TET2 contributes to gluconeogenesis and pathology of type 2 diabetes

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Abstract

The control of gluconeogenesis is critical for glucose homeostasis and the pathology of type 2 diabetes (T2D). Here, we uncover a novel function of TET2 in the regulation of gluconeogenesis. In mice, both fasting and a high-fat diet (HFD) stimulate the expression of TET2, and TET2 knockout impairs glucose production. Mechanistically, FBP1, a rate-limiting enzyme in gluconeogenesis, is positively regulated by TET2 in liver cells. TET2 is recruited by HNF4α, contributing to the demethylation of FBP1 promoter and activating its expression in response to glucagon stimulation. Moreover, metformin treatment increases the phosphorylation of HNF4α on Ser313, which prevents its interaction with TET2, thereby decreasing the expression level of FBP1 and ameliorating the pathology of T2D. Collectively, we identify an HNF4α-TET2-FBP1 axis in the control of gluconeogenesis, which contributes to the therapeutic effect of metformin on T2D and provides a potential target for the clinical treatment of T2D.

Introduction

Loss of glucose homeostasis can lead to type 2 diabetes (T2D). A hallmark of T2D is persistently elevated blood glucose levels, which can contribute to various complications, such as kidney failure and neuropathy. Given that abnormal gluconeogenic activity accounts for the primary hepatic glucose production (HGP) (1, 2), which is the main source of increased blood glucose concentration in T2D, targeting gluconeogenesis is a reasonable strategy to maintain blood glucose homeostasis.

Fructose 1,6-bisphosphatase (FBP1), a rate-controlling enzyme in gluconeogenesis, catalyzes the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate. Interestingly, it has been reported that a point mutation in FBP1 can significantly reduce the efficacy of metformin, a first-line drug for the treatment of T2D, suggesting that FBP1 is a major contributor to the therapeutic effect of metformin (3). However, it remains unclear how FBP1 responds to metformin treatment.

Ten-Eleven Translocation 2 (TET2) belongs to the TET family, which includes TET1, TET2, and TET3. These enzymes function as DNA dioxygenases, catalyzing the successive oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (4), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC) (5, 6). Finally, thymine-DNA glycosylase (TDG) mediates the removal of 5caC, leading to an unmodified cytosine, which participates in DNA demethylation and gene expression regulation (6).

Recently, it was reported that the expression of FBP1 can be regulated by promoter methylation (7, 8), leading us to hypothesize that TET2 may play a role in regulating FBP1 expression and gluconeogenesis.

Mutations in TET2 frequently occur in various myeloid cancers. Somatic alterations in TET2 are observed in 50% of patients with chronic myelomonocytic leukemia and are associated with poor outcomes (9). The frequency of TET2 mutations in patients with myelodysplastic syndromes is 19% (10). Moreover, reversing TET2 deficiency facilitates hematopoietic stem and progenitor cell (HSPC) differentiation, blunts HSPC self-renewal, and blocks leukemia progression (11). It has also been reported that TET2 represses mTORC1 and HIF1α signaling, thus suppressing tumor growth in hepatocellular carcinoma (HCC) and clear cell renal cell carcinoma (ccRCC), respectively (12, 13). These studies focused on the function of TET2 in cancer development; however, it is unclear whether TET2 is involved in T2D progression. In this study, we demonstrated that TET2 is recruited by HNF4α to the FBP1 promoter and activates FBP1 expression by demethylation, contributing to gluconeogenesis and T2D pathology, and identified HNF4α-TET2-FBP1 axis as a target of metformin treatment, suggesting that targeting the HNF4α-TET2-FBP1 axis might be a potential strategy for T2D treatment.

Results

TET2 contributes to gluconeogenesis and T2D

To explore the role of TET2 in gluconeogenesis, we developed three mouse models:

one subjected to overnight fasting for 16 hours prior to tests, and two subjected to high-fat feeding (HFD) for 11 days and 12 weeks, respectively. The results showed that both fasting and HFD increased the mRNA and protein levels of TET2 in mouse livers compared to the normal chow group (Fig. 1A-E), indicating that TET2 may play an important role in gluconeogenesis. To test this hypothesis, we examined the effect of TET2 on glucose output and found that TET2 overexpression promoted glucose output in HepG2 cells and primary mouse hepatocytes (Fig. 2A and B). Consistent with this, *Tet2* knockout (4) impaired gluconeogenesis in HepG2 cells and mouse hepatocytes, even under glucagon treatment (Fig. 2C and D). Together, these data demonstrated that TET2 contributes to gluconeogenesis, prompting us to investigate whether TET2 is involved in T2D progression. Pyruvate tolerance tests (PTT), glucose tolerance tests (GTT), and insulin tolerance tests (ITT) were conducted, and interestingly, the results showed that *Tet2* KO markedly increased glucose tolerance and insulin sensitivity compared to the control mice (Fig. 2E-G). In summary, the loss of *Tet2* function decreased gluconeogenesis in the liver and may contribute to the treatment of T2D.

TET2 upregulates FBP1 expression in liver cells

Next, we explored the potential mechanism by which TET2 increases gluconeogenesis. Fructose-1,6-bisphosphatase 1 (FBP1), a rate-limiting enzyme in gluconeogenesis, is crucial for its regulation and was recently identified as a target of metformin (3). This led us to hypothesize that FBP1 might participate in TET2-mediated regulation of gluconeogenesis. The results showed that glucagon significantly increased both TET2 and FBP1 expression levels in HepG2 and primary mouse liver cells (Fig. 3A-C).

Additionally, fasting and high-fat diet (HFD) also upregulated FBP1 mRNA levels in mouse livers (Fig. 3D and E). Notably, Pearson correlation analysis revealed a positive correlation between TET2 and FBP1 expression in both control and fasting groups (Fig. 3F). To confirm this, Gene Expression Profiling Interactive Analysis (GEPIA) (14) was used to analyze the correlation between TET2 and FBP1 expression in human liver tissue. Consistent with the mouse data, FBP1 expression levels positively correlated with TET2 levels in human livers (Fig. 3G). These findings prompted us to examine whether TET2 regulates FBP1 expression. The results showed that TET2 overexpression promoted FBP1 expression in primary mouse hepatocytes and HepG2 cells (Fig. 3H), while *Tet2* KO (4) significantly decreased FBP1 levels in HepG2 and LO-2 cells (Fig. 3I and J). Furthermore, *Tet2* KO abolished the glucagon-induced upregulation of FBP1 (Fig. 3K), suggesting that TET2 is required for glucagon-induced FBP1 upregulation. Taken together, these data indicate that TET2 regulates FBP1 in the livers.

