Deletion of RNF186 expression suppresses diet-induced hepatic steatosis by regulating insulin activity

Highlights

- RNF186 deficiency on high-fat diet alleviates liver steatosis and insulin tolerance
- RNF186 increased hepatic TG accumulation and impaired insulin sensitivity
- RNF186 ablation suppresses hepatic inflammation associated with high-fat diet
- RNF186 maybe a potential regulator of NAFLD in obesity
Deletion of RNF186 expression suppresses diet-induced hepatic steatosis by regulating insulin activity

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SUMMARY
RING finger protein 186 (RNF186) is dramatically upregulated in steatotic livers. The physiological role of RNF186 in non-alcoholic fatty liver disease (NAFLD) remains obscure. Here, we found that hepatocyte-specific RNF186 knockout (RNF186LKO) mice were protected from HFD-induced obesity. RNF186 ablation in liver suppressed inflammatory responses and ER stress and alleviated insulin tolerance, leading to improved glucose and lipid metabolism under HFD conditions. RNA-seq and western blot analyses revealed a significant downregulation of peroxisome proliferator-activated receptor γ, stearoyl-CoA desaturase 1, and cluster of differentiation 36 in the liver of RNF186 knockout mice consuming HFD. RNF186 deletion in liver results in less weight gain during HFD feeding and is associated with reduced liver fat, inflammation, and improved glucose and insulin tolerance. In contrast, upregulation of RNF186 in C57BL/6J mice impaired lipid metabolism and insulin tolerance. The collective results suggest that RNF186 may be a potential regulator of NAFLD in obesity.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease globally (Ahmed et al., 2015; Cohen et al., 2011) with an incidence of approximately 25% (Younossi et al., 2016). The progression of steatosis in NAFLD ranges from simple steatosis to steatosis with inflammation (steatohepatitis), more advanced fibrosis, liver cirrhosis, and even hepatocellular carcinoma (Cohen et al., 2011; Loomba et al., 2021). The increasing morbidity rate will continue to increase economic burdens and pose a serious threat to health. Many factors lead to the development of NAFLD. These include chronic low-grade inflammation, insulin resistance, dyslipidemia, and obesity (Temple et al., 2016). Steady progress has been made in elucidating the pathogenesis of NAFLD, identifying therapeutic targets, and advancing drug development. However, the specific mechanism and effective therapeutic method remain largely unknown.

RING finger protein 186 (RNF186) is a RING-type ubiquitin E3 ligase that contains a RING finger domain (Nakamura, 2011; Wang et al., 2013). More than 200 RNF family genes have been identified. Many have diverse functions in different biological and pathological processes (Okamoto et al., 2020). Several dedicated ubiquitin ligases have critical roles in the regulation of hepatic metabolism. For example, RNF5 is important in the regulation of liver cholesterol synthesis by regulating the expression of sterol regulatory element-binding protein 2 (SREBP2) (Kuan et al., 2020). RNF20 may be involved in the regulation of triglyceride (TG) synthesis by promoting polyubiquitination and degradation of SREBP1c, leading to a decrease in the expression of lipogenic genes (Lee et al., 2014). Several lines of evidence suggest that RNF186 is involved in the pathogenesis of intestinal inflammation by participating in the regulation of ER stress (Fujimoto et al., 2017; Rivas et al., 2016). Recently, RNF186 was reported to be essential for the control of nutrient sensing through the ubiquitination of Sestrin-2, a critical cellular process controlling metabolism (Lear et al., 2019). We previously demonstrated that RNF186 impairs insulin sensitivity by inducing ER stress in vitro (Tong et al., 2018). However, while this enables RNF186 to regulate insulin signaling, the role of RNF186 in hepatocyte metabolism remains largely unknown.

