabolism in mature adipocytes. Importantly, these findings proved  
437 that *FYGL*, a proteoglycan, could improve metabolic disorders *in vivo* by targeting both  
438 preadipocytes and mature adipocytes. The mechanistic profile of *FYGL* in the treatment  
439 of diabetes-associated obesity is shown in Figure 10. *FYGL* could be used as a  
440 promising agent to treat lipid metabolism disorders and obesity in the clinic.

441  
442

#### 443 **Declarations**

444 **Ethics approval and consent to participate:** The study was conducted in accordance  
445 with the Declaration of Helsinki and approved by the Ethics Committee of Fudan  
446 University (No. FE21038, 5 March 2021).

447

448 **Consent for publication:** Not applicable.

449

450 **Availability of data and materials:** The data presented in this study are available  
451 within the article.

452

453 **Competing interests:** The authors declare that they have no competing interests.

454

455 <sup>1</sup> **Funding:** This research was funded by the National Natural Science Foundation of  
456 China (Nos. 21374022 and 81374032); National Health Commission of the People's  
457 Republic of China (No. 2017ZX09301006); Science and Technology Commission of  
458 Shanghai Municipality (No. 17401902700); Clinical Research Plan of SHDC (No.  
459 SHDC12019124); <sup>49</sup> and Shanghai Collaborative Innovation Center of Industrial  
460 Transformation of Hospital TCM Preparation.

461  
462 **Authors' contributions:** Y.W: design, <sup>14</sup> acquisition, analysis and interpretation of data,  
463 writing of the draft and final manuscript version. F.Y: interpretation of data, revision of  
464 the draft. X.Z: revision of the draft. J.L: analysis of data. Z.Z: <sup>66</sup> interpretation of data.  
465 Q.Z: <sup>66</sup> revision of the draft. J.C: <sup>66</sup> analysis of data. Y.H: interpretation of data, funding  
466 support. H.Y: design of the work, funding support. P.Z: design of the work, funding  
467 support, <sup>39</sup> writing, reviewing and editing of the manuscript. All authors read and  
468 approved the final manuscript.