To explore the mechanism by which TET2 regulates FBP1, ChIP-qPCR was performed to investigate whether TET2 could bind to the FBP1 promoter and catalyze the conversion of 5mC to 5hmC in HepG2 cells under glucagon treatment. We found that glucagon treatment promoted TET2 binding to the FBP1 promoter in HepG2 cells, which increased 5hmC levels and reduced 5mC levels (Fig. 3L-N). Importantly, *Tet2* KO blocked this process and caused the abnormal accumulation of 5mC in the FBP1 promoter (Fig. 3L-N). These data demonstrate that TET2 mediates the transcriptional activation of FBP1 in response to glucagon stimulation.

HNF4α is required for TET2 mediated transcriptional activation of FBP1

Given that TET2 binds to DNA without sequence specificity, we sought to understand how TET2 specifically activates FBP1 expression upon glucagon treatment. Recent genome-wide methylation and transcriptome analyses identified hepatocyte nuclear factor 4 alpha (HNF4α) as a master gluconeogenic transcription factor, playing a critical role in the pathogenesis of diabetic hyperglycemia (15). More importantly, ChIP-seq data suggested that HNF4α binds to the FBP1 promoter and participates in the regulation of gluconeogenesis in adult mouse hepatocytes (16, 17). To determine whether HNF4α is involved in TET2-mediated FBP1 expression, we performed immunofluorescence to examine the colocalization of TET2 and HNF4α with or without glucagon treatment in HepG2 cells. The results showed that the colocalization of TET2 and HNF4α significantly increased under glucagon treatment (Fig. 4A). Consistently, we observed that glucagon increased the interaction between TET2 and HNF4α in HepG2 cells (Fig. 4B). Furthermore, we knocked down HNF4α in HepG2 cells using siRNA (Fig. 4C), which inhibited glucagon-induced TET2 binding to the FBP1 promoter (Fig. 4D), thereby suppressing TET2-mediated FBP1 expression (Fig. 4E) and impairing glucose output under glucagon treatment (Fig. 4F). Notably, the expression of both HNF4α and FBP1 also increased in mouse livers under fasting or HFD treatment compared to the control group (Fig. 4G-I). In conclusion, these results support the notion that TET2-mediated gluconeogenesis is dependent on HNF4α.

HNF4α phosphorylation affects its binding to TET2 and FBP1 expression

Our results demonstrated that HNF4α recruits TET2 to the FBP1 promoter and activates FBP1 expression through demethylation, playing a crucial role in the regulation of hepatic glucose output. However, it remains unclear whether the HNF4α-TET2-FBP1 axis responds to metformin treatment. Metformin, a first-line antidiabetic drug widely used to treat hyperglycemia in type 2 diabetes, is also a well-established adenosine 5'-monophosphate-activated protein kinase (AMPK) activator (18). Interestingly, one study revealed that AMPK phosphorylates HNF4α at Ser 313, reducing its transcriptional activity (19). Combining these studies with our findings, we wonder whether metformin can affect the HNF4α-TET2-FBP1 axis. To explore the role of metformin-induced AMPK phosphorylation of HNF4α in FBP1 expression, we treated cells with metformin and assessed the interaction between HNF4α and TET2. The results showed that metformin administration impaired HNF4α's ability to bind to TET2 (Fig. 5A), leading to a significant reduction in TET2 binding to the FBP1 promoter (Fig. 5B). Consistently, metformin treatment significantly decreased the expression level of FBP1 (Fig. 5C). Notably, metformin also induced high levels of HNF4α phosphorylation at Ser 313 (Fig. 5C). To determine the effect of HNF4α phosphorylation on HNF4α-TET2-FBP1 axis, we transfected HepG2 cells with wild type HNF4α and Ser 313 mutants. The results showed that the phosphomimetic mutation (S313D) of HNF4α impaired its ability to bind to TET2 (Fig. 5D), prevented TET2 from binding to the FBP1 promoter (Fig. 5E), and reduced FBP1 expression at both mRNA and protein levels (Fig. 5F). In contrast, the phosphoresistant mutation (S313A) showed higher activity in interacting with TET2, recruiting TET2 to the FBP1

promoter, and activating its expression (Fig. 5D-F). Taken together, these data demonstrate that metformin-mediated HNF4α phosphorylation suppresses FBP1 expression by preventing TET2 recruitment to the FBP1 promoter by HNF4α.

Targeting TET2 improves the efficacy of metformin in glucose metabolism *in vivo*

Increased rates of gluconeogenesis, derived from continuously excessive hepatic glucose production (HGP), result in abnormal glucose homeostasis in type 2 diabetes (T2D). Fasting or high-fat diet (HFD)-induced T2D mice showed increased levels of TET2 and FBP1 in hepatocytes (Fig. 1A-E). However, it remains unclear whether knockdown of TET2, in combination with metformin, would decrease glucose production, thereby improving T2D treatment outcomes. HFD-induced diabetic mice were infused with AAV-scr or AAV-si*Tet2*. To confirm the efficiency of *Tet2* knockdown *in vivo*, *Tet2* mRNA levels in mouse livers were examined, revealing that TET2 expression significantly decreased upon AAV-si*Tet2* treatment (Fig. 6A). Notably, fasting blood glucose and insulin levels were markedly lower in siTet2 group than the control group in response to metformin treatment (Fig. 6B and C). PTT performed on HFD mice indicated that TET2 suppression sharply decreased hepatic gluconeogenesis (Fig. 6D), which was consistent with lower protein levels of FBP1 in mouse livers (Fig. 6E). Moreover, GTT and ITT showed that *Tet2* knockdown in HFD mice significantly enhanced glucose tolerance and insulin sensitivity compared to AAV-scr-infused mice (Fig. 6F and G). Of note, the beneficial effects of *Tet2* knockdown alone were comparable to those of metformin in lowering glucose levels and improving insulin sensitivity from a therapeutic perspective. Interestingly, the combination of metformin

and *Tet2* knockdown in HFD mice exhibited better glucose-lowering effects and insulin sensitivity than either *Tet2* knockdown or metformin alone. Collectively, these data demonstrated that suppressing TET2 synergizes with metformin to lower glucose production and enhance insulin sensitivity by inhibiting FBP1 expression.

Discussion

Our study revealed the function of TET2 in the regulation of gluconeogenesis. Notably, gluconeogenesis levels in *Tet2* knockout HepG2 cells and primary mouse hepatocytes significantly decreased even under glucagon treatment, demonstrating that TET2 is required for glucagon-induced upregulation of glucose output. Our findings link the novel function of TET2 to the gluconeogenic process. However, the role of TET2 in the pathophysiology of type 2 diabetes (T2D) remains unclear and requires further research. Additionally, the mechanisms by which TET2 is upregulated during gluconeogenesis deserve further exploration.