The major goal of the current study was to explore the function of liver RNF186 in the regulation of insulin tolerance and obesity-associated NAFLD. The present findings demonstrate the involvement of RNF186 in
the regulation of hepatic insulin action in vivo. Deletion of RNF186 alleviated insulin resistance and ER stress induced by a high-fat diet (HFD), leading to a decrease in the expression of lipogenic genes. These findings suggest that RNF186 may play an important role in liver lipid metabolism by regulating insulin signaling pathways.

RESULTS

Liver-RNF186 KO mice display improved glucose tolerance and insulin tolerance

We next examined whether liver-specific ablation of RNF186 influenced glucose metabolism in HFD-fed mice. Fasting glucose levels and plasma insulin levels of RNF186LKO mice were markedly lower than those of control mice fed the HFD for 18 weeks (Figures 3A and 3B). Glucose tolerance tests (GTTs) results indicated a significantly lower plasma glucose level in RNF186 KO mice compared with the control group after 8 weeks (Figures 3C and 3D). Moreover, pyruvate tolerance tests (PTTs) data confirmed that pyruvate tolerance was improved in RNF186LKO mice compared with RNF186f/f controls (Figure 3G). Insulin tolerance tests (ITTs) findings demonstrated that the decrease in the plasma glucose level was significantly lower in RNF186 KO mice than in control mice after insulin challenge (Figure 3H). These results suggest that liver-specific knockout of RNF186 can significantly improve glucose tolerance and insulin tolerance induced by obesity.

Gene enrichment analyses from liver explore a comparable role of RNF186 deletion in lipid metabolism with HFD treatment

To systemically elucidate the underlying mechanism by which RNF186 affects glucose and lipid metabolism in vivo, we performed RNA-seq on liver tissue from RNF186LKO and control mice.
Figure 1. Hepatocyte RNF186 KO mice are protected from high-fat-diet (HFD)-induced obesity

(A) The representative gross morphology of control and RNF186\textsuperscript{LKO} mice after 18 weeks of the HFD (n = 7 per genotype).

(B) Body weights of control and RNF186\textsuperscript{LKO} mice were measured weekly from weeks 1–18 (n = 7 per genotype).

(C) Fat body weight and lean body weight of control and RNF186\textsuperscript{LKO} mice at 18 weeks of age fed the HFD (n = 7 per genotype).

(D) Food intake of control and RNF186\textsuperscript{LKO} mice fed with HFD (n = 7 per genotype).

(E) Gross morphology of different fat tissue mass of control and RNF186\textsuperscript{LKO} mice after 18 weeks of the HFD (n = 7 per genotype).

(F–G) The weight of different fat pads in control and RNF186\textsuperscript{LKO} mice (n = 7 per genotype). (H) H&E staining of epiWAT and subWAT of RNF186\textsuperscript{eff} and RNF186\textsuperscript{LKO} mice; scare bars, 50 μm (n = 7 per genotype). Data are presented as means ± SEM *p < 0.05, **p < 0.01 by Student’s test.
analysis separated three KO samples (red) from three control samples. A total of 21,878 genes remained for analysis after filtering out genes with little or no expression. A total of 124 genes were differentially expressed as shown in the Volcano plot in Figure 4A. Kyoto Encyclopedia of Genes and Genomes (KEGG)

Figure 2. Protection against hepatic steatosis in RNF186^{LKO} mice
(A and B) Representative results of (A) morphology, (B) H&E staining of sections (top panel), and Oil Red O staining (bottom panel) of livers from control and RNF186^{LKO} mice after the 18-week HFD; scale bars, 50 μm (n = 7 per genotype).
(C) The ratio of the liver weight to body weight in wild type and KO mice (n = 7 per genotype).
(D and E) Hepatic (D) and serum (E) TG levels in control and RNF186^{LKO} mice after 18 weeks of the HFD and following a 6-h fast (n = 7 per genotype).
(F and G) Hepatic (F) and serum (G) TC levels in control and RNF186^{LKO} mice after 18 weeks of the HFD and following a 6-h fast (n = 7 per genotype).
(H) Serum NEFA levels in control and RNF186^{LKO} mice after 18 weeks of the HFD and following a 6-h fast (n = 7 per genotype). Data are present as means ± SEM *p < 0.05, **p < 0.01 by Student’s test.
pathway analysis showed that deletion of RNF186 in liver significantly altered the fat metabolism and the peroxisome proliferator-activated receptor (PPAR) signaling pathway (Figure 4B).