469  
470 **Acknowledgements:** Not applicable.  
471

472 **References**

- 473 1. Chatterjee S, Khunti K, Davies MJ: **Type 2 diabetes**. *The Lancet* 2017, **389**:2239-2251.
- 474 2. Tong Y, Xu S, Huang L, Chen C: **Obesity and insulin resistance: Pathophysiology and**  
475 **treatment**. *Drug Discovery Today* 2022, **27**:822-830.
- 476 3. Nathan DM: **Diabetes Advances in Diagnosis and Treatment**. *Jama-Journal of the American*  
477 *Medical Association* 2015, **314**:1052-1062.
- 478 4. Dhurandhar NV: **What is obesity?** *International Journal of Obesity* 2022, **46**:1081-1082.
- 479 5. Czech MP: **Mechanisms of insulin resistance related to white, beige, and brown adipocytes**.  
480 *Molecular Metabolism* 2020, **34**:27-42.
- 481 6. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U: **Insulin resistance and impaired**  
482 **adipogenesis**. *Trends in Endocrinology & Metabolism* 2015, **26**:193-200.
- 483 7. Dhurandhar NV: **What is obesity?** *International Journal of Obesity* 2022.
- 484 8. Romao JM, Guan LL: **Chapter 21 - Adipogenesis and Obesity**. In *MicroRNA in Regenerative*  
485 *Medicine*. Edited by Sen CK. Oxford: Academic Press; 2015: 539-565
- 486 9. Hansen Jacob B, Kristiansen K: **Regulatory circuits controlling white versus brown**  
487 **adipocyte differentiation**. *Biochemical Journal* 2006, **398**:153-168.
- 488 10. Yang FT, Stanford KI: **Batokines: Mediators of Inter-Tissue Communication (a Mini-**  
489 **Review)**. *Current Obesity Reports* 2022, **11**:1-9.
- 490 11. Wu M, Liu D, Zeng R, Xian T, Lu Y, Zeng G, Sun Z, Huang B, Huang Q: **Epigallocatechin-3-**  
491 **gallate inhibits adipogenesis through down-regulation of PPAR $\gamma$  and FAS expression**  
492 **mediated by PI3K-AKT signaling in 3T3-L1 cells**. *European Journal of Pharmacology* 2017,  
493 **795**:134-142.
- 494 12. Linges A, Paul D, Naidu VGM, Satheeshkumar N: **AMPK activating and anti adipogenic**  
495 **potential of Hibiscus rosa sinensis flower in 3T3-L1 cells**. *Journal of Ethnopharmacology*  
496 2019, **233**:123-130.
- 497 13. Otto TC, Lane MD: **Adipose development: from stem cell to adipocyte**. *Crit Rev Biochem*  
498 *Mol Biol* 2005, **40**:229-242.
- 499 14. Tang QQ, Lane MD: **Adipogenesis: from stem cell to adipocyte**. *Annu Rev Biochem* 2012,  
500 **81**:715-736.
- 501 15. Cristancho AG, Lazar MA: **Forming functional fat: a growing understanding of adipocyte**  
502 **differentiation**. *Nature Reviews Molecular Cell Biology* 2011, **12**:722-734.
- 503 16. Lehrke M, Lazar MA: **The Many Faces of PPAR $\gamma$** . *Cell* 2005, **123**:993-999.
- 504 17. Sun Z, Gong J, Wu L, Li P: **Chapter 14 - Imaging Lipid Droplet Fusion and Growth**. In  
505 *Methods in Cell Biology*. Volume 116. Edited by Yang H, Li P: Academic Press; 2013: 253-268
- 506 18. Bosch M, Parton RG, Pol A: **Lipid droplets, bioenergetic fluxes, and metabolic flexibility**.  
507 *Seminars in Cell & Developmental Biology* 2020, **108**:33-46.
- 508 19. Klemm RW, Ikonen E: **The cell biology of lipid droplets: More than just a phase**. *Seminars*  
509 *in Cell & Developmental Biology* 2020, **108**:1-3.
- 510 20. Herzig S, Shaw RJ: **AMPK: guardian of metabolism and mitochondrial homeostasis**.  
511 *Nature Reviews Molecular Cell Biology* 2018, **19**:121-135.
- 512 21. López M: **EJE PRIZE 2017: Hypothalamic AMPK: a golden target against obesity?**  
513 *European Journal of Endocrinology* 2017, **176**:R235-R246.
- 514 22. Aaseth J, Ellefsen S, Alehagen U, Sundfør TM, Alexander J: **Diets and drugs for weight loss**  
515 **and health in obesity – An update**. *Biomedicine & Pharmacotherapy* 2021, **140**:111789.