A recent study showed that metformin treatment can decrease liver glucose production by targeting FBP1 (3), suggesting that FBP1 may be a promising target for ameliorating diabetes. However, the regulation of FBP1 is poorly understood, although several studies have reported that the promoter methylation mediates FBP1 expression silencing in cancer (20, 21). Here, we found that TET2 positively regulates FBP1 upon glucagon treatment, indicating that targeting TET2 may be a promising strategy for treating T2D.

We further explored how TET2 specifically regulates FBP1 expression in response

to glucagon stimulation. Like most chromatin-modifying enzymes, which require DNA sequence-specific binding proteins, such as DNA transcription factors, to help them be recruited and regulate specific gene expression (22), TET2 also requires binding partners to regulate particular pathways in a context-dependent manner. This is supported by findings that Wilms tumor protein (WT1) (23) and Smad Nuclear Interacting Protein 1 (SNIP1) (24) are indispensable for TET2 to suppress leukemia cell proliferation and regulate the cellular DNA damage response, respectively. Our data demonstrated that TET2 upregulates FBP1 and further increases gluconeogenesis in an HNF4α-dependent manner. Furthermore, metformin-induced HNF4α phosphorylation at Ser 313 impairs its binding ability to TET2 and reduces TET2 recruitment to the FBP1 promoter, leading to decreased FBP1 expression. More importantly, TET2-FBP1 inhibition has a synergistic effect with metformin in HFD mice.

Intriguingly, a clinical investigation study revealed that TET2 mutation occurred more frequently in the diabetes mellitus (DM) group than the non-DM, which suggested TET2 might be connected with insulin resistance (25). Additionally, inactivating mutations of epigenetic regulator TET2 led to metabolic dysfunction including clonal hematopoiesis, aggravate age- and obesity-related insulin resistance in mice (26). For the HNF4α variants, an analysis study using exome sequencing data showed that human genetic variations in HNF4α destroyed the protein structure and function, impaired insulin secretion and reduced sensitivity to insulin, and increased the risk of T2D in individuals (27, 28). Furthermore, it was reported that a point mutation in FBP1 can reduce the efficacy of metformin treatment (3), providing a genetic evidence for the

role of TET2-FBP1 axis in the therapeutic effect of metformin. In summary, our findings uncovered a previously unknown function of TET2 in gluconeogenesis. TET2, together with HNF4α, facilitates FBP1 expression by maintaining the hypomethylation of the FBP1 promoter, which leads to an increase in the gluconeogenic process. Thus, targeting the HNF4α-TET2-FBP1 axis may be a potential strategy to lower blood glucose in T2D.

Methods

Cell Culture and Transfection

HepG2, 293T, and primary mouse hepatocytes were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and incubated at 37°C in a 5% CO2 atmosphere. For transfection with Tet2 plasmids or siRNA, FuGENE HD (Roche) and Lipofectamine 2000 (Invitrogen) were utilized respectively. For lentivirus production, polyethyleneimine (PEI) from EZ Trans, Liji Shengwu, was the preferred agent. All transfection procedures were conducted according to the manufacturers' instructions. The sequences of HNF4α siRNA were: 5'-AAUGUAGUCAUUGCCUAGGTT-3' and 5'-UCUUGUCUUUGUCCACCACTT-3' (21).

Isolation of Primary Mouse Hepatocytes

Primary mouse hepatocytes were isolated by perfusing the liver with pre-warmed Hank's Balanced Salt Solution (HBSS) at a flow rate of 5-7 mL/minute for 5 minutes after anesthetizing the mouse. Once the liver appeared pale, the perfusion solution was

switched to a digestion buffer, maintained at the same flow rate and duration. The liver was then transferred to a 10 cm dish containing 10 mL of digestion medium. Hepatocytes were released by gently cutting and agitating the liver, after which 20 mL of cold PBS was added to halt the trypsin digestion. The cell suspension was filtered through a 70 μm nylon mesh (BD Falcon), centrifuged at 50g for 2 minutes, and the cell pellet was washed twice with cold PBS at 50g for 5 minutes each. The hepatocytes were then ready for subsequent experiments.

qPCR and ChIP-qPCR

Total RNA was extracted using an RNA purification kit (B0004D, EZBioscience). Real-time PCR was conducted using SYBR Green following cDNA synthesis. β-actin was used as an internal control. Chromatin immunoprecipitation (ChIP) assays (Millipore, 17-408) were carried out per the manufacturer's instructions. Chromatin was sheared by sonication at 4°C. Two micrograms of TET2 antibody (Millipore, MABE 462) were employed to immunoprecipitate chromatin fragments. The ChIP-enriched DNA was subsequently analyzed by qPCR. Primer sequences are provided in the supplementary data.

Western Blot and Immunoprecipitation

Cell lysates were prepared using NP40 lysis buffer. Proteins were separated by SDS-PAGE. The following primary antibodies were used: Tubulin (Proteintech, 66031-1-Ig), TET2 (CST, 18950S), HNF4α (SAB, 32591), FBP1 (Sigma, HPA005857), and HA (Shanghai Genomics Technology). Secondary antibodies included anti-rabbit (SAB,

L3012) and anti-mouse (SAB, L3032). For immunoprecipitation (10), total protein was incubated with protein A beads (Shanghai Genomics Technology) and TET2 antibody (Millipore, MABE 462) for 3 hours at 4°C, followed by western blot analysis.

Gene Knockout

The CRISPR/Cas9 system was utilized to generate Tet2 knockout cells. Lentiviruses were produced by co-transfecting HEK293T cells with plasmids containing sgRNA (8μg), psPAX2 (6μg), and pMD2.G (2μg) in a 10 cm dish. The collected supernatant was used to infect the target cells. Following selection with puromycin, Western blot analysis was conducted to assess the efficiency of the knockout. The targeting sequence for TET2 sgRNA was 5'-GATTCCGCTTGGTGAAAACG-3'.

Immunofluorescence

For immunofluorescence, antibodies against TET2 and HNF4α were combined and applied to the slides, which were then incubated at 4°C overnight. Subsequently, the slides were stained with secondary antibodies (Alexa Fluor 555 and 488), followed by DAPI staining. Confocal images were captured using a Leica fluorescence microscope.

