Trilobatin Ameliorates Inflammatory Response Through Mir375-Mediated FABP4 Expression In Adipocytes

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Research Article

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Abstract

Fatty acid binding protein 4 (FABP4) is predominantly expressed in adipose tissue and functions as an important mediator of inflammation. However, small molecules that regulate the expression of FABP4 in adipocytes have not been well characterized. In this work, we found that, together with decreasing the mRNA expression and secretion of proinflammatory cytokines, including tumor necrosis factor α (TNFα) and interleukin -6 (IL-6). Treatment with trilobatin dose-dependently attenuated the mRNA and protein levels of FABP4 in lipopolysaccharide (LPS)-induced differentiated 3T3-L1 adipocytes. Meanwhile, administration of 10 mg/kg trilobatin for 4 weeks (*i. g.*) also decreased the mRNA and protein levels of FABP4 and the mRNA expression of TNFα and IL-6 in epididymal white adipose tissue (eWAT) and the contents of TNFα and IL-6 in serum of ob/ob mice. In addition, we screened and identified that trilobatin protected against the decrease of microRNA precursors including premiR-375, premiR-338 and premiR-129, but only miR-375 inhibitor prohibited the role of trilobatin on the expression of FABP4, hinted that miR-375 might be involved in trilobatin regulating FABP4 expression in LPS-treated differentiated 3T3-L1 adipocytes. The findings of this study suggested that trilobatin might be a promising compound for the management of obesity because of its anti-inflammatory activity by suppressing the expression of FABP4 in adipocytes.

Introduction

With the increasing prevalence, obesity has become a matter of great concern worldwide, which has been identified as the key risk factor for a number of metabolic disorders, including nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM) and atherosclerosis [1]. Beside with the accumulation of body fat caused by the imbalance between food intake and energy expenditure, obesity is also accompanied by a low-grade chronic inflammatory state because of the increase of proinflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β) and interleukin − 6 (IL-6) secreted by adipocytes and macrophages [2, 3]. So, inhibition of chronic inflammatory response would be beneficial to improve obesity.

Fatty acid-binding protein 4 (FABP4), also known as adipocyte protein 2 (aP2), is one of the most abundant proteins in adipocytes, which plays crucial roles in regulation of fatty acids storge and lipolysis [4]. It has been shown that circulating FABP4 is elevated in metabolic disorders including obesity, and addition of exogenous FABP4 interfered with the differentiation and lipolysis of adipocytes, through interactions with hormone sensitive lipase (HSL) and p38/NFκB mediated inflammation *in vitro* and *in vivo* [5]. Silencing of FABP4 had been proven to be effective strategy for metabolic recovery and body weight loss in high-fat diet-induced mice [6]. A clinical study had confirmed the importance of genetic deletion of FABP4 for the treatment of obesity-induced inflammation [7]. So, screening for inhibitors for FABP4 and investigating their associated molecular mechanisms might be important strategies for the management of obesity.

Trilobatin, a natural sweetener, is a glycosylated dihydrochalcone isolated from the leaves of *Lithocarpus polystachyus* Rehd [8]. The chemical structure of trilobatin was shown as in **supplementary Fig. 1.** Trilobatin has been shown to exhibit anti-diabetic properties in high-fat diet (HFD) and streptozotocin (STZ) induced diabetic mice [9]. In addition, trilobatin had been reported to decrease cerebral ischemia/reperfusion-induced neuroinflammation and oxidative injury via suppressing Toll-like receptor 4 (TLR4)/nuclear factor-kappa B and Nrf2/Kelch-like ECH-associated protein 1 (Keap-1) signaling pathways *in vitro* and *in vivo* [10]. Our previous works demonstrated that trilobatin ameliorated insulin resistance and improved the expression and translocation of glucose transporter 4 (GLUT4) via insulin receptor substrates (IRS)/ phosphatidylinositol 3 kinase (PI3K) signaling pathway in C2C12 myotubes and the muscle tissues of ob/ob mice [11]. However, the effect of trilobatin on adipose tissues of obesity and the mechanisms of actions remain unclear.

In this study, we confirmed the relevance between FABP4 and the chronic inflammatory state of obesity with the integrated bioinformatic analysis, and the results indicated that FABP4 was involved in inflammatory response of obesity (**supplementary Fig. 2**). Moreover, we evaluated the effect of trilobatin on the inflammatory response and the expression of FABP4 in lipopolysaccharide (LPS)-induced differentiated 3T3-L1 adipocytes and the epididymal white adipose tissue (eWAT) of ob/ob mice. Last but not least, we probed into the molecular mechanisms of trilobatin regulating the expression of FABP4 gene in adipocytes. Our data revealed that miR-375 played a critical role for trilobatin in regulating the expression of FABP4 and attenuating the inflammatory response in LPS-induced adipocytes and the eWAT of ob/ob mice.

Materials And Methods

Materials

Lipopolysaccharides (LPS), insulin, isobutylmethylxanthine (IBMX), dexamethasone and trilobatin were obtained from Sigma (St Louis, MO, USA). FABP4 (15872-1) and β-actin (20536-1) antibodies were purchased from Proteintech (Wuhan Sanying, Wuhan, China). iScript cDNA synthesis kits (1708891) were from Bio-Rad (Hercules, CA, USA). TNFα (EMC102a) and IL-6 (EMC005) ELISA kits were purchased from Neobioscience (Beijing, China). miRNA inhibitors were purchased from RiboBio (Guangzhou, China). DAPI, and other chemicals were purchased from Beyotime (Shanghai, China).