A heatmap of the differential expression analysis revealed the gene expression signatures of the most differentially expressed genes in these samples. The heatmap summary of the results of the partial differential expression analysis revealed the expression characteristics of genes that were differentially related to lipid metabolism in these samples. Among these differential genes, those related to lipid synthesis, such as stearoyl-CoA desaturase 1 (SCD1), peroxisome proliferator-activated receptor gamma (PPARγ), and Hmgcs1, were reduced by RNF186 deficiency (Figure 4C). The findings suggested that deletion of the RNF186 gene may downregulate lipid synthesis in the liver. Consistent with the RNA-seq data, qRT-PCR results further validated

Figure 3. Ablation of RNF186 improves glucose tolerance and hepatic insulin tolerance

(A) Blood glucose levels of control and RNF186LKO mice after 18 weeks of the HFD followed by a 6-h fast (n = 7 per genotype).
(B) Serum insulin levels for control and RNF186LKO mice after 18 weeks of the HFD followed by a 6-h fast (n = 7 per genotype). (C–F) GTTs analyses were performed in control and KO mice after 4 (C), 8 (D), and 18 (E and F) weeks of the HFD (n = 6 per genotype). (G–H) PTTs (G) and ITTs (H) analyses were performed in control and KO mice after 18 weeks of the HFD (n = 6 per genotype). Data are present as means ± SEM *p < 0.05, **p < 0.01, ***p < 0.001 by Student’s t-test.
Figure 4. RNF186 regulates hepatic TG content in a lipogenesis-dependent manner
(A) Scatterplot of differentially expressed genes of RNA-seq in livers from RNF186^{f/f} and RNF186^{LKO} mice after 18 weeks of the HFD (n = 3 per genotype).
(B) KEGG pathway analysis showing downregulated pathways by RNA-seq.
(C) Heatmap showing the expression of genes involved in hepatic lipid metabolism of RNA-seq (n = 3 per genotype).
Deletion of RNF186 in liver alleviates insulin signaling in diet-induced obesity

Our previous studies confirmed that RNF186 can regulate insulin sensitivity in vitro (Tong et al., 2018). Therefore, in the present study, we further investigated the effect of RNF186 on insulin signaling in vivo. We injected insulin or saline into obese mice of different genotypes through the inferior vena cava after a 16-h fast and quickly removed the liver and adipose tissue to analyze the insulin signaling pathway. The results confirmed that the phosphorylation levels of AKT increased in the liver RNF186 KO mice fed the HFD relative to control mice (Figures 5A and 5B). Consistent with this result, glycogen synthase kinase 3 beta (GSK-3beta) phosphorylation was increased in the liver of RNF186 LKO mice compared with control mice (Figures 5A and 5B). We also identified the decreased mRNA and protein levels of cluster of differentiation 36 (CD36), the fatty acid translocase protein, in hepatic tissue of RNF186 KO mice compared with the control groups (Figures 4D and 4E). Consistent with the decreased blood glucose levels, the expression of gluconeogenic genes and phosphoenolpyruvate carboxykinase (PEPCK) was decreased in the livers of RNF186 LKO mice (Figure 4F). However, the mRNA levels of SREBP-1c and its target genes, including fatty acid synthase and acetyl-CoA carboxylase, were unchanged (Figure 4F). Additionally, there were not obvious alterations of the activity of genes related to fatty acid oxidation (Figure 4F). These data suggest that deletion of RNF186 can downregulate lipogenic gene expression due to HFD-induced obesity and protect against liver steatosis.