Glucose Production

Cells were washed three times with PBS before the medium was replaced with glucose-free DMEM (Gibco, A14430-01) supplemented with 20 mM sodium lactate and 2 mM sodium pyruvate. After an 8-hour incubation, glucose levels in the supernatants were measured using a glucose assay kit (ThermoFisher, A22189).

Pyruvate Tolerance Test (PTT), Glucose Tolerance Test (GTT), and Insulin Tolerance Test (ITT)

For the PTT and GTT, mice underwent fasting for 16 hours and 12 hours, respectively, before receiving an intraperitoneal (i.p.) injection of either pyruvate (2g/kg) or glucose (2g/kg). In the ITT, mice fasted for 6 hours in the morning prior to receiving an insulin injection (1U/kg, i.p.). Blood glucose levels were measured at intervals of 0, 15, 30, 45, 60, 90, and 120 minutes after injection using a glucometer during tail vein bleeding.

Animal Study Protocols

All experimental protocols involving animals were evaluated and approved by the Fudan University Animal Care and Use Committee and conducted according to the guidelines. Male C57BL/6J mice, aged 6-8 weeks, were utilized for this study. These mice were housed in specific-pathogen-free (SPF) conditions at 25°C with a 12-hour light/dark cycle and were fed either a normal chow or a high-fat diet (HFD) (Research Diets, 45% calories from fat). To induce insulin resistance, wild-type C57BL/6J mice were placed on a HFD for 2 weeks. For the experiments with AAV-scr or AAV-siTet2, HFD mice were infected with the respective virus for 10 days.

Statistical Analysis

The Student's t-test was used for comparing two groups, while one-way ANOVA followed by Tukey's post hoc test was conducted for multiple comparisons when more than two groups were analyzed. Data analysis was performed using SPSS or GraphPad Prism. A p-value of less than 0.05 was considered statistically significant.

References

1. Basu R, Barosa C, Jones J, Dube S, Carter R, Basu A, et al. Pathogenesis of prediabetes: role of the liver in isolated fasting hyperglycemia and combined fasting and postprandial hyperglycemia. . *The Journal of clinical endocrinology and metabolism.* 2013;98(3):E409–E17.
2. Magnusson I, Rothman DL, Katz LD, Shulman RG, and Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *The Journal of clinical investigation.* 1992;90(4):1323–7.
3. Hunter RW, Hughey CC, Lantier L, Sundelin EI, Peggie M, Zeqiraj E, et al. Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nature medicine.* 2018;24(9):1395–406.
4. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science (New York, NY).* 2009;324(5929):930–5.
5. He Y-F, Li B-Z, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science (New York, NY).* 2011;333(6047):1303–7.
6. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science (New York, NY).* 2011;333(6047):1300–3.
7. Hirata H, Sugimachi K, Komatsu H, Ueda M, Masuda T, Uchi R, et al. Decreased Expression of Fructose-1,6-bisphosphatase Associates with Glucose Metabolism and Tumor Progression in Hepatocellular Carcinoma. *Cancer research.* 2016;76(11):3265–76.
8. Dong C, Yuan T, Wu Y, Wang Y, Fan TWM, Miriyala S, et al. Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer cell.* 2013;23(3):316–31.
9. Kosmider O, Gelsi-Boyer V, Ciudad M, Racoeur C, Jooste V, Vey N, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. *Haematologica.* 2009;94(12):1676–81.
10. Delhommeau F, Dupont S, Valle VD, James C, Trannoy S, Massé A, et al. Mutation in TET2 in myeloid cancers. *New England journal of medicine.* 2009;360(22):2289-301.
11. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, et al. Restoration of TET2 Function Blocks Aberrant Self-Renewal and Leukemia Progression. *Cell.* 2017;170(6):1079–95.e20.
12. He J, Lin M, Zhang X, Zhang R, Tian T, Zhou Y, et al. TET2 is required to suppress mTORC1 signaling through urea cycle with therapeutic potential. *Cell discovery.* 2023;9(1):84.
13. Zhang X, Li S, He J, Jin Y, Zhang R, Dong W, et al. TET2 Suppresses VHL Deficiency-Driven Clear Cell Renal Cell Carcinoma by Inhibiting HIF Signaling. *Cancer research.* 2022;82(11):2097–109.
14. Tang Z, Li C, Kang B, Gao G, Li C, and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic acids research.* 2017;45(W1):W98–W102.
15. Zhang N, Geng T, Wang Z, Zhang R, Cao T, Camporez JP, et al. Elevated hepatic expression of H19 long noncoding RNA contributes to diabetic hyperglycemia. *JCI insight.* 2018;3(10):e120304.
16. Rhee J, Inoue Y, Yoon JC, Puigserver P, Fan M, Gonzalez FJ, et al. Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(7):4012–7.

17. Alder O, Cullum R, Lee S, Kan AC, Wei W, Yi Y, et al. Hippo signaling influences HNF4A and FOXA2 enhancer switching during hepatocyte differentiation. *Cell reports.* 2014;9(1):261–71.
18. Aroda VR, Knowler WC, Crandall JP, Perreault L, Edelstein SL, Jeffries SL, et al. Metformin for diabetes prevention: insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. *Diabetologia.* 2017;60(9):1601–11.
19. Hong YH, Varanasi US, Yang W, and Leff T. AMP-activated protein kinase regulates HNF4alpha transcriptional activity by inhibiting dimer formation and decreasing protein stability. *The Journal of biological chemistry.* 2003;278(30):27495–501.
20. Dong Y, Huaying S, Danying W, Chihong Z, Ruibin J, Xiaojiang S, et al. Significance of Methylation of FBP1 Gene in Non-Small Cell Lung Cancer. *BioMed research international.* 2018;2018:3726091.
21. Chen M, Zhang J, Ning Li, Qian Z, Zhu M, Li Q, et al. Promoter hypermethylation mediated downregulation of FBP1 in human hepatocellular carcinoma and colon cancer. *PloS one.* 2011;6(10):e25564.
22. Smith E, and Shilatifard A. The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. *Molecular cell.* 2010;40(5):689–701.
23. Wang Y, Xiao M, Chen X, Chen L, Xu Y, Lv L, et al. WT1 recruits TET2 to regulate its target gene expression and suppress leukemia cell proliferation. *Molecular cell.* 2015;57(4):662–73.
24. Chen L-L, Lin H-P, Zhou W-J, He C-X, Zhang Z-Y, Cheng Z-L, et al. SNIP1 Recruits TET2 to Regulate c-MYC Target Genes and Cellular DNA Damage Response. *Cell reports.* 2018;25(6):1485–500.e4.
25. Xu F, Jin J, Guo J, Xu F, Chen J, Liu Q, et al. The clinical characteristics, gene mutations and outcomes of myelodysplastic syndromes with diabetes mellitus. *Journal of cancer research and clinical oncology.* 2024;150(2):71.
26. Fuster JJ, Zuriaga MA, Zorita V, MacLauchlan S, Polackal MN, Viana-Huete V, et al. TET2-Loss-of-Function-Driven Clonal Hematopoiesis Exacerbates Experimental Insulin Resistance in Aging and Obesity. *Cell reports.* 2020;33(4):108326.
27. Ellard S, and Colclough K. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1 alpha (HNF1A) and 4 alpha (HNF4A) in maturity-onset diabetes of the young. *Human mutation.* 2006;27(9):854–69.
28. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, et al. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature.* 1996;384(6608):458–60.
29. Tang Z, Li C, Kang B, Gao G, Li C, and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 2017;45(W1):W98-W102.