Cell culture

Mouse-derived 3T3-L1 preadipocytes were purchased from the China Center for Type Culture Collection (Wuhan, China), and routinely maintained in high glucose (25 mM) Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (V/V) fetal bovine serum (FBS) at 37°C in a humidified incubator with a 5% CO_2 atmosphere. To induce 3T3-L1 preadipocytes differentiating into adipocyte-like cells, the cells were firstly incubated with 0.5 mM isobutylmethylxanthine (IBMX, ab120840) and 1 μM dexamethosone (ab120743) for 3 days, and then, the media were replaced with complete media plus 10 μg/mL insulin (ab123768) and the cells were continued to culture another 3 days.

Animals

Male ob/ob mice and wild-type littermates (C57BL/6J background) (8–10 weeks old) were purchased from Tengxin Biotechnology Co. (Chongqing, China), which were allowed ad libitum access to food and water unless otherwise stated. The animals were maintained at 22°C and 50% humidity on a 12-h light/dark cycle. The mice were administrated with 10 mg/kg trilobatin (*i. g.*) once for each day for continued 4 weeks, and the control mice were treated with same volume of in phosphate-buffered saline (PBS). Before dissection, the mice were euthanatized with CO_2 inhalation, and the epididymal white adipose tissues (eWAT) were isolated, froze in liquid nitrogen, and stored at -80°C.

RNA isolation, reverse transcription and real-time PCR

Total RNA was extracted from the treated cells or tissues using Trizol reagent (Invitrogen). The integrity of RNA was confirmed spectrophotometrically. First-strand cDNA was generated using the random hexamer primer provided with an iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). The qPCR experiments were conducted on Step-One plus real-time PCR system and carried out using a final volume of 10 µL, containing 1 ng of reverse-transcribed total cDNA, 2 nM of forward and reverse primers, and SYBR green PCR master mixture, which was performed in 96-well plates using the CFX96 real-time PCR system (Bio-Rad). The PCR conditions consisted of 40 cycles with 15 s denaturation at 95°C, 30 s annealing at 55°C, and 60 s extensions at 72°C. The fold change in mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method using β-actin as the reference gene to normalize the data for all samples. The primer sequences for PCR are shown in **supplementary Table 1**.

Immunofluorescence staining

After the mice were administrated with 10 mg/kg trilobatin for 4 weeks (once for each day), the eWATs were isolated and fixed (10% formalin solution in 0.1 M PBS), frozen at -80°C overnight, and cut into 10 µm sections on a freezing microtome (Leica, Nussloch, Germany). The sections were permeabilized in 0.1% Triton X-100 in PBS and blocked in 1% BSA in PBS before incubation with the primary antibody. After stained with FABP4 antibody (Proteintech) and together with DAPI nuclear stain (Invitrogen). Fluorescence images were acquired using a confocal microscope (Nikon, Tokyo, Japan).

Western blot

Equal amounts of proteins (20–30 µg each) were separated on a 10% SDS-PAGE and transferred to a PVDF membrane (Immobilon P; Millipore, MA, USA). The blots were blocked for 1 h at room temperature with 5% skimmed milk powder in TBST (20 mM Tris, 150 mM NaCl, 2.7 mM KCl, 0.1% Tween 20, pH 7.4). The membranes were next immunoblotted with FABP4 primary antibodies (Proteintech) at dilutions of 1:2000 for overnight at 4°C. Subsequently, blots were washed with TBST three times and incubated for one hour with a horseradish peroxidase-conjugated secondary antibody at 1:10000 dilution with 5% skimmed milk powder in TBST. Excess antibody was washed off with TBST, and immunoreactivity was detected using ECL western blotting reagent (Millipore). Signal bands were quantified by densitometric

analysis using Image J software (available from NIH at http://imagej.nih.gov/ij/) after scanning the blotted membrane.

FABP4 gene interruption

When the differentiated 3T3-L1 adipocytes reached 60% confluence, they were transfected with 50 nM siRNA targeting mouse FABP4 or a scrambled negative siRNA respectively by using Lipofectamine 3000 reagent (Thermo Fisher, CA, USA) according to the protocol from manufacture. Six hours later, the medium was replaced with complete growth medium, and the cells were cultured for 24 h. The interfering efficiency was identified with qRT-PCR and western blot assays. All the siRNA sequences for mouse FABP4 gene are listed in **Supplementary Table 2**.

Statistical analysis

All the data are expressed as the mean ± SD. Significant differences between groups were analyzed using one-way ANOVA with Dunnet's multiple comparisons test, and a Two-tailed, unpaired Student's *t*-test was performed for comparison of two groups. $p < 0.05$ was considered to represent a significant difference. Statistical analysis was performed with GraphPad Prism 8.01 software (La Jolla, CA, USA).

Results

Trilobatin reduced the inflammatory response in LPS-treated 3T3-L1 adipocytes.

As shown in Fig. 1a, after differentiated 3T3-L1 adipocytes were treated with 0.1, 1.0 and 10 μM trilobatin for 24 hours, the cell viability was 101.6 ± 4.5, 98.6 ± 5.3 and 97.7 ± 3.9% respectively, compared to the mean of 0.01% DMSO group (control = 100%), suggested that trilobatin at concentrations ranging from 0.1 μM to 10 μM had no significant cytotoxicity on differentiated 3T3-L1 adipocytes. Meanwhile, we also evaluated the effect of trilobatin on the cell viability in LPS-induced 3T3-L1 adipocytes, the results showed that preincubation with 0.1, 1.0 and 10 μM trilobatin increased the cell viability about 4.5, 12.4 and 21.3% respectively (Fig. 1b).