- 516 23. Lingvay I, Sumithran P, Cohen RV, Le Roux CW: **Obesity management as a primary**  
517 **treatment goal for type 2 diabetes: time to reframe the conversation.** *The Lancet* 2021.
- 518 24. Liu TT, Liu XT, Chen QX, Shi Y: **Lipase Inhibitors for Obesity: A Review.** *Biomedicine and*  
519 *Pharmacotherapy* 2020, **128**.
- 520 25. Drucker DJ: **Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-**  
521 **1.** *Cell Metab* 2018, **27**:740-756.
- 522 26. Foretz M, Guigas B, Viollet B: **Understanding the glucoregulatory mechanisms of**  
523 **metformin in type 2 diabetes mellitus.** *Nature Reviews Endocrinology* 2019, **15**:569-589.
- 524 27. Ali Esmail A-S, Muayad Hussein A, Kareema Helal S: **Medicinal plants for the treatment of**  
525 **obesity and overweight: A review.** *World Journal of Biology Pharmacy and Health Sciences*  
526 2022, **10**:001-010.
- 527 28. Huang HL, Hong YW, Wong YH, Chen YN, Chyuan JH, Huang CJ, Chao PM: **Bitter melon**  
528 **(*Momordica charantia* L.) inhibits adipocyte hypertrophy and down regulates lipogenic**  
529 **gene expression in adipose tissue of diet-induced obese rats.** *Br J Nutr* 2008, **99**:230-239.
- 530 29. Ramachandra Shobha C, Prashant V, Akila P, Chandini R, Nataraj Suma M, Basavanagowdappa  
531 H: **Fifty Percent Ethanolic Extract of *Momordica charantia* Inhibits Adipogenesis and**  
532 **Promotes Adipolysis in 3T3-L1 Pre-Adipocyte Cells.** *Rep Biochem Mol Biol* 2017, **6**:22-32.
- 533 30. Parray HA, Yun JW: **Cannabidiol promotes browning in 3T3-L1 adipocytes.** *Molecular and*  
534 *Cellular Biochemistry* 2016, **416**:131-139.
- 535 31. Subhasree PDBaRS: **The Sacred Mushroom “Reishi”-A Review.** *American-Eurasian Journal*  
536 *of Botany* 2008, **1 (3): 107-110, 200**.
- 537 32. Bishop KS, Kao CHJ, Xu Y, Glucina MP, Paterson RRM, Ferguson LR: **From 2000years of**  
538 ***Ganoderma lucidum* to recent developments in nutraceuticals.** *Phytochemistry* 2015,  
539 **114**:56-65.
- 540 33. Teng BS, Wang CD, Zhang D, Wu JS, Pan D, Pan LF, Yang HJ, Zhou P: **Hypoglycemic effect**  
541 **and mechanism of a proteoglycan from *ganoderma lucidum* on streptozotocin-induced**  
542 **type 2 diabetic rats.** *Eur Rev Med Pharmacol Sci* 2012, **16**:166-175.
- 543 34. Pan D, Wang L, Chen C, Hu B, Zhou P: **Isolation and characterization of a hyperbranched**  
544 **proteoglycan from *Ganoderma Lucidum* for anti-diabetes.** *Carbohydrate Polymers* 2015,  
545 **117**:106-114.
- 546 35. Yu F, Wang Y, Teng Y, Yang S, He Y, Zhang Z, Yang H, Ding C-F, Zhou P: **Interaction and**  
547 **Inhibition of a *Ganoderma lucidum* Proteoglycan on PTP1B Activity for Anti-diabetes.**  
548 *ACS Omega* 2021, **6**:29804-29813.
- 549 36. Teng B-S, Wang C-D, Yang H-J, Wu J-S, Zhang D, Zheng M, Fan Z-H, Pan D, Zhou P: **A**  
550 **Protein Tyrosine Phosphatase 1B Activity Inhibitor from the Fruiting Bodies of**  
551 ***Ganoderma lucidum* (Fr.) Karst and Its Hypoglycemic Potency on Streptozotocin-Induced**  
552 **Type 2 Diabetic Mice.** *Journal of Agricultural and Food Chemistry* 2011, **59**:6492-6500.
- 553 37. Yang Z, Wu F, He Y, Zhang Q, Zhang Y, Zhou G, Yang H, Zhou P: **A novel PTP1B inhibitor**  
554 **extracted from *Ganoderma lucidum* ameliorates insulin resistance by regulating IRS1-**  
555 **GLUT4 cascades in the insulin signaling pathway.** *Food & Function* 2018, **9**:397-406.
- 556 38. Yang Z, Zhang Z, Zhao J, He Y, Yang H, Zhou P: **Modulation of energy metabolism and**  
557 **mitochondrial biogenesis by a novel proteoglycan from *Ganoderma lucidum*.** *RSC*  
558 *Advances* 2019, **9**:2591-2598.
- 559 39. Vohra MS, Ahmad B, Serpell CJ, Parhar IS, Wong EH: **Murine in vitro cellular models to**

