Research Square

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Fat Mass and Obesity–Associated Protein Promotes Liver Steatosis By Targeting PPARα

Xiaohui Wei
Shanghai Jiao Tong University School of Medicine
Jielei Zhang
The First Affiliated Hospital of Zhengzhou University
Min Tang
Shanghai Jiao Tong University School of Medicine
Xuejiao Wang
Shanghai Jiao Tong University School of Medicine
Nengguang Fan
Shanghai Jiao Tong University School of Medicine
Yongde Peng (yongdepeng0908@126.com)
Shanghai Jiao Tong University School of Medicine

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. The fat mass and obesity–associated protein (FTO) has been shown to be involved in obesity; however, its role in NAFLD and the underlying molecular mechanisms remain largely unknown.

Methods: FTO expression was first examined in the livers of patients with NAFLD and animal and cellular models of NAFLD using quantitative real-time polymerase chain reaction and western blotting. Next, its role in lipid accumulation in hepatocytes was assessed both in vitro and in vivo via gene overexpression and knockdown studies.

Results: FTO expression was increased in the livers of mice and humans with hepatic steatosis, probably due to its decreased ubiquitination. FTO overexpression in HepG2 cells induced triglyceride accumulation, whereas FTO knockdown exerted an opposing effect. Consistent with the findings of in vitro studies, adeno-associated viruses 8 (AAV8)-mediated FTO overexpression in the liver promoted hepatic steatosis in C57BL/6J mice. Mechanistically, FTO inhibited the mRNA expression of peroxisome proliferator-activated receptor α (PPARα) in hepatocytes. Activation of PPARα by the PPARα agonist GW7647 reversed lipid accumulation in hepatocytes induced by FTO overexpression.

Conclusions: Overall, FTO expression is increased in NAFLD, and it promotes hepatic steatosis by targeting PPARα.

Background

Nonalcoholic fatty liver disease (NAFLD), characterized by lipid accumulation in the liver, has become the most prevalent liver disease affecting approximately 1.7 billion individuals worldwide. The disease spectrum ranges from simple steatosis to steatohepatitis, fibrosis, cirrhosis, and even carcinoma, and has become a leading cause of liver transplantation. In addition, NAFLD is closely associated with the development of insulin resistance, type 2 diabetes, and cardiovascular diseases [1, 2]. However, to date, there is a lack of approved pharmacotherapies for the treatment of NAFLD, partially due to an unclear understanding of its mechanisms [3-5]. Thus, further studies are needed to identify key targets for the pathogenesis of NAFLD and for the development of effective therapeutic strategies.

N6-methyladenosine (m6A), the most prevalent mRNA modification, is implicated in diverse biological processes including fat accumulation and energy metabolism [6-8]. The m6A modification of RNA is regulated by methyltransferases (METTL3, METTL14, and WTAP) and demethylases (ALKBH5 and FTO) [7]. Fat mass and obesity–associated protein (FTO), first identified by a genome-wide association study in an obesity study, is widely expressed throughout the body including in the brain, adipose tissue, and liver [9-11]. FTO, an m6A demethylase, decreases the m6A modification of mRNA and plays a crucial role in determining RNA splicing, translation, and degradation [12, 13]. FTO overexpression in mice increases fat mass, whereas its deficiency protects mice from obesity, suggesting a key role of FTO in fat metabolism and obesity [13, 14]. Furthermore, FTO promotes adipogenesis via its m6A demethylation activity [11].

Recently, the role of FTO in hepatic fat metabolism and NAFLD has been investigated. FTO expression is increased in the liver of patients with NAFLD and animal models of NAFLD [15, 16]. Enhanced FTO expression leads to a reduction of the m6A level, induces the expression of lipogenic genes, and increases triglyceride accumulation in hepatocytes [17]. However, a mutant FTO lacking demethylase activity fails to produce these effects [17]. Similarly, in another study, FTO was found to promote the maturation of the sterol regulatory element-binding protein-1c (SREBP1c) and enhance the transcription of the cell death-inducing DFFA-like effector C during lipid accumulation in HepG2 cells [18]. Taken together, FTO appears to be a key player in fat accumulation in hepatocytes. However, the targets of FTO in hepatocytes and its role in NAFLD in vivo remain largely unknown.

In the present study, we found that FTO protein expression was increased in the livers of patients and mice with NAFLD, and that this increase may be related to its decreased ubiquitination. We also found that FTO contributed to lipid accumulation both in hepatocytes in vitro and the livers in mice in vivo. Mechanistically, FTO expression decreased the mRNA level of peroxisome proliferator-activated receptor α (PPARα). Activation of PPARα reversed the lipid accumulation induced by FTO, indicating that PPARα is a downstream target of FTO.

Methods

Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco (Carlsbad, CA, USA). Oil Red O, palmitic acid, low fatty acid bovine serum albumin (BSA), cycloheximide (CHX), GW7647, and MG132, as well as the anti-FLAG M2 affinity gel and antibodies against FLAG, hemagglutinin (HA), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were procured from Sigma-Aldrich (St. Louis, MO, USA). The antibody against FTO was purchased from Abcam (Cambridge, MA, USA) and the anti-PPARα antibody was from ProteinTech Group (Rosemont, IL, USA). Horseradish peroxidase-conjugated antibodies against rabbit or goat IgG were purchased from Jackson Laboratories (West Grove, PA, USA).

Subjects and liver samples

Liver samples were obtained from eight subjects who visited the surgical department of the Shanghai General Hospital for surgeries for a non-hepatocellular primary tumor or colorectal cancer liver metastasis. Subjects with viral hepatitis, excessive ethanol consumption (> 140 g for men or > 70 g for women, per week), or a history of drug-induced liver injury were excluded. The human study was approved by the Institutional Review Board of Shanghai General Hospital affiliated to Shanghai Jiao Tong University School of Medicine and performed in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from all subjects.