To evaluate the anti-inflammatory activity, we determined the effect of trilobatin on the TNFα and IL-6 mRNA levels in LPS-treated differentiated 3T3-L1 adipocytes. Data indicated that, incubation with 1 μg/mL induced a remarkable increase in TNFα mRNA expression by 2.1-fold and IL-6 mRNA by 2.7-fold of the control group, whereas trilobatin (0.1–10 μM) decreased the mRNA levels of TNFα and IL-6 in a dose-dependent manner (Fig. 1c and **d**). Consistent with the results of mRNA expression, trilobatin treatment (0.1–10 μM) concentration-dependently inhibited the secretion of TNFα and IL-6, and the inhibitory rates of 10 μM trilobatin treatment were 42.5% for TNFα and 47.3% for IL-6 in LPS-treated 3T3-L1 adipocytes (Fig. 1e and **f**).

FABP4 played a critical role in the anti-inflammatory activity of trilobatin.

The results on qRT-PCR and western blot indicated that treatment with trilobatin decreased the expression of FABP4 induced by LPS in a dose-dependent manner in differentiated 3T3-L1 adipocytes, and

incubation with 10 μM trilobatin for 24 h decreased the mRNA and protein levels by 2.5- and 3.5-folds respectively in LPS-treated 3T3-L1 adipocytes (Fig. 2a, b).

To assess the role of FABP4 in trilobatin against inflammatory response induced by LPS, we used siRNA transfection to interrupt the expression of FABP4 in differentiated 3T3-L1 adipocytes. Our data indicated that treatment with 50 nM siRNA for 24 h decreased the mRNA and protein levels of FABP4 to 30% and 20% respectively (Fig. 2c, d). Furthermore, we also found that, in FABP4 gene interrupted 3T3-L1 adipocytes, trilobatin had no significant effect on mRNA and protein levels of TNFα and IL-6 induced by LPS (Fig. 2e-h).

Trilobatin reduced the expression of FABP4 and inhibited the inflammatory response eWAT of ob/ob mice.

Several publications had reported that circulating FABP4 might be linked to obesity, inflammation and metabolic syndrome [5, 12], but the expression of FABP4 in eWAT and its role in inflammation of ob/ob mice remain unknown. In the current study, we determined the mRNA and protein levels of FABP4 in eWAT of ob/ob mice. The data from qRT-PCR, western blot and immunofluorescent staining assay revealed that, compared to control group (saline solution), the mRNA and protein levels of FABP4 in eWAT of ob/ob mice were increased 7.6-fold and 1.5-fold, and administration of 10 mg/kg trilobatin for 4 weeks respectively decreased the mRNA and protein levels of FABP4 by about 39% and 36% in the eWAT of ob/ob mice (Fig. 3a-c). Furthermore, data from qRT-PCR assay showed that, compared to C57BL/6 mice, the mRNA levels of TNFα and IL-6 in ob/ob mice increased by 4.5-fold and 5.6-fold, respectively. Administration of 10 mg/kg trilobatin for 4 weeks dramatically decreased the mRNA levels of TNFα (Fig. 3d) and IL-6 (Fig. 3e). Using ELISA assay, we confirmed that administration of trilobatin also significantly decreased the protein levels of TNFα (Fig. 3f) and IL-6 (Fig. 3g) in serum of ob/ob mice.

miR-375 was involved in trilobatin regulating the expression of FABP4.

A growing body of evidence indicates that obesity-induced chronic inflammation plays a fundamental role in the pathogenesis of metabolic syndrome, and miRNAs are largely dysregulated in obesity [13]. To elucidate the molecular mechanisms associated with trilobatin regulating the expression of FABP4, we screened the miRNAs which could bind with the 3'-UTR sequence or coding sequence of FABP4 using http://mirwalk.umm.uni-heidelberg.de/ and http://www.targetscan.org/ and other resources online. Results showed that there were twelve miRNAs including miR-129, miR-338, miR-375, miR-205, miR-347, miR-350, miR-126a, miR-103, miR-503, miR-330, miR-236 and miR-206 possibly interacted with FABP4 gene (Fig. 4a). And then, we used qRT-PCR to confirm the mRNA expression and determine the effect of trilobatin on these mRNAs in LPS-treated differentiated 3T3-L1 adipocytes and the eWAT of ob/ob mice. Data showed that only pre-miR129 (pmiR-129), pre-miR-338 (pmiR-338) and pre-miR-375 (pmiR-375) were decreased in the cellular and animal inflammatory models, most important, trilobatin significantly increased the mRNA levels of pmiR-129, pmiR-338 and pmiR-375 (Fig. 4b, c). Furthermore, we found that miR-375 inhibitor, but not the miR-129 and miR-338 inhibitors prevented the action of trilobatin on mRNA expression of FABP4 induced by LPS in differentiated 3T3-L1 adipocytes (Fig. 4d).

Discussion

Trilobatin is one of the primary active components in the leaves of *Lithocarpus polystachyus* Rehd, which has been shown anti-obesity activity by regulating gut microbiota in high-fatty diet-induced SD (Sprague-Dawley) rats [8], and anti-inflammatory action by decreasing the levels of inflammatory cytokines such as TNFα, IL-1β and IL-6 in LPS-induced RAW264.7 macrophages [14]. In the present study, we determined the anti-inflammatory activity of trilobatin in LPS-treated differentiated 3T3-L1 adipocytes. Our data indicated that treatment with trilobatin (from 0.1 to 10 μM) inhibited the mRNA levels of TNFα and IL-6 and secretion of the two pro-inflammatory cytokines in a dose-dependent manner in LPS-treated differentiated 3T3-L1 adipocytes. Meanwhile, administration of trilobatin for 4 weeks also inhibited the expression of TNFα and IL-6 mRNAs in eWAT and attenuated the contents of TNFα and IL-6 in serum of ob/ob mice, all these finding suggested that trilobatin exhibited significant anti-inflammatory activity *in vitro* and *in vivo*.