RNF186 deficiency protects mice from HFD-induced liver inflammation

Because HFD-induced obesity is associated with the development of inflammation, we further analyzed whether RNF186 LKO can alleviate inflammation induced by an HFD. RNF186 KO mice displayed lower levels of serum alanine transaminase (ALT) and aspartate transaminase (AST) in serum than the levels in the control group after consumption of the HFD (Figure 6A). We also measured serum concentrations of tumor necrosis factor-alpha (TNFalpha), which has postulated roles in obesity and insulin action (Khan et al., 2019). RNF186 KO mice displayed lower serum TNFalpha levels than in control mice (Figure 6B). In addition, we performed immunohistochemical analysis of liver sections from two genotypes mice, using an antibody specific for F4/80 antigen, a pan-macrophage marker. The number of F4/80 positive cells was markedly decreased in the livers of RNF186 KO mice compared with control group (Figure 6C). Western blot analysis confirmed that the phosphorylation levels of nuclear factor kappa B (NF-kappa B) were reduced in the livers of RNF186 LKO mice compared to the levels in control mice (Figure 6D). Consistent with this, downregulation of TNFalpha, interleukin-6 (IL-6), IL-1beta, and monocyte chemoattractant protein-1 (MCP1) due to RNF186 deficiency was identified by RT-PCR in liver tissue (Figure 6E). Our previous study demonstrated that overexpression of RNF186 induces ER stress and increases inflammation in vitro (Tong et al., 2018). Here, deletion of RNF186 decreased inositol-requiring kinase 1 (IRE1) and eukaryotic initiation factor 2alpha (eIF2alpha) phosphorylation levels in the livers of mice fed the HFD compared with control mice (Figure 6F). However, the protein level of X-box binding protein 1 (XBP-1s) showed no change between two groups (Figure 6F). These results suggest that knockout of RNF186 in the liver may have a protective effect on HFD-induced inflammation.

Overexpression of RNF186 promotes TG accumulation and inflammation in the liver of C57BL/6J mice

To further explore the role of RNF186 in the progression of NAFLD, we upregulated RNF186 expression in the liver of C57BL/6J mice by tail vein injection of Ad-RNF186. qPCR and western blots showed that adenovirus-mediated RNF186 was effectively expressed in the liver compared with control adenovirus Ad-GFP-treated mice (Figures 5A and 5B). Overexpression of RNF186 increased the fasting glucose levels compared with control groups (Figure 7A). Meanwhile, overexpression of RNF186 led to a markedly upregulation in hepatic TG content in C57BL/6J mice (Figure 7B), whereas serum or hepatic total TC and FFAs were comparable...
between two groups (data not shown). The liver weight was obviously higher in RNF186 overexpression mice than in control mice (Figure 7C). Furthermore, we found that Ad-RNF186-infected mice significantly increased the serum insulin and TNFα levels compared with the levels in control group (Figures 7D and 7E). GTTs and ITTs indicated that hepatic overexpression of RNF186 had no effect on glucose tolerance and impaired insulin tolerance compared with the control mice (Figures 7F and 7G). Additionally, the mRNA expression of SCD1 and PPARγ was significantly higher in mice infected with Ad-RNF186 than in mice infected with Ad-GFP (Figures 7H and 7I). We also observed the expression of mRNA proinflammatory factors genes, including TNFα, IL6, and MCP1, was increased in the livers of mice infected with Ad-RNF186 compared with those of control mice. Our previous study has been reported that overexpression of RNF186 induced ER stress and increased the phosphorylation levels of IRE1 and eIF2α in primary hepatocytes (Tong et al., 2018). Consistently, the phosphorylation of IRE1 and eIF2α was significantly higher in the liver of mice infected with Ad-RNF186 than the control mice (Figure 7K). These results indicate that upregulation of RNF186 impairs lipid metabolism and induced inflammation in the liver of C57BL/6J mice.