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Figure legends

Figure 1, TET2 expression increases in fasting and HFD mouse livers.

A, B, qRT-PCR analysis of TET2 mRNA levels in mouse livers after 16 hours overnight fasting (A) or 11 days high-fat diet (B). n=7. **C-D,** WB analysis of TET2 protein levels in mouse livers following the treatment described in A and B (C and D). n=5. **E**, WB analysis of TET2 protein levels in mouse livers after 12 weeks high-fat diet. n=5.

Figure 2, TET2 boosts gluconeogenesis.

A-D, Glucose production assays were performed after TET2 overexpression in HepG2 cells (A) and mouse primary hepatocytes (B), or pre-treated with glucagon in WT and TET2 KO HepG2 cells (C) and mouse primary hepatocytes (D). **E-G,** PTT(E), GTT(F) and ITT(G) were performed in WT and Tet2 KO mice as described in methods.

Figure 3, TET2 up-regulates FBP1 expression in liver cells

A, B, qRT-PCR were performed to analyze TET2 and FBP1 expression levels after glucagon (20nM) treatment for 48 hours in HepG2 cells (A) and primary mouse hepatocytes (B). **C**, WB analysis of Tet2 and Fbp1 protein levels after glucagon (20nM) treatment in mouse primary liver cells. **D, E,** qRT-PCR analysis of Fbp1 mRNA levels in mouse livers following the treatment described in Figure 1A (D) and 1B (E). **F**, Data from Figure 1A were reused to analyze the correlation between Tet2 and Fbp1 levels in mouse livers with or without the treatment. **G**, The correlation between TET2 and FBP1 levels in human livers was analyzed. Data were collected from GEPIA(29). **H**, WB analysis was performed to detect TET2 and FBP1 expression after overexpression

TET2 in HepG2 cells and mouse primary hepatocytes. **I, J**, qRT-PCR (I) and WB (J) analysis of FBP1 expression in CRISPR/Cas9-mediated TET2 knockout liver cells. **K**, TET2 and FBP1 protein levels were determined by WB in WT and Tet2 KO mouse primary hepatocytes and HepG2 cells treated with or without glucagon. **L-N**, TET2(L), 5hmC(M) and 5mC(N) ChIP-qPCR were performed to measure the ability of TET2 binding to and demethylating FBP1 promoter in response to glucagon stimulation. Each ChIP Ct value is normalized to IgG Ct value.

Figure 4, HNF4α is necessary for TET2 mediated FBP1 up-regulation

A, Immunofluorescence analysis of TET2 and HNF4α co-localization in HepG2 cells after glucagon treatment for 48 h. **B**, The interaction between TET2 and HNF4α was determined in HepG2 cells treated with or without glucagon using IgG and TET2 antibody followed by WB. **C**, Two siRNAs targeting HNF4α were transfected into HepG2 and the knockdown efficiency were determined by qRT-PCR and WB analysis. **D**, ChIP analysis of TET2 binding to FBP1 promoter was performed following the treatment with siRNA targeting HNF4α and glucagon as indicated. **E**, FBP1 protein levels were examined by WB after the treatment with TET2 overexpression and siRNA targeting HNF4α as indicated. **F**, Glucose production levels were examined after the treatment with glucagon and HNF4α siRNA. **G-I**, Hnf4α and Fbp1 protein levels in mouse livers were examined by WB after the treatment with 16 hours overnight fasting (G) or 11 days high-fat diet (H), or 12 weeks high-fat diet (I). n=5.