To understand the mechanism of trilobatin inhibiting the production of pro-inflammatory cytokines, we used bioinformatic analysis to screen the relative genes associated with the chronic low-grade inflammation in eWAT of high-fat diet-fed mice (GSE39549) and expression profiling of obese adipocytes (GSE133099). The results indicated that FABP4 might be involved in the chronic low-grade inflammation in adipose tissues of obesity.

Although FABP4 is one of the most abundant proteins in mature adipocytes, but the biological role of FABP4 is not well understood. The increasing evidence supports that, as a central regulator of whole-body metabolic control, FABP4 has been linked to insulin sensitivity, lipid metabolism and inflammation [15]. Dysregulated FABP4 has been proved to be associated with the development of insulin resistance, metabolic syndrome and cardiovascular diseases [12]. Additionally, because macrophages are one of important sites of FABP4 action, total or macrophage specific FABP4-deficiency leads to a marked defense against early and advanced atherosclerosis [16].

In this study, we determined the expression of FABP4 in LPS-treated differentiated 3T3-L1 adipocytes. Our data confirmed that LPS induced a significant increase of FAPB4 expression, and trilobatin dose-dependently attenuated the mRNA and protein levels of FABP4 in LPS-treated differentiated 3T3-L1 adipocytes. In addition, FABP4 gene interruption prevented the action of trilobatin on the inflammatory cytokines such as TNFα and IL-6 in differentiated 3T3-L1 adipocytes challenged by LPS. Furthermore, together with decreasing the mRNA and protein levels of FABP4, trilobatin also reduced the inflammatory response by decreasing the levels of TNFα and IL-6 in eWAT and the serum of ob/ob mice. All these data suggested that FABP4 might play a critical role in trilobatin preventing inflammatory response in differentiated 3T3-L1 adipocytes.

MicroRNAs (miRNAs) are small non-coding RNA molecules that modulate the expression of multiple protein-encoding genes by inducing cleavage of mRNAs or via inhibiting protein translation. Recently, miRNAs have been recognized as powerful regulators of numerous genes and pathways in the pathogenesis of inflammation [17]. To determine the molecular mechanisms of trilobatin regulating FABP4 expression, we screened the miRNAs which could bind with the 3'-UTR sequence or coding

sequence of FABP4, and determined the effect of trilobatin on the expression of miRNAs. Our data showed that, although miR-375, miR-129 and miR-338 were decreased in LPS-treated 3T3-L1 adipocytes and the eWAT of ob/ob mice, but only miR-375 inhibitor prohibited the expression of FABP4 gene in LPS-treated 3T3-L1 adipocytes, suggesting that miR-375 might have been involved in regulating the expression of FABP4 by trilobatin.

Although how trilobatin regulates the expression of miR-375 is still not fully understood, together with our previous finding that trilobatin improved insulin resistance in skeletal muscle of ob/ob mice [11], the results of the current study suggested that trilobatin might be a promising lead compound for the management of metabolic syndrome because of its anti-inflammatory activity.

Abbreviations

DMEM, Dulbecco's modified Eagle's medium; eWAT, epididymal white adipose tissue; FABP4, fatty acid binding protein 4; HFD, high-fat diet; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; STZ, streptozotocin; TLR4, Toll-like receptor 4; TNFα, tumor necrosis factor

Declarations

Fundings

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Competing Interest

The authors declare that they have no competing interests.

Authors' contributions

LJH and YL designed the project and contributed the research questions, LXG, WYC, performed research and analyzed results. YF performed statistical analysis. LJH, YF, and YL wrote and edited the manuscript.

Data Availability

The data and materials of the current study are available from the corresponding author on reasonable request.

Ethics Approval

The protocols of all animal experiments were reviewed and approved by the Animal Ethical and Welfare Committee of Chongqing University of Technology, and all procedures involved in animal experiments

were carried out in accordance with the principles and guidelines of the Chinese Council of Animal Care.

References

1. Yudkin JS (2007) Inflammation, obesity, and the metabolic syndrome. Horm Metab Res 39:707-9.http://doi.org/ 10.1055/s-2007-985898
2. Wu H and Ballantyne CM (2020) Metabolic Inflammation and Insulin Resistance in Obesity. Circ Res 126:1549–1564.http://doi.org/ 10.1161/CIRCRESAHA.119.315896
3. Stolarczyk E (2017) Adipose tissue inflammation in obesity: a metabolic or immune response? Curr Opin Pharmacol 37:35–40.http://doi.org/ 10.1016/j.coph.2017.08.006
4. Zhang M, Zhu W and Li Y (2014) Small molecule inhibitors of human adipocyte fatty acid binding protein (FABP4). Med Chem 10:339 – 47.http://doi.org/ 10.2174/1573406411309666004 5
5. Dou HX, Wang T, Su HX, Gao DD, Xu YC, Li YX and Wang HY (2020) Exogenous FABP4 interferes with differentiation, promotes lipolysis and inflammation in adipocytes. Endocrine 67:587–596.http://doi.org/ 10.1007/s12020-019-02157-8
6. Won YW, Adhikary PP, Lim KS, Kim HJ, Kim JK and Kim YH (2014) Oligopeptide complex for targeted non-viral gene delivery to adipocytes. Nat Mater 13:1157–64.http://doi.org/ 10.1038/nmat4092
7. Steen KA, Xu H and Bernlohr DA (2017) FABP4/aP2 Regulates Macrophage Redox Signaling and Inflammasome Activation via Control of UCP2. Mol Cell Biol 37.http://doi.org/ 10.1128/MCB.00282 – 16
8. Shen H, Huang L, Dou H, Yang Y and Wu H (2021) Effect of Trilobatin from Lithocarpus polystachyus Rehd on Gut Microbiota of Obese Rats Induced by a High-Fat Diet. Nutrients 13:891.http://doi.org/ 10.3390/nu13030891
9. Wang J, Huang Y, Li K, Chen Y, Vanegas D, McLamore ES and Shen Y (2016) Leaf Extract from Lithocarpus polystachyus Rehd. Promote Glycogen Synthesis in T2DM Mice. PLoS One 11:e0166557.http://doi.org/ 10.1371/journal.pone.0166557
10. Gao J, Chen N, Li N, Xu F, Wang W, Lei Y, Shi J and Gong Q (2020) Neuroprotective Effects of Trilobatin, a Novel Naturally Occurring Sirt3 Agonist from Lithocarpus polystachyus Rehd., Mitigate Cerebral Ischemia/Reperfusion Injury: Involvement of TLR4/NF-kappaB and Nrf2/Keap-1 Signaling. Antioxid Redox Signal 33:117–143.http://doi.org/ 10.1089/ars.2019.7825
11. Liu M, Wang L, Li X, Wu Y, Yin F and Liu J (2020) Trilobatin ameliorates insulin resistance through IRS-AKT-GLUT4 signaling pathway in C2C12 myotubes and ob/ob mice. Chin Med 15:110–122.http://doi.org/ 10.1186/s13020-020-00390-2
12. Thumser AE, Moore JB and Plant NJ (2014) Fatty acid binding proteins: tissue-specific functions in health and disease. Curr Opin Clin Nutr Metab Care 17:124–9.http://doi.org/ 10.1097/MCO.0000000000000031
13. Zhang XM, Guo L, Chi MH, Sun HM and Chen XW (2015) Identification of active miRNA and transcription factor regulatory pathways in human obesity-related inflammation. BMC Bioinformatics