**DISCUSSION**

It has long been known that the common feature of NAFLD is insulin resistance. Insulin resistance increases the levels of insulin in the serum, which causes hepatocytes to promote hepatic lipid synthesis through several
insulin-sensitive signaling factors and induces a vicious cycle of inflammation (Farese et al., 2012; Tilg and Moschen, 2008). Growing evidence indicates that adipose tissue dysfunction/inflammation is crucial in NAFLD pathogenesis. Studies have revealed the metabolic crosstalk between adipose tissue and the liver. Previously, we reported that the protein expression of RNF186 was upregulated in the fatty liver compared with the control group and that RNF186 impairs insulin sensitivity by increasing ER stress and promotes TG production from primary hepatocytes (Tong et al., 2018). However, the precise molecular mechanisms of RNF186 in the regulation of hepatic fat metabolism in vivo have not been established. In the present study, we demonstrate that RNF186 is crucial in exacerbating HFD-induced lipid accumulation, inflammation, and insulin resistance in hepatocytes. Hepatocyte-specific RNF186 KO mice were protected from hepatic insulin resistance induced by HFD feeding for 18 weeks. It is manifested as an increase in the phosphorylation level of AKT in the liver of RNF186-KO mice. In addition, we also found a significant improvement in pyruvate tolerance in KO mice, and pyruvate serves as a major substrate for hepatic gluconeogenesis (Wang et al., 2019).

**Figure 6. RNF186 deficiency protects mice from HFD-induced hepatic inflammation**

(A) Hepatic deletion RNF186 decreased serum ALT and AST levels (n = 7 per genotype).
(B) Serum TNFα levels for control and RNF186LKO mice after 18 weeks of the HFD followed by a 6-h fast (n = 7 per genotype).
(C) Histological F4/80 staining in liver sections from RNF186f/f and RNF186LKO mice fed the HFD for 18 weeks; scale bars, 25 μm (n = 4 per genotype).
(D) Western blot analysis of NF-κB protein and phosphorylation levels in liver of RNF186f/f and RNF186LKO mice after 18 weeks of the HFD (n = 5 per genotype).
(E) qPCR analysis of genes involved in inflammation response in the livers of wild-type and RNF186LKO mice after 18 weeks of the HFD (n = 5 per genotype). Data are present as means ± SEM *p < 0.05, **p < 0.01, ***p < 0.001 by Student’s test.
Figure 7. Overexpression of RNF186 in the liver of C57BL/6J mice exacerbates lipid metabolism

(A) Blood glucose level in control C57BL/6J mice injected with Ad-GFP or Ad-RNF186 9 days after injection under fasting conditions (n = 7 per group).

(B) Change of hepatic TG levels in C57BL/6J mice infected with Ad-GFP or Ad-RNF186 after 9 days under fasting conditions (n = 7 per group).

(C) Hepatic RNF186 overexpression significantly increased the liver weight in mice infected with Ad-RNF186 compared with the control mice.

(D and E) Change of serum insulin (D) and TNFα (E) levels in C57BL/6J mice infected with Ad-GFP or Ad-RNF186 under fasting conditions (n = 7 per group).

(F and G) GTTs (F) and ITTs (G) in control C57BL/6J mice injected with Ad-GFP or Ad-RNF186 5 days after injection (n = 5 per group).
TG synthesis in vitro tissue of RNF186 increased release of glucose (Jornayvaz and Shulman, 2012). The present findings demonstrate that the accompanied by decreases in glycogen synthesis and gluconeogenesis inhibition, which will lead to an oratively promote NAFLD progression (Tilg and Moschen, 2008; Wang et al., 2016). Insulin resistance is anisms. Interactions among insulin resistance, aberrant lipid metabolism, and hepatic inflammation collab.