Figure 5, Metformin impairs the ability of HNF4α binding to TET2 and FBP1

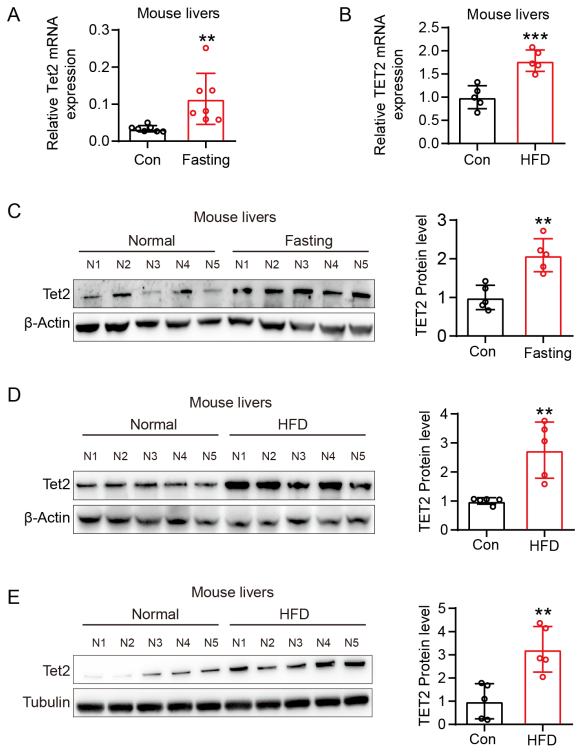
expression

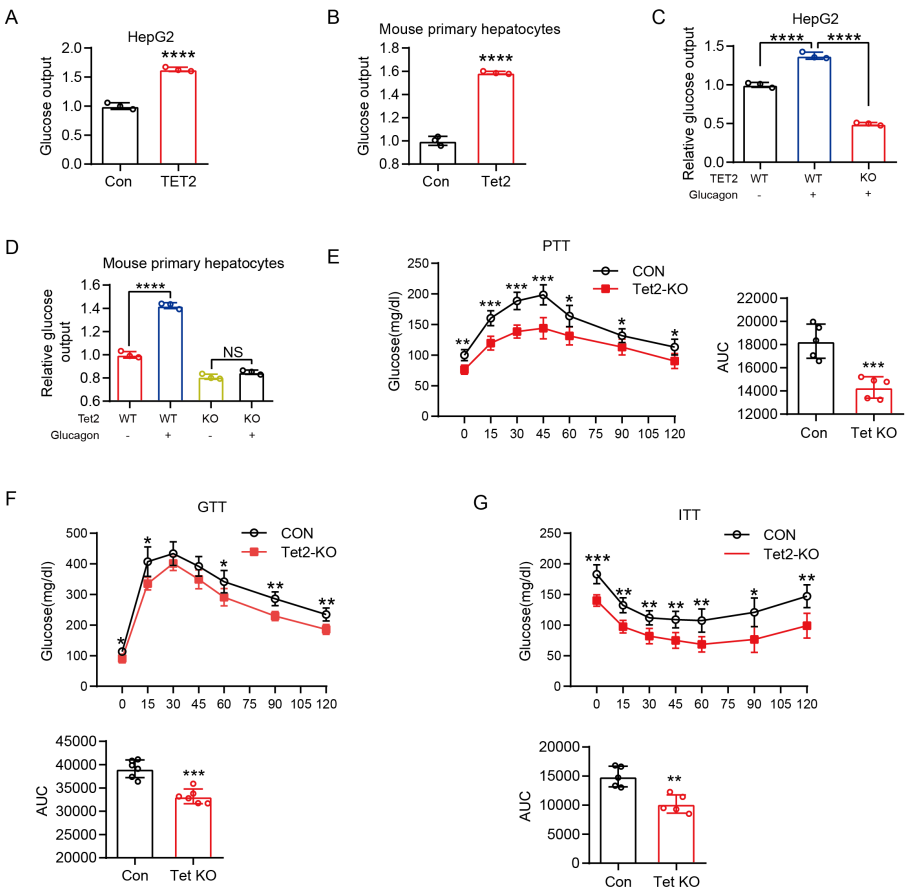
A, The interaction between HNF4α and TET2 with or without metformin treatment was determined by immunoprecipitation using IgG and TET2 antibody followed by WB. **B**, TET2 ChIP-qPCR was used to detect the ability of TET2 binding to FBP1 promoter with or without metformin treatment. **C**, WB analysis of HNF4α, HNF4α phosphorylation at Ser 313 and FBP1 levels after metformin treatment. +:5mM, ++：10mM. **D**, The interaction between TET2 and HNF4α wildtype and mutants was determined by WB. **E**, TET2 ChIP-qPCR was performed to determine the binding of TET2 to FBP1 promoter after transfection of HNF4α wildtype and mutants as indicated. **F**, qRT-PCR and WB analysis of FBP1 levels after transfection of HNF4α wildtype and mutants as indicated.

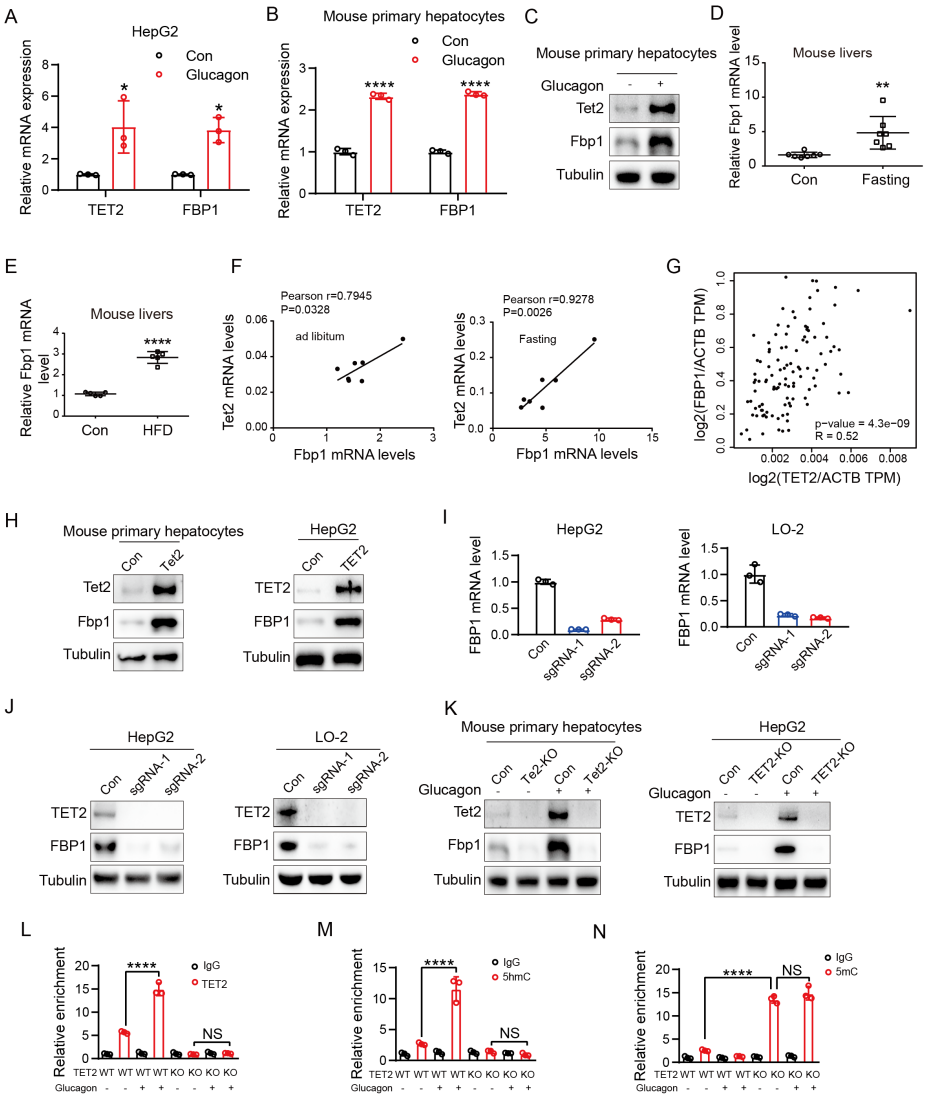
Figure 6, Targeting TET2 improves the efficacy of metformin in glucose metabolism *in vivo*