16:76.http://doi.org/ 10.1186/s12859-015-0512-5

14. Fan X, Zhang Y, Dong H, Wang B, Ji H and Liu X (2015) Trilobatin attenuates the LPS-mediated inflammatory response by suppressing the NF-kappaB signaling pathway. Food Chem 166:609–615.http://doi.org/ 10.1016/j.foodchem.2014.06.022
15. Queipo-Ortuno MI, Escote X, Ceperuelo-Mallafre V, Garrido-Sanchez L, Miranda M, Clemente-Postigo M, Perez-Perez R, Peral B, Cardona F, Fernandez-Real JM, Tinahones FJ and Vendrell J (2012) FABP4 dynamics in obesity: discrepancies in adipose tissue and liver expression regarding circulating plasma levels. PLoS One 7:e48605.http://doi.org/ 10.1371/journal.pone.0048605
16. Fu Y, Luo L, Luo N and Garvey WT (2006) Lipid metabolism mediated by adipocyte lipid binding protein (ALBP/aP2) gene expression in human THP-1 macrophages. Atherosclerosis 188:102 –11.http://doi.org/ 10.1016/j.atherosclerosis.2005.10.041
17. Singh RP, Massachi I, Manickavel S, Singh S, Rao NP, Hasan S, Mc Curdy DK, Sharma S, Wong D, Hahn BH and Rehimi H (2013) The role of miRNA in inflammation and autoimmunity. Autoimmun Rev 12:1160–5.http://doi.org/ 10.1016/j.autrev.2013.07.003