- 560 **better understand adipogenesis and its potential applications. *Differentiation* 2020, **115**:62-  
561 84.**
- 562 40. Zhang Y, Pan Y, Li J, Zhang Z, He Y, Yang H, Zhou P: **Inhibition on  $\alpha$ -Glucosidase Activity  
563 and Non-Enzymatic Glycation by an Anti-Oxidative Proteoglycan from *Ganoderma  
564 lucidum*.** *Molecules* 2022, **27**:1457.
- 565 41. Pan Y, Yuan S, Teng Y, Zhang Z, He Y, Zhang Y, Liang H, Wu X, Li J, Yang H, Zhou P:  
566 **Antioxidation of a proteoglycan from *Ganoderma lucidum* protects pancreatic  $\beta$ -cells  
567 against oxidative stress-induced apoptosis in vitro and in vivo.** *International Journal of  
568 Biological Macromolecules* 2022, **200**:470-486.
- 569 42. Zebisch K, Voigt V, Wabitsch M, Brandsch M: **Protocol for effective differentiation of 3T3-  
570 L1 cells to adipocytes.** *Analytical Biochemistry* 2012, **425**:88-90.
- 571 43. Liang H, Pan Y, Teng Y, Yuan S, Wu X, Yang H, Zhou P: **A proteoglycan extract from  
572 *Ganoderma Lucidum* protects pancreatic beta-cells against STZ-induced apoptosis.**  
573 *Bioscience, Biotechnology, and Biochemistry* 2020, **84**:2491-2498.
- 574 44. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng  
575 Y-H, Doria A, et al: **Identification and Importance of Brown Adipose Tissue in Adult  
576 Humans.** *New England Journal of Medicine* 2009, **360**:1509-1517.
- 577 45. Stock MJ, Cinti S: **ADIPOSE TISSUE | Structure and Function of Brown Adipose Tissue.**  
578 In *Encyclopedia of Food Sciences and Nutrition (Second Edition)*. Edited by Caballero B.  
579 Oxford: Academic Press; 2003: 29-34
- 580 46. Virtanen KA: **Adipose Tissue: Structure and Function of Brown Adipose Tissue.** In  
581 *Encyclopedia of Food and Health*. Edited by Caballero B, Finglas PM, Toldrá F. Oxford:  
582 Academic Press; 2016: 30-34
- 583 47. Lidell ME, Betz MJ, Leinhard OD, Heglind M, Elander L, Slawik M, Mussack T, Nilsson D,  
584 Romu T, Nuutila P, et al: **Evidence for two types of brown adipose tissue in humans.** *Nature  
585 Medicine* 2013, **19**:631-634.
- 586 48. Ouellet V, Routhier-Labadie A, Bellemare W, Lakhali-Chaieb L, Turcotte E, Carpentier AC,  
587 Richard D: **Outdoor Temperature, Age, Sex, Body Mass Index, and Diabetic Status  
588 Determine the Prevalence, Mass, and Glucose-Uptake Activity of 18F-FDG-Detected BAT  
589 in Humans.** *The Journal of Clinical Endocrinology & Metabolism* 2011, **96**:192-199.
- 590 49. Kajimura S, Bruce, Seale P: **Brown and Beige Fat: Physiological Roles beyond Heat  
591 Generation.** *Cell Metabolism* 2015, **22**:546-559.
- 592 50. Kane JP, Pullinger CR, Goldfine ID, Malloy MJ: **Dyslipidemia and diabetes mellitus: Role of  
593 lipoprotein species and interrelated pathways of lipid metabolism in diabetes mellitus.**  
594 *Current Opinion in Pharmacology* 2021, **61**:21-27.
- 595 51. Ceperuelo-Mallafré V, Ejarque M, Serena C, Duran X, Montori-Grau M, Rodríguez MA, Yanes  
596 O, Núñez-Roa C, Roche K, Puthanveetil P, et al: **Adipose tissue glycogen accumulation is  
597 associated with obesity-linked inflammation in humans.** *Molecular Metabolism* 2016, **5**:5-  
598 18.
- 599 52. Wallace M, Metallo CM: **Tracing insights into de novo lipogenesis in liver and adipose  
600 tissues.** *Seminars in Cell & Developmental Biology* 2020, **108**:65-71.
- 601 53. Munday MR: **Regulation of mammalian acetyl-CoA carboxylase.** *Biochem Soc Trans* 2002,  
602 **30**:1059-1064.
- 603 54. Hsiao Y-S, Jogl G, Esser V, Tong L: **Crystal structure of rat carnitine palmitoyltransferase**