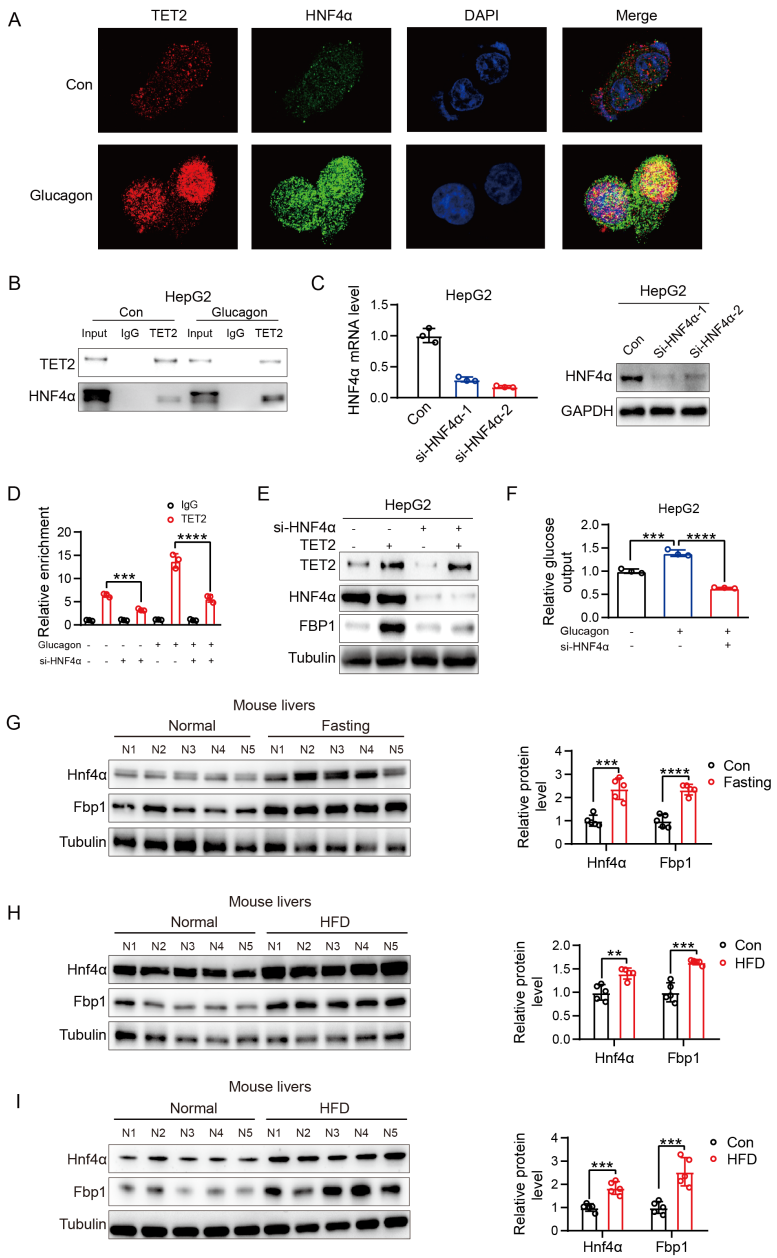
A, The mRNA levels of *Tet2* were examined by qPCR in HFD mice infected with AAV-scr or AAV-*siTet2* for 10 days. **B**, **C**, Fasting blood glucose (B) and insulin (C) levels were determined in HFD mice infected with AAV-scr or AAV-*siTet2* for 10 days, and treated with or without metformin for another 10 days. n = 6. **D**, PTT was examined in HFD mice infected with AAV-scr or AAV-*siTet* for 10 days, and treated with or without metformin for another 10 days. n = 6. **E**, WB analysis of Tet2 and Fbp1 levels in livers of HFD mice infected with AAV-scr or AAV-*siTet,* and treated with or without metformin for another 10 days. n = 6. **F**, **G,** GTT (F) and ITT (G) were examined in HFD mice infected with AAV-scr or AAV-*siTet* for 10 days, and treated with or without

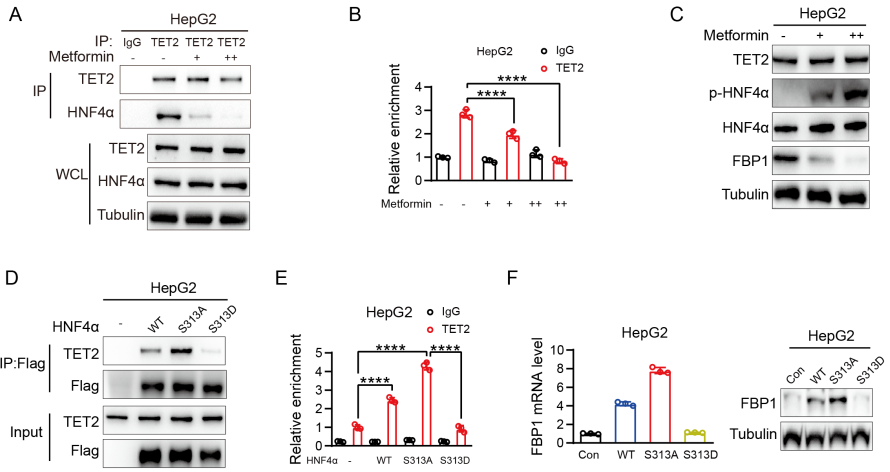
metformin for another 10 days. n = 6.

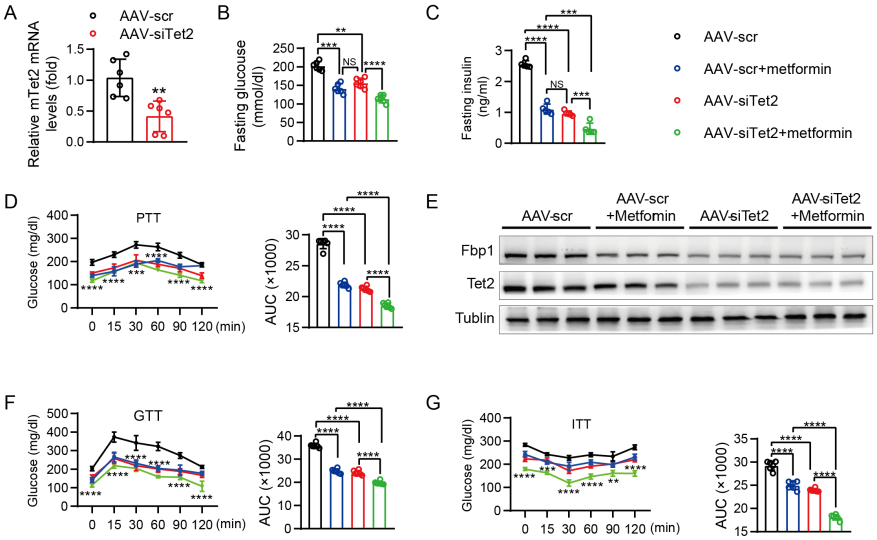

A
Mouse livers
Relative Tet2 mRNA expression
0.3
0.2
0.1
0.0
**
Con
Fasting
B
Mouse livers
Relative TET2 mRNA expression
2.5
2.0
1.5
1.0
0.5
0.0
***
Con
HFD
C
Mouse livers
Normal
Fasting
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Tet2
β-Actin
TET2 Protein level
3
2
1
0
**
Con
Fasting
D
Mouse livers
Normal
HFD
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Tet2
β-Actin
TET2 Protein level
4
3
2
1
0
**
Con
HFD
E
Mouse livers
Normal
HFD
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Tet2
Tubulin
TET2 Protein level
5
4
3
2
1
0
**
Con
HFD