Figures

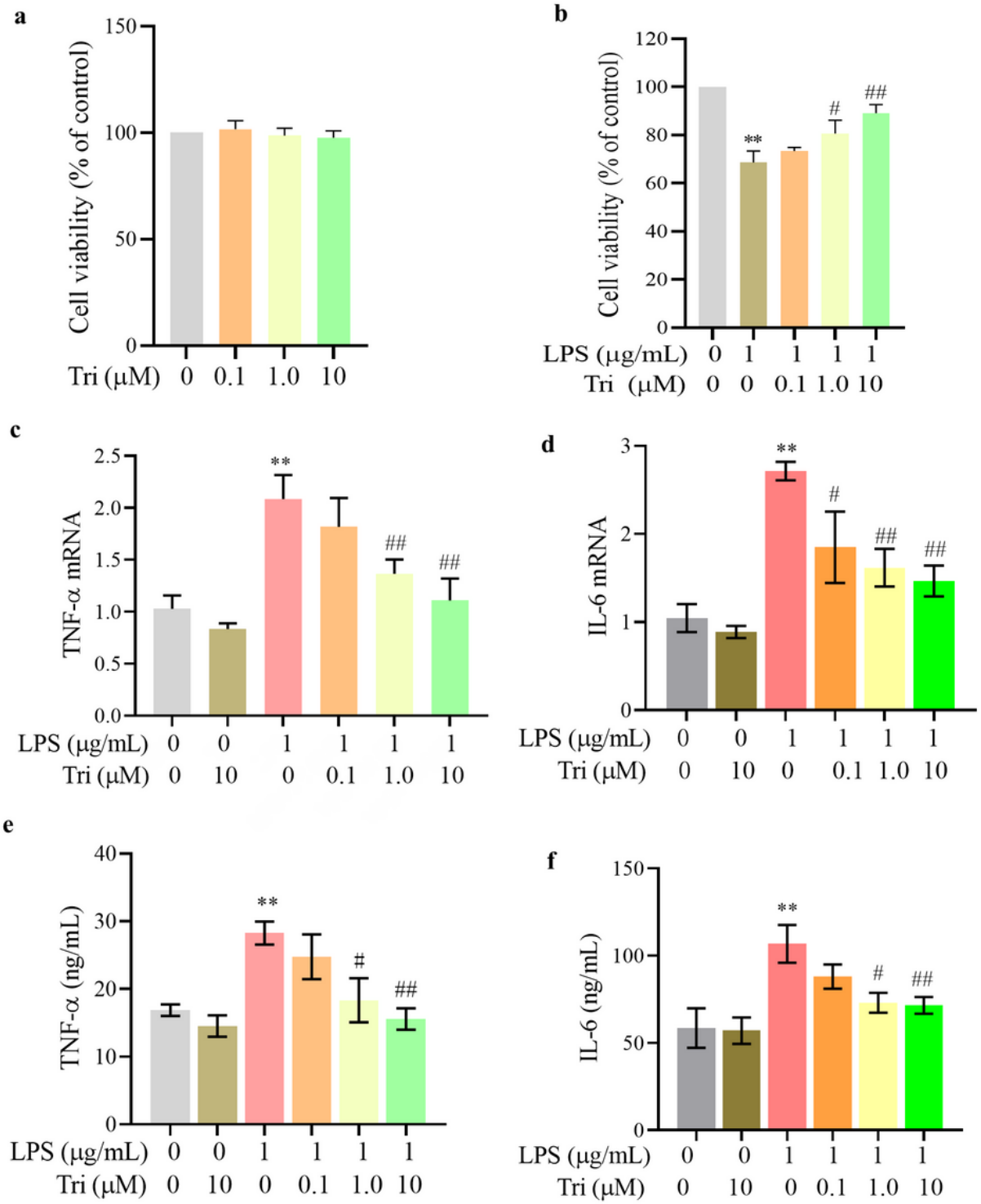

Figure 1

Trilobatin inhibited the inflammatory response in LPS-treated 3T3-L1 adipocytes.

a and **b**: After 3T3-L1 preadipocytes were induced to differentiate into adipocyte-like cells according to the protocol showed in Methods, the cells were incubated with 0.1, 1.0 and 10 μM trilobatin (Tri) for 24 hours in the absence (**a**) or presence (**b**) of 1 μg/mL LPS, the cell viability was determined with MTT. Data

are presented as mean ± SD (n = 4). *, p < 0.05, **, p < 0.01 vs. control (0.01% DMSO), and #, p < 0.05, ##, p < 0.01 vs the group of LPS alone.

c-f: After the differentiated 3T3-L1 adipocytes were preincubated with 1 μg/mL LPS for 8 h, and then the indicated concentrations of trilobatin (Tri) were added and continued to incubate 24 h. The mRNA and protein levels of TNFα and IL-6 were determined with qRT-PCR and ELISA assays according to the protocol from the supplier. Data are shown as mean ± SD (n = 6). **, p < 0.01 vs. control, and #, p < 0.05, ##, p < 0.01 vs. the group of LPS alone.