Insulin is the master regulator of hepatic glucose and lipid metabolism through direct and indirect mech-

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Herein, we demonstrate that the deletion of RNF186 leads to downregulation of the expressions of hepatic SCD1, PPARY, and HMGS1. SCD1 and PPARY are important regulators of hepatic de novo lipogenesis and TG synthesis in vitro and in vivo (Mendez-Sanchez et al., 2007). Hepatic overexpression of PPARY-2 using adenovirus was sufficient to increase liver TG, along with an increase in mRNA levels of SREBP-1c and other lipo-

The mechanism is still unclear and needs further study. We hypothesized that liver-specific knockout of RNF186 might cause changes in adipose tissue energy consumption.

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Limitations of the study
Altogether, RNF186 KO mice are protected against HFD-induced weight gain and associated fatty liver, insulin resistance, and hyperglycemia. These results may suggest a direct role, but are confounded by the differences in body weight in the models presented and will require further study. It is possible that liver-specific knockout of RNF186 may affect the secretion of related factors in mouse liver and act on adipose tissue, and regulate the accumulation of triglycerides in adipose tissue or affect energy expenditure. In the future, we will further explore the role of RNF186 in the metabolism of adipose tissue by constructing adipose tissue-specific RNF186 knockout mice.

STAR★METHODS
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SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.103859.

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AUTHOR CONTRIBUTIONS
XH, CG, YC, and HZ conceived of the experimental plan. XH, QFZ, and MG performed experiments and analyzed the data. QY, XT QZ, LL, and LZ performed the experiments. SL and XL analyzed data analysis. XH, MG, and HZ wrote the manuscript. CG, YC, and HZ analyzed data analysis and critical revision of the manuscript.

DECLARATION OF INTERESTS
The authors declare no conflict of interest.

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**STARMETHODS**

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Rabbit anti-SCD-1   | ABclonal | Cat# A16429; RRID: AB_2772150 |
| Rabbit anti-PPARγ    | Cell Signaling Technology | Cat# 2435T; RRID: AB_2166051 |
| Rabbit anti-CD36     | Abclonal | Cat# A5792; RRID: AB_2766544 |
| Rabbit anti-β-actin | Cell Signaling Technology | Cat# 4970S; RRID: AB_2223172 |
| Rabbit anti-AKT      | Cell Signaling Technology | Cat# 9272S; RRID: AB_329827 |
| Rabbit anti-p-AKT    | Cell Signaling Technology | Cat# 9271S; RRID: AB_329825 |
| Rabbit anti-GSK-3β   | Cell Signaling Technology | Cat# 9315S; RRID: AB_490890 |
| Rabbit anti-p-GSK-3β | Cell Signaling Technology | Cat# 5558S; RRID: AB_10013750 |
| Rabbit anti-NF-κB    | Cell Signaling Technology | Cat# 8242T; RRID: AB_10859369 |
| Rabbit anti-p-NF-κB  | Cell Signaling Technology | Cat# 3033T; RRID: AB_331284 |
| Rabbit anti-IRE1α    | Cell Signaling Technology | Cat# 3294T; RRID: AB_823545 |
| Rabbit anti-p-IRE1α  | ABclonal | Cat# AP0878; RRID: AB_2771207 |
| Rabbit anti-eIF-2α   | Cell Signaling Technology | Cat# 5324T; RRID: AB_10692650 |
| Rabbit anti-p-eIF-2α | ABclonal | Cat# 3398T; RRID: AB_2096481 |
| Rabbit anti-XBP1     | BOSTER | Cat# PB9463 |
| Rabbit anti-RNF186   | Sangon | Cat# YS-2692R |
| Rabbit anti-F4/80    | Abcam | Cat# ab6640; RRID: AB_1140040 |
| Bacterial and virus strains |        |            |
| Ad-GFP              | Tong et al. (2018) | https://doi.org/10.1016/j.cellsig.2018.09.008 |
| Ad-RNF186           | Tong et al. (2018) | https://doi.org/10.1016/j.cellsig.2018.09.008 |
| Chemicals, peptides, and recombinant proteins |        |            |
| Insulin             | Novo Nordisk | Cat# B-0212-03 |
| PVDF membranes      | Millipore | Cat# IPVH00010 |
| Oil Red O           | Solarbio | Cat# O8010 |
| Sodium Pyruvate     | Sigma-Aldrich | Cat# P2256 |
| Mouse Insulin ELISA Kit | Lengton Bioscience | Cat# BPE20352 |
| Mouse TNFα ELISA Kit | R&D Systems | Cat# MHSTA50 |
| TRizol reagent      | Life Technologies | Cat# 15596026 |
| BSA                 | Solarbio | Cat# A8850 |
| iScript Reverse Transcription mix | Thermo Fisher | Cat# K1622 |
| iQ SYBR Green Supermix | Promega | Cat# A6100A |
| Phosphatase inhibitor cocktail | Bestbio | Cat# 33110A |
| Protease inhibitor cocktail | Beyotime | Cat# ST506-2 |
| Tyloxpolic          | Sigma-Aldrich | Cat# 25301-02-4 |
| Formalin            | Servicebio | Cat# G1101 |
| Cholesterol reagent | Applygen | Cat# E1015 |
| Triglyceride reagent | Applygen | Cat# E1013 |
| Free fatty acid reagent | Sigma-Aldrich | Cat# O7501, P9767 |
| Alanine transaminase reagent | Roche | Cat# 05850797190 |
| Aspartate transaminase reagent | Roche | Cat# 05850819190 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Huabing Zhang (slzhang1977@163.com & huabingzhang@ahmu.edu.cn).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- RNA seq data have been deposited at SRA and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mouse models
Liver specific RNF186 knockout (KO) mice were created by breeding floxed mice (Nanjing Biomedical Research Institute of Nanjing University, Nanjing, China). RNF186 flox/flox mice (RNF186\textsuperscript{f/f}) were created using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 methods (Ji et al., 2020). These mice were then crossed with albumin-Cre mice to generate liver specific RNF186 knockout (RNF186\textsuperscript{LKO}) mice. The KO efficiency was confirmed in liver tissue and other tissues by western blot analysis using anti-RNF186 antibodies. Unless otherwise noted, 8-week-old male mice were used for all experiments. All animal experiments conformed to the guidelines of the Animal Center of Anhui Medical University. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Anhui Medical University.
Mouse experiments