Animals

Male C57BL/6J mice (6 weeks of age) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). Mice were housed in a pathogen-free barrier facility with a 12 h light/12 h dark cycle and allowed free access to a standard chow diet and water. Animal procedures were approved by the Committee on the Ethics of Animal Experiments of Shanghai Jiao Tong University and were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Shanghai Jiao Tong University.

To establish the NAFLD mouse model, six male mice, aged 8 weeks, were fed a high-fat diet (HFD) (D12492; Research Diets, New Brunswick, NJ, USA) for 16 weeks, whereas six control mice were fed a standard chow diet. After 16 weeks, livers of the mice were snap-frozen in liquid nitrogen immediately after resection and stored at −80 °C until use for the detection of FTO expression.

To overexpress FTO in livers, an adeno-associated virus 8-expressing FTO (AAV8-FTO) vector and its control were constructed by Jiman (Shanghai, China). Male mice, aged 8 weeks, received a single tail vein injection of 1 × 1011 genome copies of the AAV or control vector, and were then fed the HFD for 8 weeks. Body weights were recorded weekly.

Tissue processing

Liver tissues were fixed with 10% formalin solution immediately after resection, and embedded in paraffin. Liver sections (5 μm) were stained with hematoxylin and eosin, and immunohistochemically for FTO, according to standard protocols. Frozen sections of liver tissues were prepared for Oil Red O staining.

Cell culture and treatment

HepG2 cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO2 humidified atmosphere at 37 °C. For FTO overexpression and knockdown, the lentivirus-FTO vector and its short hairpin (sh)RNA vector were constructed by Heyuan (Shanghai, China). These vectors were transfected into HepG2 cells to establish stable FTO-expressing or FTO-knockdown cell lines. HepG2 cells transfected with an empty lentivirus vector were used as the control.

Before treatment, hepatocytes were serum starved in DMEM containing 0.25% BSA for 8 h. Next, palmitic acid (0.5 mM) and 2% BSA were added to the culture medium for 24 h. The HepG2 cells were then fixed with 4% paraformaldehyde and stained with Oil Red O solution (0.2 mg/mL). The Triglyceride Quantification Colorimetric/Fluorometric Kit (BioVision, K622 Milpitas, CA, USA) was used to quantify the cell triglyceride content according to the manufacturer's protocol.

To determine whether the FTO protein was degraded by proteasomes, HepG2 cells were treated with 10 μg/mL of the protein synthesis inhibitor CHX, with or without 10 μM of the proteasome inhibitor MG132 for 12 h.

RNA preparation, quantitative real-time polymerase chain reaction (qRT-PCR), and RNA sequencing

Total RNA was extracted from liver tissue or cells with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. One microgram of RNA was reverse-transcribed into first-strand cDNA using the PrimeScript RT reagent kit (Takara, Otsu, Japan). The qRT-PCR was then performed in triplicate using the Thunderbird SYBR Green qPCR Mix reagent (Toyobo, Osaka, Japan) on an ABI 7500 Real-Time PCR System (Perkin-Elmer Applied Biosystems, Warrington, UK). The primers are listed in Table 1. Gene expression was normalized to GAPDH using the $2-\Delta\Delta Ct$ method. The RNA transcriptome sequencing analysis was performed by Lianchuan Biotechnology (Hangzhou, China) using the Illumina HiSeq 2500 platform.

Western blotting analyses

Liver tissues and cell samples were washed three times with phosphate-buffered saline (PBS) and collected with sodium dodecyl sulfate (SDS) lysis buffer, followed by centrifugation at 9000 x g for 20 min at 4 °C. Protein lysates were separated via 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (EMD Millipore, Bedford, MA, USA), which were then blocked with 5% fat-free milk at 25 °C for 1 h and incubated with the indicated primary antibodies overnight at 4 °C. After washing and incubating with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature, Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA) was used to visualize the immunoreactive proteins. GAPDH was used as the internal control. Protein bands were quantified via densitometric analyses using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Protein immunoprecipitation and ubiquitination assays

The HA-ubiquitin plasmid was transiently transfected into the stable cell line overexpressing FLAG-FTO with Lipofectamine 3000 (Invitrogen). Ubiquitination of FTO was induced by incubating HepG2 cells with 10 μM MG132. Briefly, cells were lysed in 1% SDS (in PBS) and diluted in 10 volumes of ice-cold cell lysis buffer (1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM Na-pyrophosphate in PBS) supplemented with 10 mM N-ethyl maleimide and complete EDTA-free protease inhibitor cocktail (Roche, Mannheim, Germany). Cell lysates were sonicated and centrifuged at 9000 x g for 20 min at 4 °C. Pre-cleared lysates were then incubated with the anti-FLAG M2 Affinity Gel overnight at 4 °C, followed by four washes with a wash buffer and elution in 2X SDS sample buffer. The immunoprecipitated proteins were resolved via SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting with specific antibodies against HA or FTO.

Statistical analyses

Statistical analyses were performed using the Prism software, version 7.0 (GraphPad Software, San Diego, CA, USA). Data are presented as means ± SEM. Non-normally distributed data were transformed logarithmically before analysis. Cellular experiments were performed in duplicate at least three times. A two-tailed unpaired Student's t-test was performed for comparisons between two groups. $P < 0.05$ was considered statistically significant.

Results

FTO expression is increased in fatty liver.

To explore the role of FTO in NAFLD, we first examined its expression in the livers of human subjects with or without NAFLD. Immunohistochemistry revealed that FTO protein levels were increased in hepatocytes of subjects with NAFLD (Fig. 1A). In contrast, FTO mRNA expression was not significantly different between the livers from patients with and without NAFLD (Fig. 1B).