- 604 **II (CPT-II)**. *Biochemical and Biophysical Research Communications* 2006, **346**:974-980.
- 605 55. Chen Y, Ren Q, Zhou Z, Deng L, Hu L, Zhang L, Li Z: **HWL-088, a new potent free fatty**  
606 **acid receptor 1 (FFAR1) agonist, improves glucolipid metabolism and acts additively with**  
607 **metformin in ob/ob diabetic mice**. *Br J Pharmacol* 2020, **177**:2286-2302.
- 608 56. Jaiswal M, Schinske A, Pop-Busui R: **Lipids and lipid management in diabetes**. *Best Practice*  
609 *& Research Clinical Endocrinology & Metabolism* 2014, **28**:325-338.
- 610 57. Taskinen M-R, Borén J: **New insights into the pathophysiology of dyslipidemia in type 2**  
611 **diabetes**. *Atherosclerosis* 2015, **239**:483-495.
- 612 58. Oguri Y, Shinoda K, Kim H, Alba DL, Bolus WR, Wang Q, Brown Z, Pradhan RN, Tajima K,  
613 Yoneshiro T, et al: **CD81 Controls Beige Fat Progenitor Cell Growth and Energy Balance**  
614 **via FAK Signaling**. *Cell* 2020, **182**:563-577.e520.
- 615 59. Cléménçon B, Babot M, Trézéguet V: **The mitochondrial ADP/ATP carrier (SLC25 family):**  
616 **Pathological implications of its dysfunction**. *Molecular Aspects of Medicine* 2013, **34**:485-  
617 493.
- 618 60. Cohen P, Kajimura S: **The cellular and functional complexity of thermogenic fat**. *Nature*  
619 *Reviews Molecular Cell Biology* 2021, **22**:393-409.
- 620 61. Hay N: **Akt isoforms and glucose homeostasis – the leptin connection**. *Trends in*  
621 *Endocrinology & Metabolism* 2011, **22**:66-73.
- 622 62. Corrêa-Giannella ML, Machado UF: **SLC2A4 gene: a promising target for**  
623 **pharmacogenomics of insulin resistance**. *Pharmacogenomics* 2013, **14**:847-850.
- 624 63. Li G, Zhang L: **miR-335-5p aggravates type 2 diabetes by inhibiting SLC2A4 expression**.  
625 *Biochemical and Biophysical Research Communications* 2021, **558**:71-78.
- 626 64. Rosen ED, Macdougald OA: **Adipocyte differentiation from the inside out**. *Nature Reviews*  
627 *Molecular Cell Biology* 2006, **7**:885-896.
- 628 65. Ali AT, Hochfeld WE, Myburgh R, Pepper MS: **Adipocyte and adipogenesis**. *Eur J Cell Biol*  
629 2013, **92**:229-236.
- 630 66. Otto TC, Lane MD: **Adipose Development: From Stem Cell to Adipocyte**. *Critical Reviews*  
631 *in Biochemistry and Molecular Biology* 2005, **40**:229 - 242.
- 632 67. Garin-Shkolnik T, Rudich A, Hotamisligil GS, Rubinstein M: **FABP4 Attenuates PPARγ and**  
633 **Adipogenesis and Is Inversely Correlated With PPARγ in Adipose Tissues**. *Diabetes* 2014,  
634 **63**:900-911.
- 635 68. Furuhashi M: **Fatty Acid-Binding Protein 4 in Cardiovascular and Metabolic Diseases**.  
636 *Journal of Atherosclerosis and Thrombosis* 2019, **26**:216-232.
- 637 69. Berndt J, Kovacs P, Ruschke K, Klötting N, Fasshauer M, Schön MR, Körner A, Stumvoll M,  
638 Blüher M: **Fatty acid synthase gene expression in human adipose tissue: association with**  
639 **obesity and type 2 diabetes**. *Diabetologia* 2007, **50**:1472-1480.
- 640 70. Grabner GF, Xie H, Schweiger M, Zechner R: **Lipolysis: cellular mechanisms for lipid**  
641 **mobilization from fat stores**. *Nature Metabolism* 2021, **3**:1445-1465.
- 642 71. Wang Y, Rodrigues B: **Intrinsic and extrinsic regulation of cardiac lipoprotein lipase**  
643 **following diabetes**. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of*  
644 *Lipids* 2015, **1851**:163-171.
- 645 72. Walther TC, Farese RV: **Lipid Droplets and Cellular Lipid Metabolism**. *Annual Review of*  
646 *Biochemistry* 2012, **81**:687-714.
- 647 73. Ahmadian M, Abbott Marcia J, Tang T, Hudak Carolyn SS, Kim Y, Bruss M, Hellerstein Marc K,

- 648 Lee H-Y, Samuel Varman T, Shulman Gerald I, et al: **Desnutrin/ATGL Is Regulated by**  
649 **AMPK and Is Required for a Brown Adipose Phenotype.** *Cell Metabolism* 2011, **13**:739-  
650 748.
- 651 74. Borén J, Taskinen M-R, Björnson E, Packard CJ: **Metabolism of triglyceride-rich lipoproteins**  
652 **in health and dyslipidaemia.** *Nature Reviews Cardiology* 2022, **19**:577-592.
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(A)

(B)

Figure 1 (A) The dominant polysaccharide sequence of *FYGL* characterized by chemical analysis and NMR spectroscopy[34]. Rs represents the carbohydrate residues of  $\rightarrow 2,4$ - $\alpha$ -L-Rhap-(1 $\rightarrow$ ),  $\rightarrow 6$ - $\beta$ -D-Galp-1 $\rightarrow$ , Araf-(1 $\rightarrow$  or  $\rightarrow 3,6$ )- $\beta$ -D-Galp-(1 $\rightarrow$ ). Protein moieties are covalently bonded with carbohydrate moieties by Ser and Thr residues in the -O- linkage. (B) The dominant sequence of the protein moieties of *FYGL* characterized by mass spectrometry.