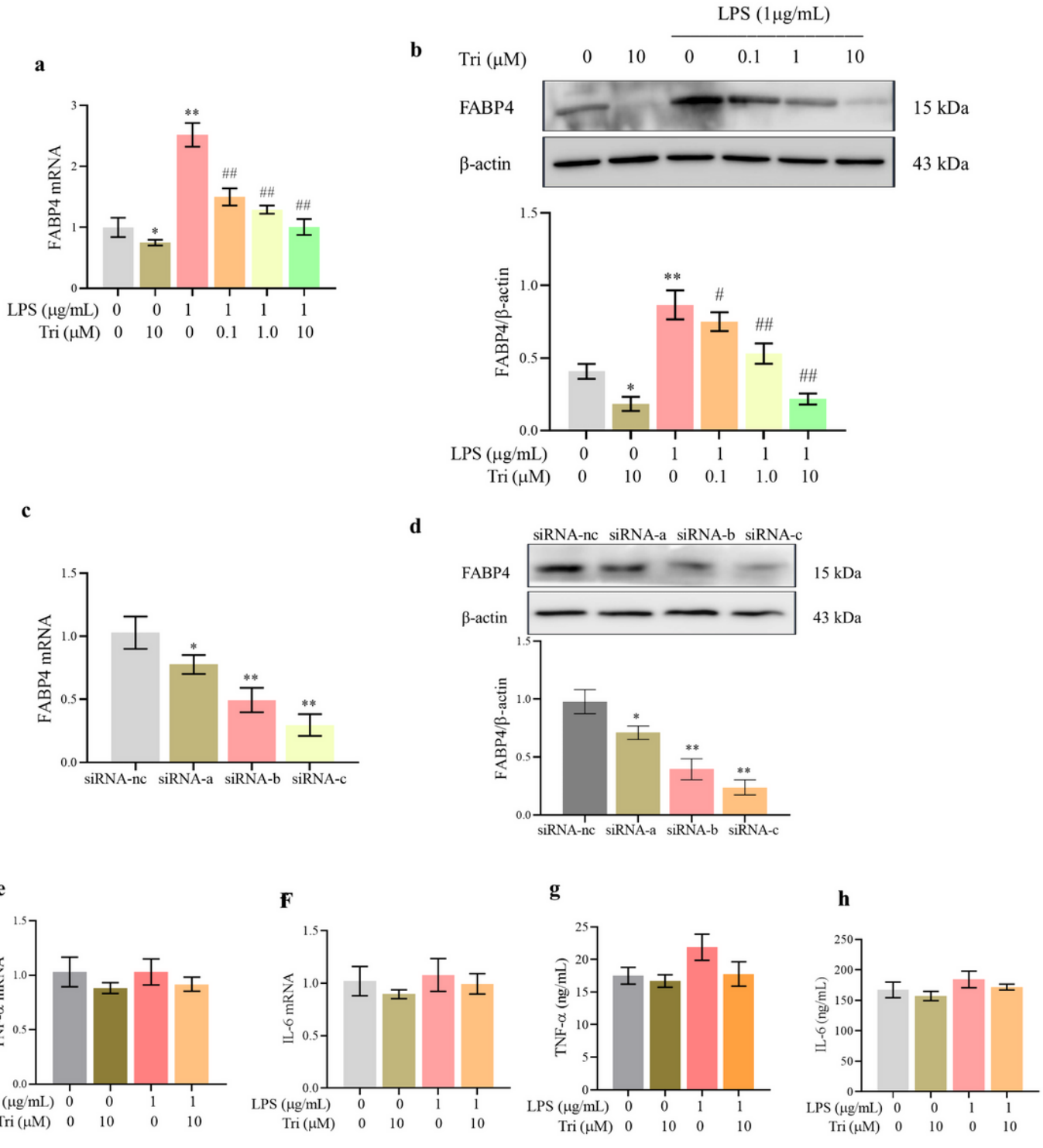

Figure 2

FAPB4 played a critical role in trilobatin inhibiting the inflammatory response.

a and **b**: After the differentiated 3T3-L1 adipocytes were preincubated with 1 μg/mL LPS for 8 h, the indicated concentrations of trilobatin (Tri) were added and continued to incubate 24 h. The mRNA and protein levels of FABP4 were determined with qRT-PCR and western blot assays.

c and **d**: After the differentiated 3T3-L1 adipocytes were transfected with 50 nM siRNA for FABP4 for 24 h, the mRNA and protein levels of FABP4 were analyzed with qRT-PCR and western blot to check the interfered efficiency.

e-h: After the FAPB4 interrupted 3T3-L1 adipocytes were preincubated with 1 μg/mL LPS for 8 h, 10 μM trilobatin (Tri) was added and continued to incubate for 24 h, the mRNA and protein levels of TNFα and IL-6 were measured with qRT-PCR and ELISA respectively.

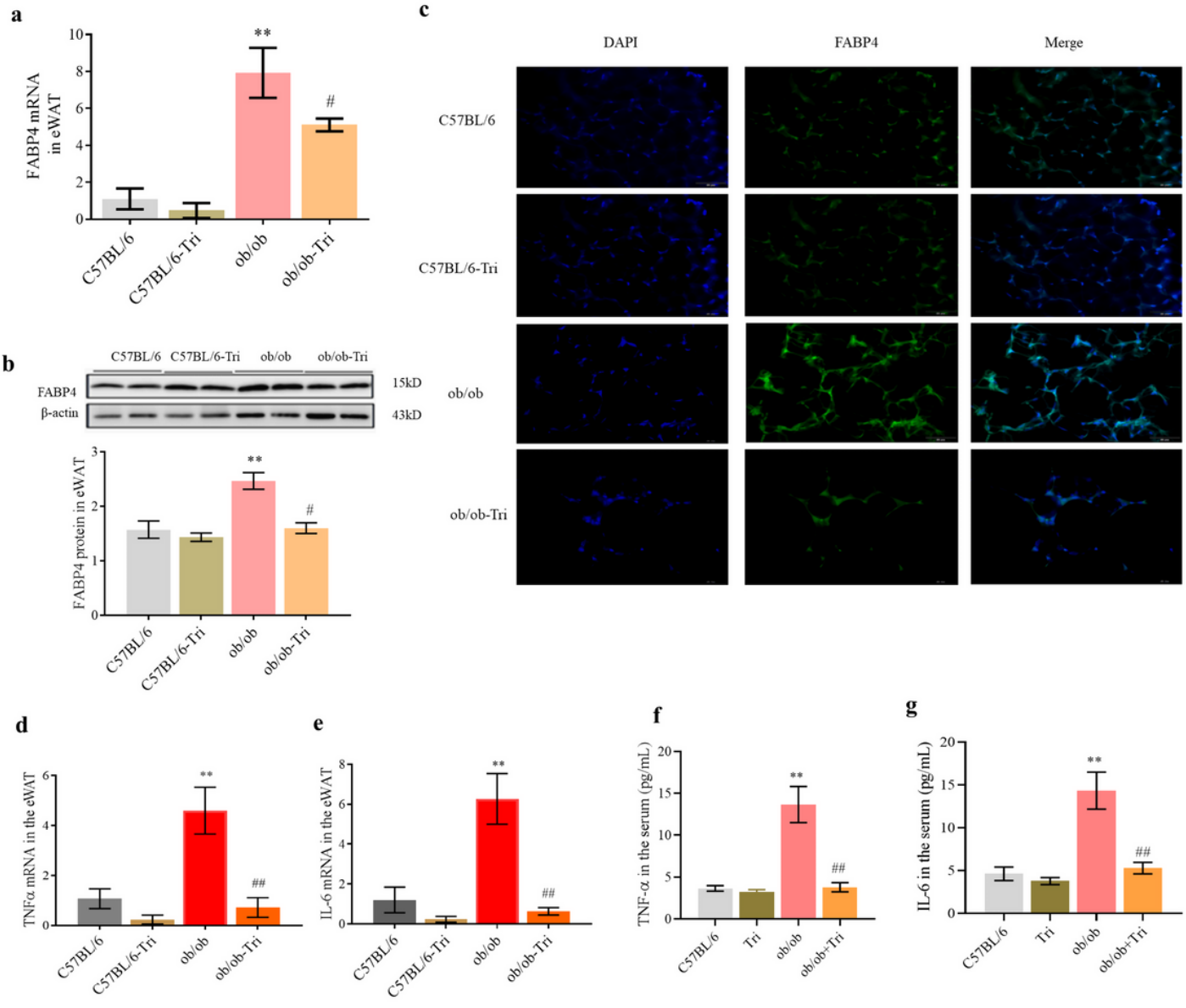

Figure 3

Trilobatin regulated FABP4 expression to attenuate the inflammatory response in ob/ob mice.