A mouse model of hepatic steatosis was established by feeding mice a HFD for 16 weeks. Consistent with our observation in humans, FTO protein expression was significantly increased in the livers of HFD-fed mice compared with that in the controls (Fig. 1C). In vitro FTO protein expression was also significantly increased in HepG2 cells challenged with palmitic acid (Fig. 1E). Consistently, the mRNA level of FTO was not distinctly changed in fatty livers of mice or in palmitic acid–treated HepG2 cells (Fig. 1D, F). This suggests that the FTO protein level in fatty liver may be regulated at the post-transcriptional level.

Palmitic acid decreases ubiquitination of FTO in hepatocytes

Previous studies have shown that FTO is modified by ubiquitination and degraded in proteasomes in HeLa cells [19]. Considering the discrepancy in changes of FTO protein and mRNA in NAFLD, we speculated that FTO may be dysregulated by the ubiquitin-proteasome system in fatty liver. Consistent with a previous study [19], we observed that MG132 (a proteasome inhibitor) could prevent the decrease of FTO protein level in HepG2 cells treated with CHX, a protein synthesis inhibitor (Fig. 2A). This indicated that FTO was degraded by proteasomes in hepatocytes. To confirm whether FTO protein upregulation in fatty liver was related to its ubiquitination, we evaluated the ubiquitination level of FTO in hepatocytes exposed to palmitic acid in an in vitro model of fatty liver. As shown in Figure 2B, the ubiquitination of FTO was markedly decreased by exposure to palmitic acid. Thus, changes in ubiquitination may be responsible for the increase in FTO protein expression in NAFLD.

FTO promotes lipid accumulation in hepatocytes

To investigate the effects of FTO on lipid metabolism in hepatocytes, we first infected HepG2 cells with a lentivirus vector-mediated FTO plasmid (Lenti-FTO) and obtained a stable cell line overexpressing FTO (Fig. 3A). Oil Red O staining showed that lipid accumulation was increased in FTO-overexpressing cells compared with that in control cells after treatment with palmitic acid (Fig. 3B-E). In addition, hepatocytes with FTO overexpression exhibited a significantly higher level of triglycerides than control cells (Fig. 3F).

To investigate the effect of FTO inhibition on lipid accumulation in hepatocytes, we generated a stable cell line with FTO knockdown by infecting HepG2 cells with a lentivirus vector-mediated FTO shRNA plasmid. In contrast to FTO overexpression, knockdown of FTO markedly inhibited lipid accumulation in hepatocytes, as revealed by Oil Red O staining and triglyceride measurements (Fig. 4).

FTO promotes HFD-induced hepatic steatosis in mice

To address the role of FTO in hepatic steatosis in vivo, we infected mice with AAV8-FTO via tail vein injection; this resulted in the specific overexpression of FTO in the liver. The mice were then fed an HFD for eight weeks, and hepatic steatosis was assessed via Oil Red O staining. As shown in Figure 5A and B, FTO overexpression had little effect on the body weights of the mice. However, the livers with FTO overexpression exhibited more prominent steatosis when compared with those of the controls (Fig. 5C-G).

FTO inhibits the expression of PPARα

FTO is a main RNA demethylase, and its demethylation function plays an essential role in the fat metabolism of hepatocytes. However, the downstream targets of FTO are not yet well-understood. To identify the targets of FTO in hepatocytes, we performed RNA sequencing on samples from FTO-overexpressing and control HepG2 cells. Genes involved in multiple metabolic pathways were identified, including PPARα (Fig. 6A). We confirmed the inhibitory role of FTO on PPARα mRNA and protein expression in HepG2 cells. As shown in Figure 6B and C, FTO overexpression in HepG2 cells reduced both PPARα mRNA and protein levels. Consistent with this finding, FTO knockdown by shFTO increased PPARα protein levels (Fig. 6D).

FTO induces lipid accumulation in hepatocytes by targeting PPARα

PPARα is a nuclear receptor that plays a central role in fatty acid oxidation and is involved in the pathogenesis of hepatic steatosis and NAFLD (Montagner et al., 2016). To test whether FTO promoted lipid accumulation by targeting PPARα, we treated FTO-overexpressing HepG2 cells with the PPARα agonist GW7647. As expected, GW7647 reversed the triglyceride accumulation in HepG2 cells induced by FTO overexpression, as revealed by Oil Red O staining and triglyceride measurements (Fig. 7).

Discussion

FTO, the first m6A demethylase identified, promotes adipogenesis and obesity. In addition, previous studies have suggested a role for FTO in lipid accumulation in hepatocytes. However, its role in NAFLD is largely unknown. In the present study, we found that FTO promoted hepatic steatosis in vivo. Moreover, FTO inhibited the expression of PPARα, which may mediate its role in hepatic steatosis.

In previous studies, FTO expression was found to be increased in the livers of patients with NAFLD and mice fed an HFD for 6–12 weeks [15, 16, 21]. However, there are inconsistencies regarding the expression of FTO in NAFLD. In one study, FTO mRNA levels were found to be reduced in genetically obese mice [22]. In another study, the expression of FTO mRNA and protein was not changed in the livers of mice fed an HFD for 17 weeks [23]. In the present study, we observed increased FTO protein levels in the livers of mice fed an HFD for 16 weeks, whereas the mRNA level was not significantly changed. Differences in species and/or the duration of HFD feeding may be responsible for these inconsistent findings. Because FTO can be modified by ubiquitination and subsequently degraded by proteosomes, we speculated that

dysregulated ubiquitination of the FTO protein was responsible for the discrepancy in changes of FTO protein and its mRNA levels in NAFLD. In support of our speculation, we observed that FTO ubiquitination was inhibited in hepatocytes challenged with palmitic acid, which may lead to decreased degradation of FTO and the upregulation of its expression in NAFLD.

The role of FTO in lipid metabolism of hepatocytes has been investigated in vitro previously. FTO contributes to lipid accumulation in HepG2 cells [17, 18]. Consistently, we observed that FTO overexpression induced triglyceride accumulation in hepatocytes, whereas FTO knockdown inhibited this accumulation. However, the role of FTO in hepatic steatosis in vivo had not yet been investigated. In the present study, we overexpressed FTO specifically in the livers of mice by AAV8. Consistent with the in vitro studies, we found that FTO promoted hepatic steatosis. Meanwhile, hepatic overexpression of FTO did not affect the body weights of mice, implying a direct role for FTO in liver steatosis. Our results provide further evidence that FTO plays a key role in the development of NAFLD.