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(A)

(B)

Figure 2 Histopathological analysis of adipocytes in brown adipose tissues. (A) Representative images of H&E-stained brown adipose tissues, magnification 100×. The scale bar represents 250 μm. (B) Semiquantitative analysis of the adipocyte number per area in BAT by Image-Pro Plus 6.0 software. The mean ± S.D. values are presented (n = 6; \*\*\* $P < 0.001$  vs. normal; # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  vs. control).

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(A)

(B)

Figure 3 Transcriptome analysis of RNA sequencing of BAT in the normal, metformin, and *FYGL* groups compared to the control group. (A) DEG counts. (B) Hierarchical clustering heatmap of the expression profile of the DEGs.

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(A)

(B)

(C)

Figure 4 GO and KEGG functional enrichment analyses based on the DEGs. (A) Bubble plot of biological processes in the GO enrichment analysis. (B) The directed acyclic graph of biological process in the GO enrichment analysis. (C) Bubble plot of pathways in the KEGG enrichment analysis.

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Figure 5 (A) Laser confocal scanning microscopy images of *FYGL* in 3T3-L1 cells at 200× magnification. 3T3-L1 cells were incubated with FITC-*FYGL* (200 µg/mL) for 4 h; blue (DAPI labelled), red (rhodamine labelled) and green (FITC labelled) represent the nucleus, cytoskeleton and *FYGL*, respectively. The scale bar represents 100 µm. (B) Flow cytometric analysis of fluorescence. (C) Geometric means calculated by FlowJo software. The data are presented as the mean ± S.D. values (n = 3). \*\*\**P* < 0.001 vs. control group. (D) Effect of *FYGL* on cell viability. 3T3-L1 cells were incubated with various concentrations of *FYGL* (0, 50, 100, 200, 400 and 800 µg·mL<sup>-1</sup>) for 24 h, and cell viability was determined by a CCK-8 assay. The mean ± S.D. values are presented (n = 6)

671

(A)

(B)

(C)

Figure 6 Effect of *FYGL* on the inhibition of lipid accumulation in mature adipocytes. Differentiated 3T3-L1 cells were incubated with *FYGL* at concentrations ranging from 0–400  $\mu\text{g}/\text{mL}$ . (A) Intracellular TG in mature adipocytes. (B) Intracellular lipid droplets stained by oil red O and visualized by polarized phase contrast microscopy (500 $\times$ ). (C) Intracellular lipid accumulation was quantitatively measured using a microplate reader at an absorbance of 490 nm. Mean  $\pm$  S.D. values are presented (n = 6).  $^{###}P < 0.001$  vs. blank control group,  $^{**}P < 0.01$ ,  $^{*}P < 0.05$  vs. model group.

672

673 Figure 7 The relative mRNA expression levels of (A) C/EBP $\alpha$ , (B) FABP-4, (C) ATGL,  
674 and (D) LPL in differentiated 3T3-L1 cells, with reference to the model group. Data are  
675 presented as the mean  $\pm$  S.D. values (n = 6). ### $P$  < 0.001, ## $P$  < 0.01, # $P$  < 0.05 vs.  
676 blank control group. \*\*\* $P$  < 0.001, \*\* $P$  < 0.01, \* $P$  < 0.05 vs. model group  
677

Figure 8 Western blot analysis of proteins involved in cellular differentiation in mature 3T3-L1 cells. (A) Images of the PPAR $\gamma$ , C/EBP $\alpha$ , and FABP-4 protein bands relative to the  $\beta$ -actin protein band. (B), (C) and (D) Relative expression of PPAR $\gamma$ , C/EBP $\alpha$ , and FABP-4, respectively, with reference to  $\beta$ -actin, and normalized to the model group. Data are presented as the mean  $\pm$  S.D. values (n = 3).  $^{##}P < 0.01$ ,  $^{\#}P < 0.05$  vs. blank control group,  $^{***}P < 0.001$ ,  $^{**}P < 0.01$ ,  $^{*}P < 0.05$  vs. model group.