Five-week-old male WT and RNF186LKO mice were fed either standard chow diet (9% fat; Lab Diet) or HFD (45% fat; Research Diets) ad libitum for 18 weeks, with free access to water. C57BL/6J mice were purchased from GemPharmatech Co., Ltd (Nanjing, China). Mice were housed and maintained in a 12-h light/12-h dark cycle clean animal facility at Anhui Medical University. For adenovirus treatment, eight-week-old male C57BL/6J mice were injected with purified adenovirus with $1.0 \times 10^9$ active viral particles in 150 μl of 0.4% NaCl solution via tail vein. Then, 7-9 days later, mice fasted for 6 h were sacrificed, and livers and plasma were collected for analysis. Body weight and fasting blood glucose levels were examined.

METHOD DETAILS

In vivo glucose, insulin, and pyruvate tolerance tests

WT and RNF186LKO mice were fed HFD (45% fat; Research Diets) ad libitum for 18 weeks. For the glucose tolerance tests (GTTs) and pyruvate tolerance tests (PTTs), mice were injected with D-glucose (1–2 g/kg body weight) or pyruvate sodium (1–2 g/kg body weight) via intraperitoneal injection after 16 h of fasting. For the insulin tolerance tests (ITTs), mice were injected with insulin (0.5–0.75 U/kg body weight) via intraperitoneal injection after 6 h (from 10:00 a.m. to 4:00 p.m.) of fasting. Blood glucose levels were tested from the tail vein with a glucometer (One Touch Ultra, LifeScan Inc.) at indicated times (0, 15, 30, 45, 60, 90, 120 min after glucose, pyruvate or insulin).