Another question was how FTO promoted lipid accumulation in hepatocytes. Previous studies proposed that SREBP1c is a downstream molecular target of FTO [18]. In addition, FTO was found to decrease the mitochondria content, induce the expression of genes associated with lipogenesis (FASN, SCD1, and MOGAT1), and decrease the expression of genes associated with lipid transport (MTTP, APOB, and LIPC) [17]. Furthermore, FTO induces lipid accumulation in hepatocytes via its demethylase function, as a mutant FTO lacking demethylase activity fails to produce these effects [17]. Our study showed that FTO decreased the mRNA level of PPARα. As PPARα is a master regulator of genes involved in fatty acid oxidation, reduction of its level would contribute to lipid accumulation in hepatocytes. As expected, activation of PPARα reversed the lipid accumulation in hepatocytes induced by FTO overexpression in our study. Altogether, FTO may promote hepatic steatosis via multiple mechanisms, including effects on de novo lipogenesis, lipid transport, and fatty acid oxidation.

Conclusion

In summary, we show here that FTO is upregulated in NAFLD and that FTO suppresses the expression of PPARα in hepatocytes, leading to hepatic steatosis. These findings provide insight into the mechanism of NAFLD and suggest that FTO may be a novel target for the treatment of NAFLD. However, whether FTO directly interacts with and demethylates PPARα remains to be determined in the future studies.

Abbreviations

NAFLD, nonalcoholic fatty liver disease; FTO, fat and obesity associated protein; PPARα, peroxisome proliferators activated receptor α; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; MOGAT1, monoacylglycerol O-acyltransferase 1; MTTP, microsomal triglyceride transfer protein; APOB, apolipoprotein B; LIPC, Lipase C.

Declarations

Acknowledgements

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Fan NG and Peng YD conceived and designed the experiments. Wei XH, Zhang JL, Wang XJ and Tang M performed the experiments and analyzed the data. All authors wrote the manuscript, read and approved the manuscript.

Ethics approval

This study was approved by the Institutional Review Board of Shanghai General Hospital affiliated to Shanghai Jiao Tong University School of Medicine, and was conducted in accordance with the Helsinki Declaration of 1975. All participants were verbally informed about the study. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Primers used in real-time PCR

Gene name	Gene ID	Species	Primer Sequence (Forward: 5’-3’)	Primer Sequence (Reverse: 5’-3’)
FTO	79068	Homo sapiens	ACTTGGCTCCCTTATCTGACC	TGTGCAGTGTGAGAAAGGCTT
PPARα	5465	Homo sapiens	TTCGCAATCCATCGGCGAG	CCACAGGATAAGTCACCGAGG
GAPDH	26330	Homo sapiens	TGGATTTGGACGCATTGGTC	TTTGCACTGGTACGTGTTGAT
FTO	26383	Mus musculus	TTCATGCTGGATGACCTCAATG	GCCAACTGACAGCGTTCTAAG
GAPDH	14433	Mus musculus	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Figures