Figure 9 Western blot analysis of proteins involved in lipolysis and the AMPK $\alpha$  signalling pathway in mature 3T3-L1 cells. (A) Image of FAS protein bands, (B) Quantification of FAS expression. (C) Images of ATGL, HSL, LPL, p-AMPK $\alpha$ , and AMPK $\alpha$  protein bands. (D), (E), (F) and (G) Quantification of ATGL, HSL, LPL, and p-AMPK $\alpha$ /AMPK $\alpha$  protein levels. The protein levels in the model group are normalized to a value of 1.0. Data are presented as the mean  $\pm$  S.D. values (n = 3). <sup>##</sup>*P* < 0.01, <sup>###</sup>*P* < 0.001 vs. blank control group, <sup>\*\*</sup>*P* < 0.01, <sup>\*</sup>*P* < 0.05 vs. model group.

Figure 10 Profile of the mechanism of *FYGL* in ameliorating diabetes-associated obesity

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- 4 Mengqing Wu, Dan Liu, Rong Zeng, Tao Xian, Yi Lu, Guohua Zeng, Zhangzetian Sun, Bowei Huang, Qiren Huang. "Epigallocatechin-3-gallate inhibits adipogenesis through down-regulation of PPAR $\gamma$  and FAS expression mediated by PI3K-AKT signaling in 3T3-L1 cells", *European Journal of Pharmacology*, 2017  
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17 Yang Jiang, Shijie Ding, Feng Li, Chen Zhang, Dongxiao Sun-Waterhouse, Yilun Chen, Dapeng Li. "Effects of (+)-catechin on the differentiation and lipid metabolism of 3T3-L1 adipocytes", Journal of Functional Foods, 2019

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18 Shilin Yuan, Yanna Pan, Zeng Zhang, Yanming He, Yilong Teng, Haohui Liang, Xiao Wu, Hongjie Yang, Ping Zhou. "Amelioration of the Lipogenesis, Oxidative Stress and Apoptosis of Hepatocytes by a Novel Proteoglycan from *Ganoderma lucidum*", Biological and Pharmaceutical Bulletin, 2020

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20 Yanna Pan, Ying Zhang, Jiaqi Li, Zeng Zhang, Yanming He, Qingjie Zhao, Hongjie Yang, Ping Zhou. "A proteoglycan isolated from *Ganoderma lucidum* attenuates diabetic kidney disease by inhibiting oxidative stress-induced renal fibrosis both in vitro and in vivo", Journal of Ethnopharmacology, 2023

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21 Ying Zhang, Yanna Pan, Jiaqi Li, Zeng Zhang, Yanming He, Hongjie Yang, Ping Zhou. "Inhibition on  $\alpha$ -Glucosidase Activity and Non-Enzymatic Glycation by an Anti-Oxidative Proteoglycan from *Ganoderma lucidum*", Molecules, 2022

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22 Zhou Yang, Fan Wu, Yanming He, Qiang Zhang, Yuan Zhang, Guangrong Zhou, Hongjie Yang, Ping Zhou. "A novel PTP1B inhibitor extracted from ameliorates

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---

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24 Junpei Yamamoto, Takumi Yamane, Yuichi Oishi, Kazuo Kobayashi-Hattori. "Perfluorooctanoic acid binds to peroxisome proliferator-activated receptor  $\gamma$  and promotes adipocyte differentiation in 3T3-L1 adipocytes", Bioscience, Biotechnology, and Biochemistry, 2014 19 words — < 1%  
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---

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- 
- 31 Bao, Qinwen, Xiaozhu Shen, Li Qian, Chen Gong, Maoxiao Nie, and Yan Dong. "Anti-diabetic activities of catalpol in db/db mice", Korean Journal of Physiology and Pharmacology, 2016.  
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---

41 Christopher Church, Mallory Brown, Matthew S Rodeheffer. "Conditional immortalization of primary adipocyte precursor cells", *Adipocyte*, 2015  
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---

42 Jinye Huang, Jun Li, Hui Chen, Chensi Shen, Yuezhong Wen. "Ecological Toxicity Alleviation of Imazethapyr to Non-target Plant Wheat: Active Regulation Between Auxin and DIMBOA", Research Square Platform LLC, 2023  
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---

43 Kang Song, Yifan Zhang, Qin Ga, Zhenzhong Bai, Ri-Li Ge. "High-altitude chronic hypoxia ameliorates obesity-induced non-alcoholic fatty liver disease in mice by regulating mitochondrial and AMPK signaling", *Life Sciences*, 2020  
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---