After ob/ob mice were administrated with 10 mg/kg trilobatin (Tri) for 4 weeks, the eWAT was collected and the mRNA and protein of FABP4 were determined with qRT-PCR (**a**), western blot (**b**) and immunofluorescence (**c**). At the same time, the inflammatory cytokines such as TNFα (**d**) and IL-6 (**e**) in eWAT and serum (**f** and **g**) were determined with qRT-PCR or ELISA assay. Data are presented as mean ± SD (n = 6). *, $p < 0.5$, **, $p < 0.01$ vs. the group of C57BL/6 mice, and #, $p < 0.05$, ##, $p < 0.01$ vs. the group of ob/ob mice.

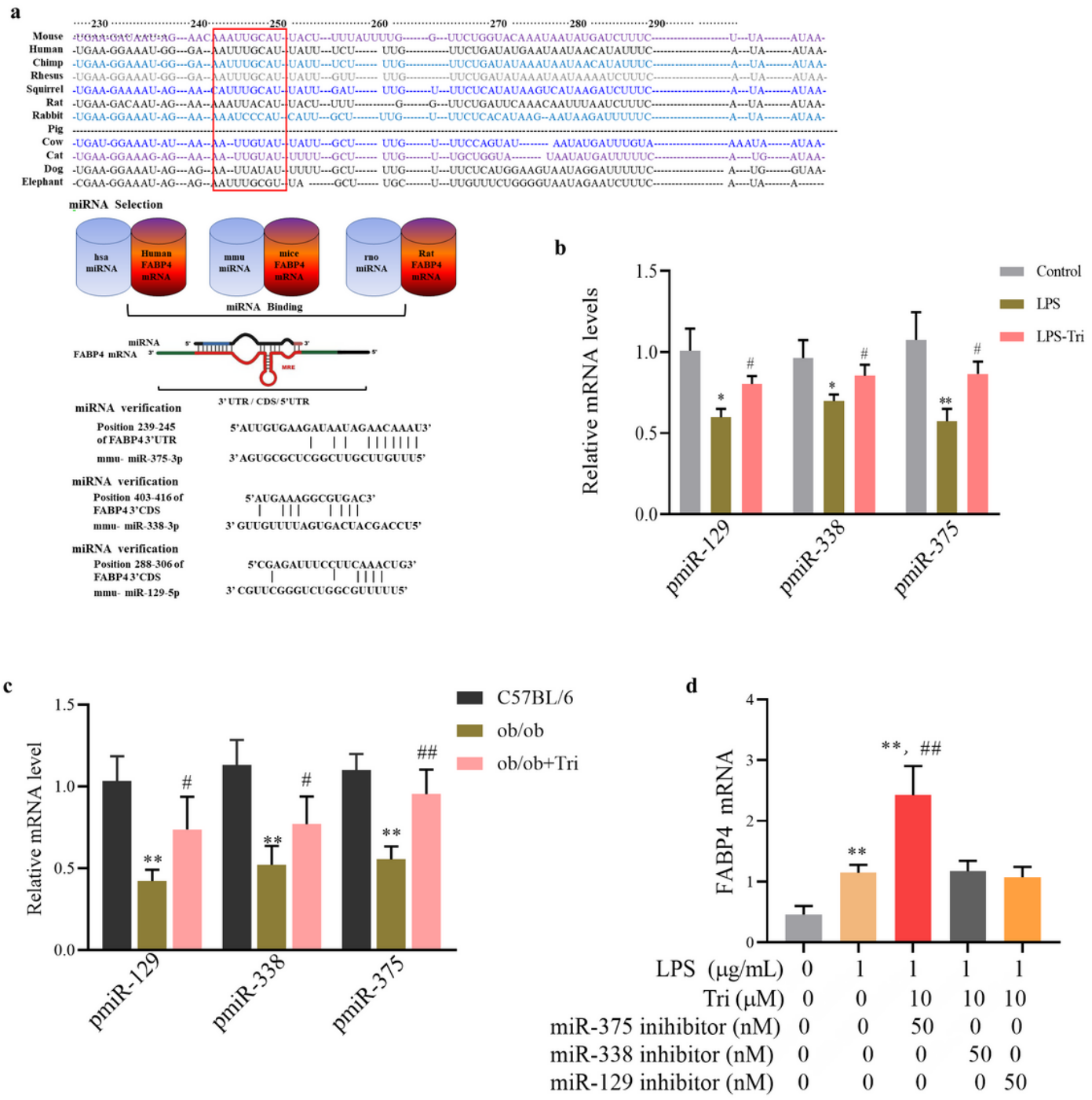

Figure 4

miR375 was involved in trilobatin regulating the expression of FABP4.

a: The interaction between miR-375, miR-129 and miR-338 and the 3' UTR sequence of FABP4 gene.

b: The mRNA levels of pmiR-338, pmiR-129 and pmiR-375 in LPS-treated differentiated 3T3-L1 adipocytes.

c: The mRNA levels of pmiR-338, pmiR-129 and pmiR-375 in eWAT of ob/ob mice.

d: After the differentiated 3T3-L1 adipocytes were preincubated with 50 nM miR-375, miR-129 and miR-338 inhibitors for 24 h, 1 μg/mL LPS and 10 μM trilobatin (Tri) were added and the cells were cultured for another 24 h, the mRNA of FABP4 was determined with qRT-PCR.

Data are shown as mean ± SD (n = 6). *, $p < 0.5$, **, $p < 0.01$ vs. control, and #, $p < 0.05$, ##, $p < 0.01$ vs. the group of ob/ob mice or LPS alone.

Supplementary Files

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- Supplementarymaterials.docx