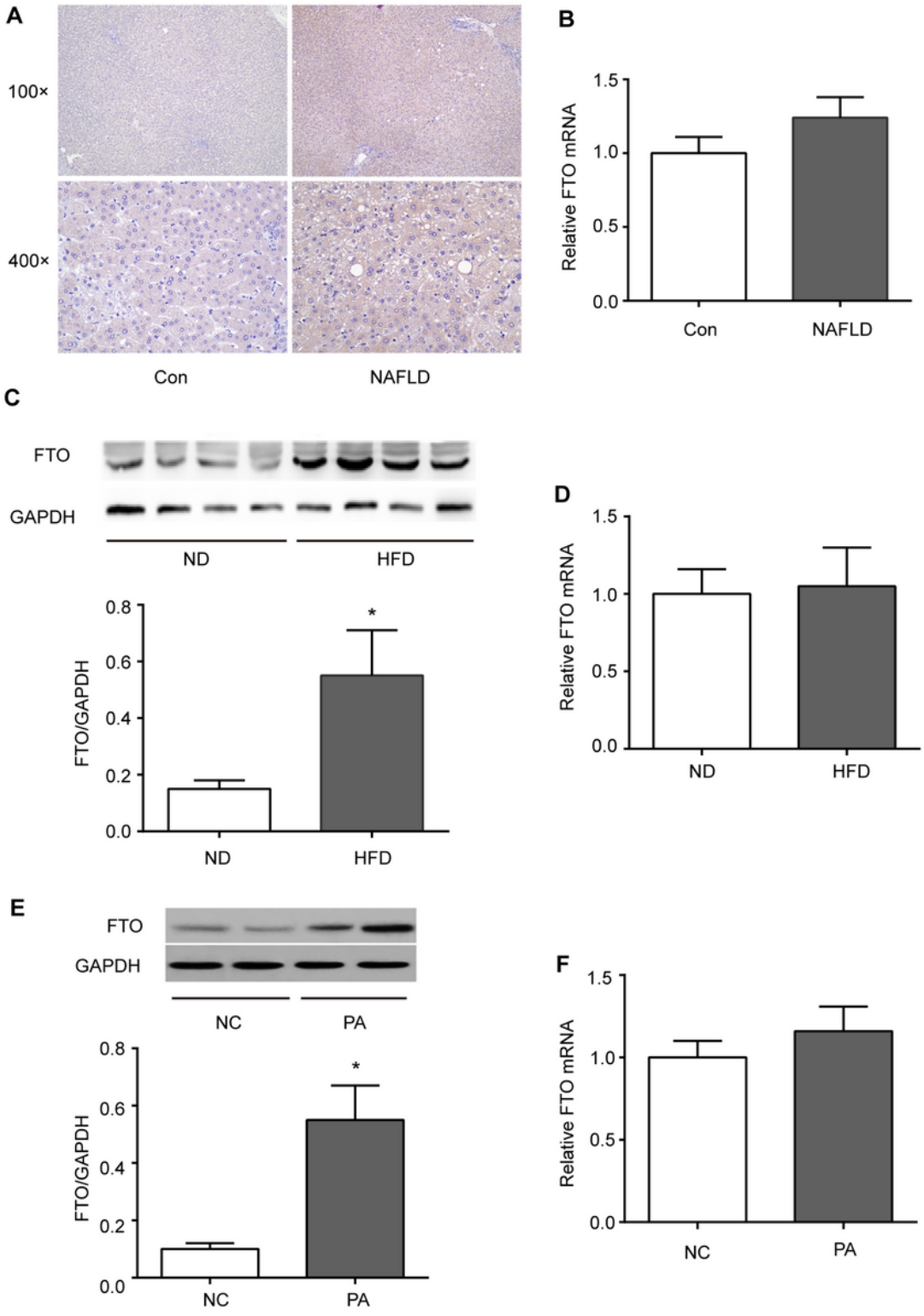

Figure 1

Upregulation of FTO in nonalcoholic fatty liver disease (NAFLD)

(A, B) Immunohistochemical and quantitative real-time polymerase chain reaction (qRT-PCR) analyses of FTO protein and mRNA expression in the livers from patients with and without NAFLD (n = 4 per group). **(C, D)** Western blotting and qRT-PCR analyses of the FTO protein and mRNA expression in the livers of

mice fed a high-fat diet (HFD) or normal diet (ND) for 16 weeks (n = 4 mice per group). Band intensities were quantified via densitometry. Relative expression was normalized to GAPDH and is expressed as the fold-change relative to the controls. **(E, F)** Western blotting and qRT-PCR analyses of the FTO protein and mRNA expression in HepG2 cells treated with palmitic acid (PA) (0.5 mM) for 24 h. Band intensities were quantified via densitometry. *P < 0.05 vs. control group.

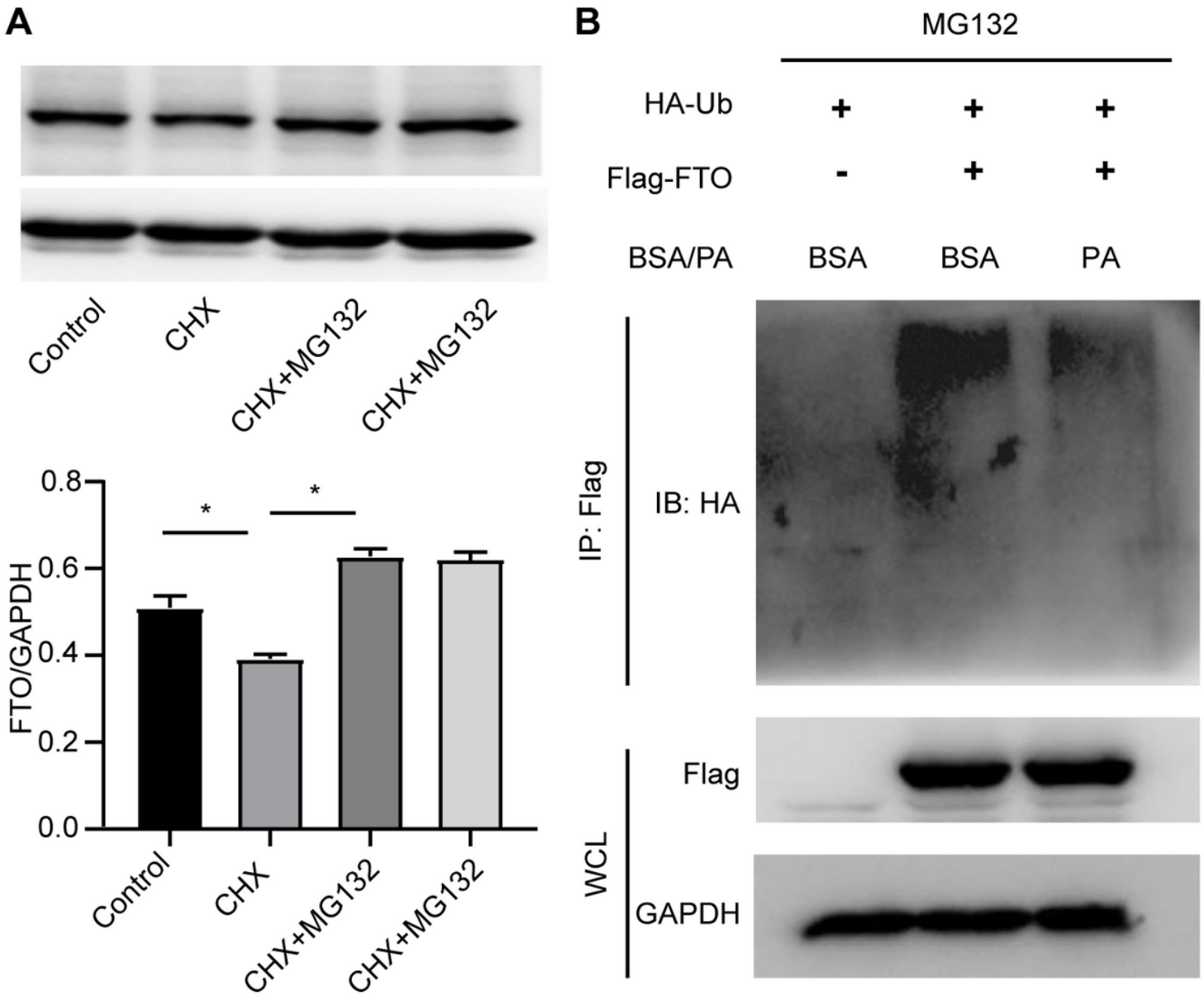

Figure 2

Palmitic acid (PA) decreases the ubiquitination of FTO in hepatocytes

(A) Western blotting analysis of FTO protein expression in HepG2 cells treated with the protein synthesis inhibitor cycloheximide (CHX, 10 μg/mL), and/or the proteasome inhibitor MG132 (10 μM) for 24 h. Band intensities were quantified via densitometry. **(B)** HepG2 cells overexpressing FLAG-FTO or negative control

vectors were transfected with HA-ubiquitin and treated with PA (0.5 mM) for 12 h. The ubiquitination of FTO was assessed via immunoprecipitation and western blotting analyses.