51 Hao Huang, Tarun Belwal, Li Li, Yanqun Xu, Ligen Zou, Xingyu Lin, Zisheng Luo. "Amphiphilic and Biocompatible DNA Origami - Based Emulsion Formation and Nanopore Release for Anti - Melanogenesis Therapy", Small, 2021 10 words — < 1%

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---

52 Lau, P., Z. K. Tuong, S.-C. Wang, R. L. Fitzsimmons, J. Goode, G. P. Thomas, G. J. Cowin, M. A. Pearen, K. Mardon, J. L. Stow, and G. E. O. Muscat. "Ror deficiency and decreased adiposity are associated with induction of thermogenic gene expression in subcutaneous white and brown adipose tissue.", AJP Endocrinology and Metabolism, 2014. 10 words — < 1%

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---

53 Menghua Zhang, Xiaoxue Liu, Zhiyao Chen, Shenhao Jiang, Lin Wang, Min Tao, Liyan Miao. "Method development and validation for simultaneous determination of six tyrosine kinase inhibitors and two active metabolites in human plasma/serum using UPLC-MS/MS for therapeutic drug monitoring", Journal of Pharmaceutical and Biomedical Analysis, 2021 10 words — < 1%

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- 59 Eva García-Escobar. "Effect of insulin analogues on 3t3-l1 adipogenesis and lipolysis : INSULIN ANALOGUES ON 3T3-L1 ADIPOGENESIS AND LIPOLYSIS", European Journal of Clinical Investigation, 09/2011 Crossref 9 words — < 1%
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63 Lei Wang, Chao Li, Qiang Huang, Xiong Fu. " Polysaccharide from Tratt Fruit Attenuates Hyperglycemia and Hyperlipidemia and Regulates Colon Microbiota in Diabetic Mice ", Journal of Agricultural and Food Chemistry, 2019  
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64 Reddy Sankaran Karunakaran, Oruganti Lokanatha, Ganjayi Muni Swamy, Chintha Venkataramaiah et al. "Anti-Obesity and Lipid Lowering Activity of Bauhiniastatin-1 is Mediated Through PPAR-γ/AMPK Expressions in Diet-Induced Obese Rat Model", Frontiers in Pharmacology, 2021  
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65 Yue Tong, Sai Xu, Lili Huang, Chen Chen. "Obesity and insulin resistance: pathophysiology and treatment", Drug Discovery Today, 2021  
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- 75 Rulifson, I. C., J. Z. Majeti, Y. Xiong, A. Hamburger, K.-J. Lee, L. Miao, M. Lu, J. Gardner, Y. Gong, H. Wu, R. Case, W.-C. Yeh, W. G. Richards, H. Baribault, and Y. Li. "Inhibition of Secreted Frizzled-Related Protein 5 Improves Glucose Metabolism", AJP Endocrinology and Metabolism, 2014.  
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- 76 Yuli Zhang, Ni Chai, Zhenzhen Wei, Zan Li et al. "YYFZBJS inhibits colorectal tumorigenesis by enhancing Tregs-induced immunosuppression through HIF-1 $\alpha$  mediated hypoxia in vivo and in vitro", Phytomedicine, 2022  
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- 77 Zhou Yang, Congheng Chen, Juan Zhao, Weijie Xu, Yanming He, Hongjie Yang, Ping Zhou. "Hypoglycemic mechanism of a novel proteoglycan, extracted from Ganoderma lucidum , in hepatocytes", European Journal of Pharmacology, 2018  
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91 "Mechanisms involved in the loss of brown adipose tissue in the AGPAT2 deficient mouse.", Pontificia Universidad Catolica de Chile, 2019

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92 "Principles of Diabetes Mellitus", Springer Science and Business Media LLC, 2017

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93 Albert Boronat-Toscano, Diandra Monfort-Ferré, Margarita Menacho, Aleidis Caro et al. "Anti-TNF Therapies Suppress Adipose Tissue Inflammation in Crohn's Disease", International Journal of Molecular Sciences, 2022

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95 Medina-Gómez, Gema. "Mitochondria and endocrine function of adipose tissue", Best Practice & Research Clinical Endocrinology & Metabolism, 2012.

Crossref

96 Min, Byulchorong, Heejin Lee, Ji Hye Song, Myung Joo Han, and Jayong Chung. "Arctiin inhibits adipogenesis in 3T3-L1 cells and decreases adiposity and body weight in mice fed a high-fat diet", Nutrition Research and Practice, 2014.

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---

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