A
HepG2
Glucose output
2.0
1.5
1.0
0.5
0.0
****
Con
TET2
B
Mouse primary hepatocytes
Glucose output
1.8
1.6
1.4
1.2
1.0
0.8
****
Con
Tet2
C
HepG2
Relative glucose output
1.5
1.0
0.5
0.0
****
****
TET2 WT WT KO
Glucagon - + +
D
Mouse primary hepatocytes
Relative glucose output
1.6
1.4
1.2
1.0
0.8
0.6
****
NS
Tet2 WT WT KO KO
Glucagon - + - +
E
PTT
CON
Tet2-KO
Glucose(mg/dl)
250
200
150
100
50
0
0 15 30 45 60 75 90 105 120
AUC
22000
20000
18000
16000
14000
12000
***
Con
Tet KO
F
GTT
CON
Tet2-KO
Glucose(mg/dl)
500
400
300
200
100
0
0 15 30 45 60 75 90 105 120
AUC
45000
40000
35000
30000
25000
20000
***
Con
Tet KO
G
ITT
CON
Tet2-KO
Glucose(mg/dl)
250
200
150
100
50
0
0 15 30 45 60 75 90 105 120
AUC
20000
15000
10000
5000
0
**
Con
Tet KO


A
HepG2
Con
Glucagon
Relative mRNA expression
TET2
FBP1
B
Mouse primary hepatocytes
Con
Glucagon
Relative mRNA expression
TET2
FBP1
C
Mouse primary hepatocytes
Glucagon
Tet2
Fbp1
Tubulin
D
Mouse livers
Relative Fbp1 mRNA level
Con
Fasting
E
Mouse livers
Relative Fbp1 mRNA level
Con
HFD
F
Pearson r=0.7945
P=0.0328
ad libitum
Tet2 mRNA levels
Fbp1 mRNA levels
Pearson r=0.9278
P=0.0026
Fasting
Tet2 mRNA levels
Fbp1 mRNA levels
G
log2(FBP1/ACTB TPM)
p-value = 4.3e-09
R = 0.52
log2(TET2/ACTB TPM)
H
Mouse primary hepatocytes
Con
Tet2
Tet2
Fbp1
Tubulin
HepG2
Con
TET2
TET2
FBP1
Tubulin
I
HepG2
FBP1 mRNA level
Con
sgRNA-1
sgRNA-2
LO-2
FBP1 mRNA level
Con
sgRNA-1
sgRNA-2
J
HepG2
Con
sgRNA-1
sgRNA-2
TET2
FBP1
Tubulin
LO-2
Con
sgRNA-1
sgRNA-2
TET2
FBP1
Tubulin
K
Mouse primary hepatocytes
Con
Tet2-KO
Con
Tet2-KO
Glucagon
Tet2
Fbp1
Tubulin
HepG2
Con
TET2-KO
Con
TET2-KO
Glucagon
TET2
FBP1
Tubulin
L
Relative enrichment
IgG
TET2
NS
TET2 WT WT WT WT KO KO KO KO
Glucagon
M
Relative enrichment
IgG
5hmC
NS
TET2 WT WT WT WT KO KO KO KO
Glucagon
N
Relative enrichment
IgG
5mC
NS
TET2 WT WT WT WT KO KO KO KO
Glucagon


A
TET2
HNF4α
DAPI
Merge
Con
Glucagon
B
HepG2
Con
Glucagon
Input IgG TET2 Input IgG TET2
TET2
HNF4α
C
HepG2
HNF4α mRNA level
Con
si-HNF4α-1
si-HNF4α-2
HepG2
Con
Si-HNF4α-1
Si-HNF4α-2
HNF4α
GAPDH
D
IgG
TET2
Relative enrichment
Glucagon
si-HNF4α
E
HepG2
si-HNF4α
TET2
TET2
HNF4α
FBP1
Tubulin
F
HepG2
Relative glucose output
Glucagon
si-HNF4α
G
Mouse livers
Normal
Fasting
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Hnf4α
Fbp1
Tubulin
Relative protein level
Con
Fasting
Hnf4α
Fbp1
H
Mouse livers
Normal
HFD
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Hnf4α
Fbp1
Tubulin
Relative protein level
Con
HFD
Hnf4α
Fbp1
I
Mouse livers
Normal
HFD
N1 N2 N3 N4 N5 N1 N2 N3 N4 N5
Hnf4α
Fbp1
Tubulin
Relative protein level
Con
HFD
Hnf4α
Fbp1


A
HepG2
IP: IgG TET2 TET2 TET2
Metformin - - + ++
IP
TET2
HNF4α
WCL
TET2
HNF4α
Tubulin
B
HepG2
IgG
TET2
Relative enrichment
****
****
Metformin - - + + ++ ++
C
HepG2
Metformin - + ++
TET2
p-HNF4α
HNF4α
FBP1
Tubulin
D
HepG2
HNF4α - WT S313A S313D
IP:Flag
TET2
Flag
Input
TET2
Flag
E
HepG2
IgG
TET2
Relative enrichment
****
****
****
HNF4α - WT S313A S313D
F
HepG2
FBP1 mRNA level
Con WT S313A S313D
HepG2
Con WT S313A S313D
FBP1
Tubulin


A
AAV-scr
AAV-siTet2
Relative mTet2 mRNA levels (fold)
B
Fasting glucouse (mmol/dl)
C
Fasting insulin (ng/ml)
AAV-scr
AAV-scr+metformin
AAV-siTet2
AAV-siTet2+metformin
D
PTT
Glucose (mg/dl)
AUC (×1000)
E
AAV-scr
AAV-scr +Metfomin
AAV-siTet2
AAV-siTet2 +Metformin
Fbp1
Tet2
Tublin
F
GTT
G
ITT
(min)