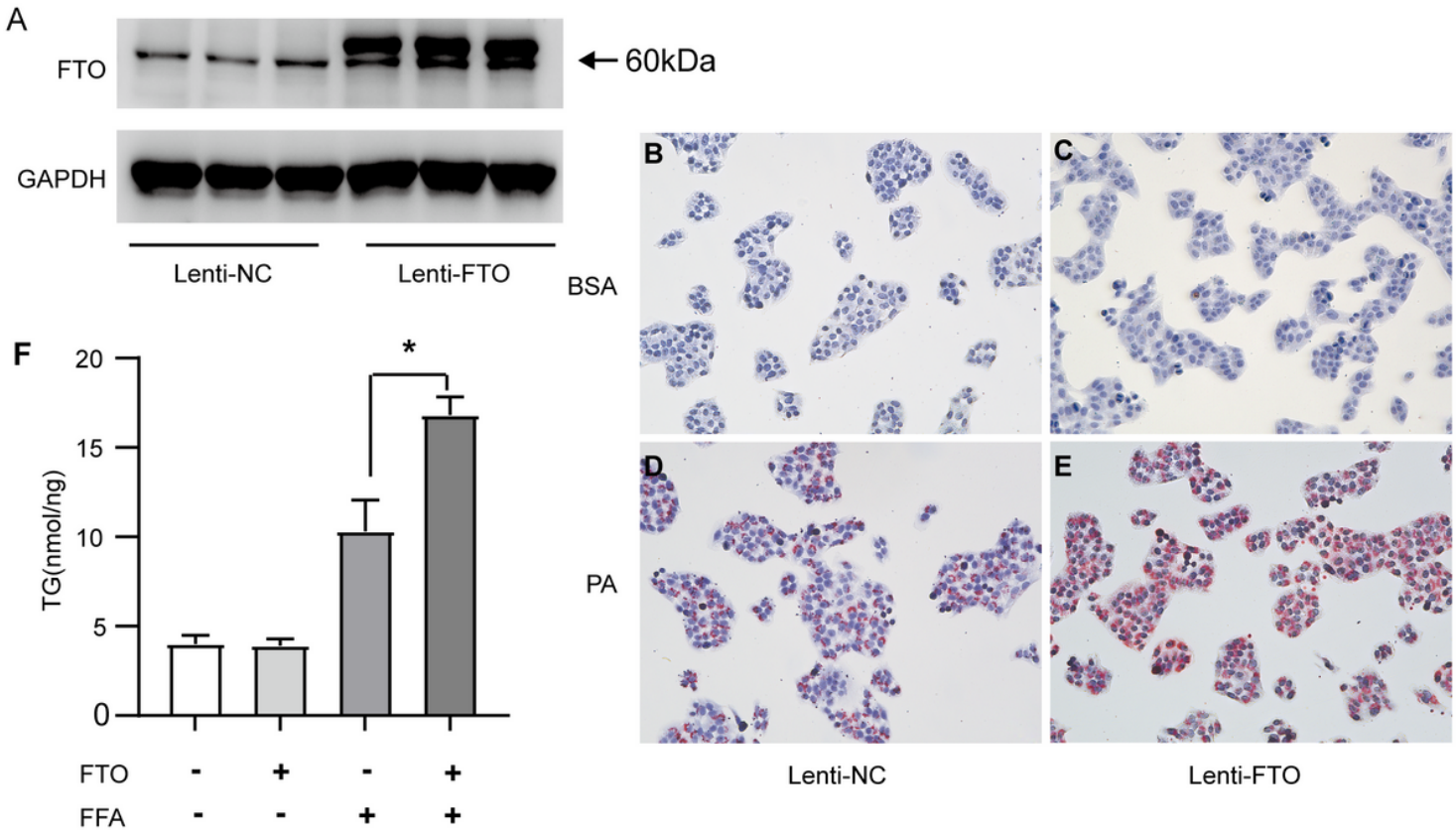

Figure 3

FTO overexpression induces lipid accumulation in hepatocytes.

(A) Western blotting analysis of the FTO protein level in HepG2 cells transfected with the FLAG-FTO lentiviral (lenti-FTO) or negative control (lenti-NC) vector. **(B-E)** Oil Red O staining of HepG2 cells overexpressing FLAG-tagged FTO or the empty vector were treated with palmitic acid (PA) (0.5 mM) or 2% bovine serum albumin (BSA) for 24 h (200x magnification). **(F)** Cellular contents of triglycerides (TG) in FTO-overexpressing and control HepG2 cells stimulated with 2% BSA or PA (0.5 mM) for 24 h. *P < 0.05 vs. control group.