In vivo insulin signaling

After an overnight fasting, mice were anesthetized with 2, 2, 2-tribromoethanol in PBS (Avertin) and injected with 5 U of regular human insulin (Novo Nordisk) via the inferior vena cava injection. Five or ten minutes after the insulin bolus, livers were removed and frozen in liquid nitrogen. Immunoblot analysis of insulin signaling molecules was performed using tissue homogenates prepared in a tissue homogenization buffer supplemented with the complete protease inhibitor cocktail (Beyotime) and Phosphatase inhibitor cocktail (Bestbio).

Preparation of expressing RNF186 recombinant adenoviruses

Recombinant overexpression of RNF186 adenoviruses were generated according to the manufacturer’s instructions (Invitrogen) and purified by the cesium chloride method as previously described (Tong et al., 2018).

RNA isolation and quantitative RT-PCR

Total RNA was extracted from tissues using a TRIzol-based method (Life Technologies, Carlsbad, California, USA). One microgram of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit and random primers according to the manufacturer’s instructions (Thermo Fisher, Waltham, MA, USA). Quantitative PCR (qPCR) was performed using the SYBR Green I qPCR kit (Promega, Madison, WI, USA) on a CFX system (Bio-Rad, Hercules, CA, USA). Gene expression data was normalized to 36B4. The specific primer sequences used for real-time PCR are listed in Table S1.

Western blotting

Whole tissue lysate was prepared with RIPA buffer supplemented with phosphatase inhibitor and protease inhibitor cocktail before the experiment. Western blotting was performed by utilizing a standard protocol as described previously [23]. The antibodies were anti-β-actin, anti-PPARγ, anti-NF-κB/phospho-NF-κB, anti-eIF2α/phospho-eIF2α, anti-AKT/phospho-AKT (Ser-473), anti-GSK-3β/phospho-GSK-3β (Cell Signaling Technology, Beverly, MA, USA), anti-SCD1, anti-CD36 (ABclonal Technology, Woburn, MA, USA) and anti-RNF186 (Sangon Biotech, Shanghai, China).

RNA-sequencing (RNA-seq) and bioinformatics analysis

Transcriptome sequencing and analyses were conducted by OE Biotech Co. Ltd. (Shanghai, China). Briefly, Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) following the manufacturer’s protocol. RNA integrity was evaluated using a model2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) $\geq 7$ were analyzed. The libraries were constructed using TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The libraries were sequenced on an Illumina sequencing platform.
(HiSeq 2500 or HiSeq X Ten) and 125bp/150bp paired-end reads were generated. The RNA-sequencing data have been deposited in NCBI under SRA accession numbers (SRA: PRJNA784646).

**Biochemical analysis and cytokine measurement**

Serum ALT, AST, TG, TC, and free fatty acid levels were determined in an automated device (Monach) in the clinical laboratory of the First Affiliated Hospital of Anhui Medical University, Hefei, China. Hepatic TG and TC contents were measured using a colorimetric diagnostic kit (Applygen Technologies, Inc., Beijing, China). Serum TNFα concentrations were determined by ELISA (R&D Systems, Minneapolis, MN, USA). Serum insulin concentrations were determined by ELISA (Lengton Bioscience Co.LTD, Shanghai, China).

**Histology and immunohistochemistry**

For hematoxylin and eosin (H&E) staining, liver tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin, and cut into 6 μm sections. The sections were stained with H&E. For Oil red O staining, liver tissue was frozen in liquid nitrogen and cut into 10 μm sections. Specimens were stained and microscopically evaluated at 100x magnification. Immunohistochemical examination was carried out to detect the expression of F4/80 (Abcam; ab6640) in liver tissues.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All data are presented as mean ± SEM for experiments including numbers of mice or duplicates as indicated in figure legends. Statistical analyses were conducted using the GraphPad Prism software version 6.0 (GraphPad Sofware, CA, USA) for Windows. The two-tailed Student’s test or analysis of variance (one-way ANOVA) was used to evaluate statistical differences; p* < 0.05 was considered statistically significant.