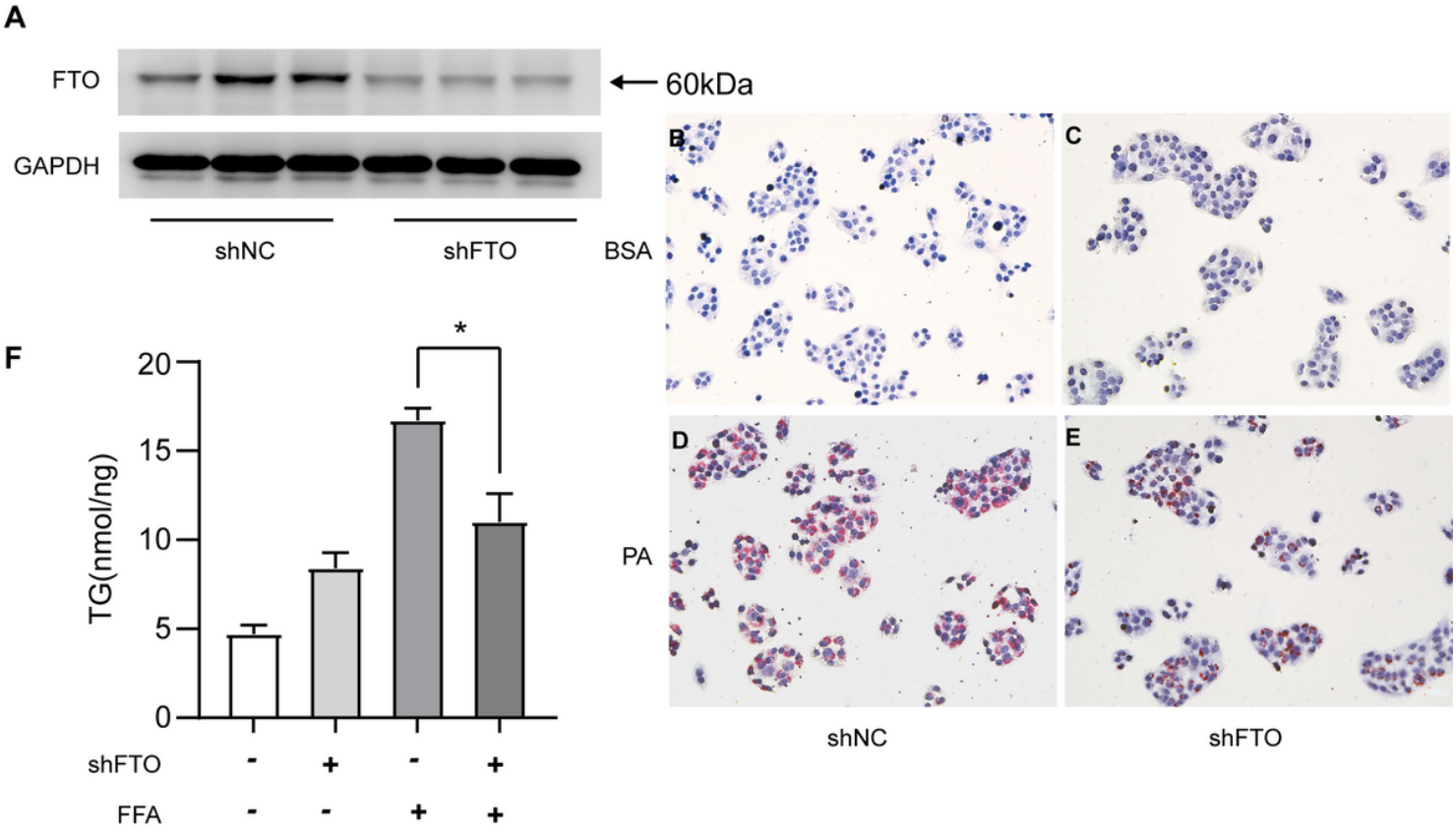

Figure 4

Knockdown of FTO inhibits lipid accumulation in hepatocytes

(A) Western blotting analysis of the FTO protein level in HepG2 cells transfected with the FTO shRNA lentiviral (shFTO) or negative control (shNC) vector. **(B-E)** Oil Red O staining of HepG2 cells transfected with shFTO or shNC after treatment with palmitic acid (PA) (0.5 mM) or 2% bovine serum albumin (BSA) for 24 h (200x magnification). **(F)** Cellular contents of triglycerides (TG) in FTO knockdown and control HepG2 cells stimulated with 2% BSA and PA (0.5 mM) for 24 h. *P < 0.05 vs. control group.

Figure 5

FTO promotes hepatic steatosis in mice

(A, B) C57BL/6J male mice were administered the AAV8-FTO (AAV8-FTO) or control (AAV8-Con) vector by tail vein injection, then fed a high-fat diet (HFD) for 8 weeks. Body weights were recorded at the indicated time points (n = 6 mice per group). **(C-F)** Representative images of Oil Red O staining (200x magnification) in the liver tissues of FTO-overexpressing and control mice fed an HFD for 8 weeks (n = 6 mice in each group). **(G)** Quantification of Oil Red O staining area in hepatocytes by the ImageJ software.

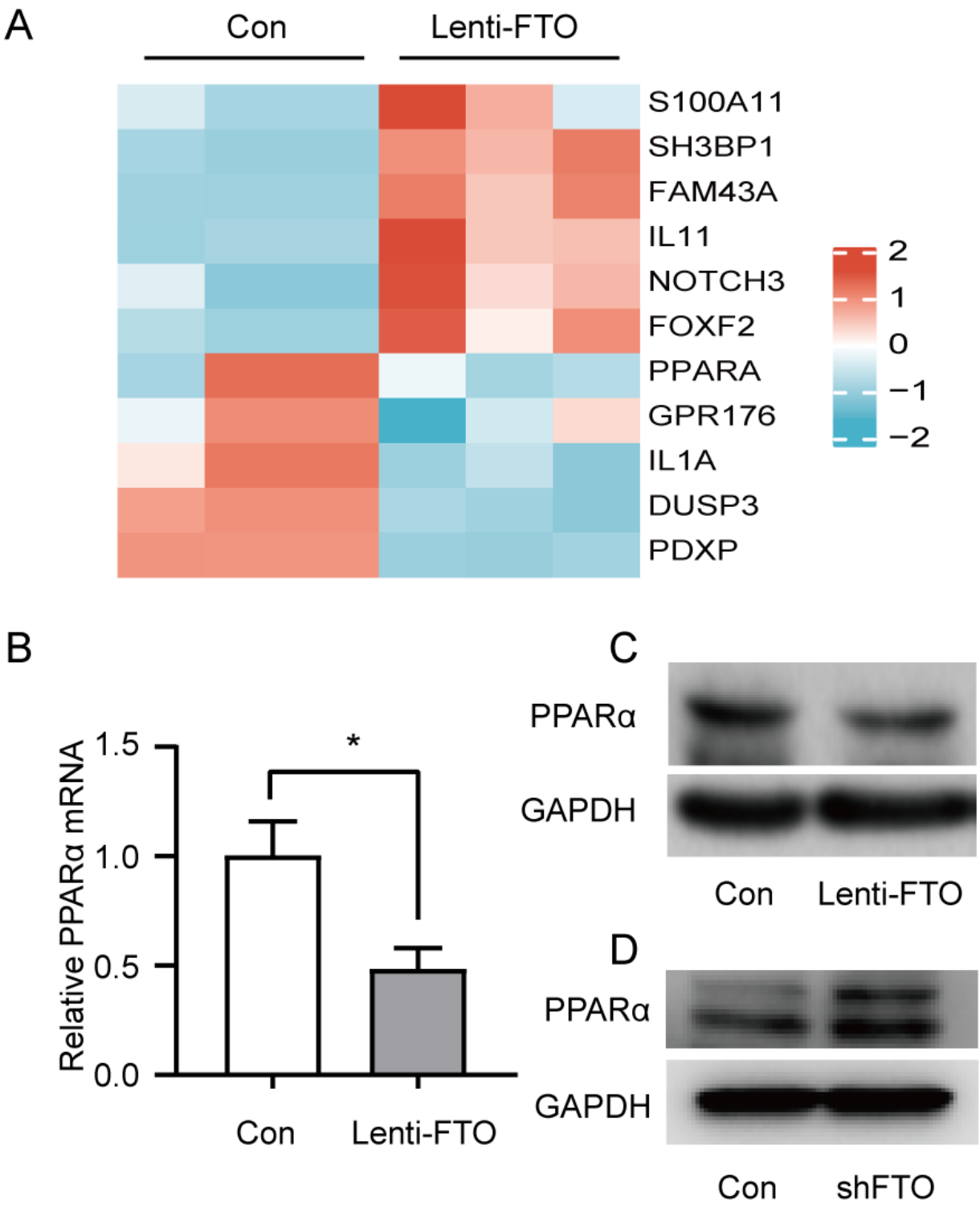

Figure 6

FTO inhibits the expression of PPARα

(A) Heat map of differentially expressed mRNAs between FTO-overexpressing and control HepG2 cells via high-throughput sequencing. **(B, C)** Quantitative real-time polymerase chain reaction and western blotting

analyses of PPARα mRNA and protein expression in FTO-overexpressing and control HepG2 cells. **(D)** Western blotting analysis of PPARα protein expression in FTO-knockdown and control HepG2 cells.

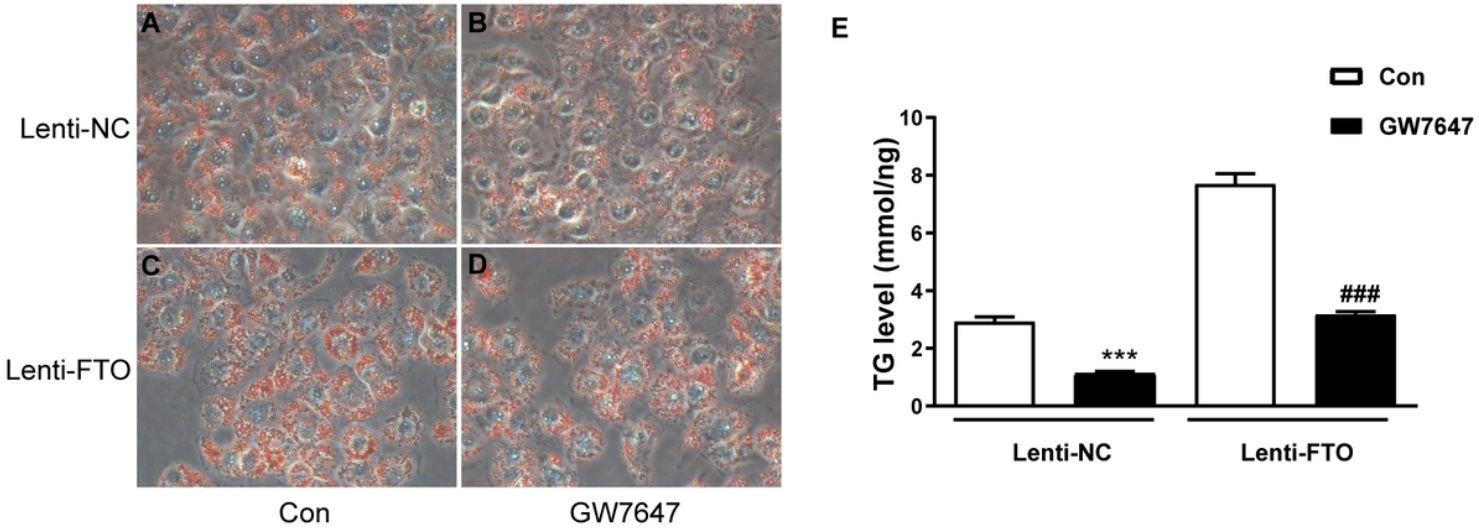

Figure 7

FTO induces lipid accumulation in hepatocytes by targeting PPARα

(A-D) FTO-overexpressing (Lenti-FTO) and control HepG2 cells (Lenti-NC) were treated with palmitic acid (PA) (0.5 mM) for 24 h without (A, C) or with (B, D) challenge with the PPARα agonist, GW7647 (10 μM). Oil Red O staining was performed and photographed at 200x magnification.