iScience, Volume *25*

Supplemental information

Deficiency of peroxisomal NUDT7 stimulates *de novo* lipogenesis in hepatocytes

Jinsoo Song, In-Jeoung Baek, Sujeong Park, Jinjoo Oh, Deokha Kim, Kyung Song, Mi Kyung Kim, Hye Won Lee, Byoung Kuk Jang, and Eun-Jung Jin

Supplement Information

Deficiency of peroxisomal NUDT7 stimulates de novo lipogenesis in hepatocytes

Jinsoo Song[1,2], In-Jeoung Baek[3], Sujeong Park[1], Jinjoo Oh[1], Deokha Kim[1], Kyung Song[4], Mi Kyung Kim[5], Hye Won Lee[6], Byoung Kuk Jang[5], Eun-Jung Jin[1, 2, 7*]

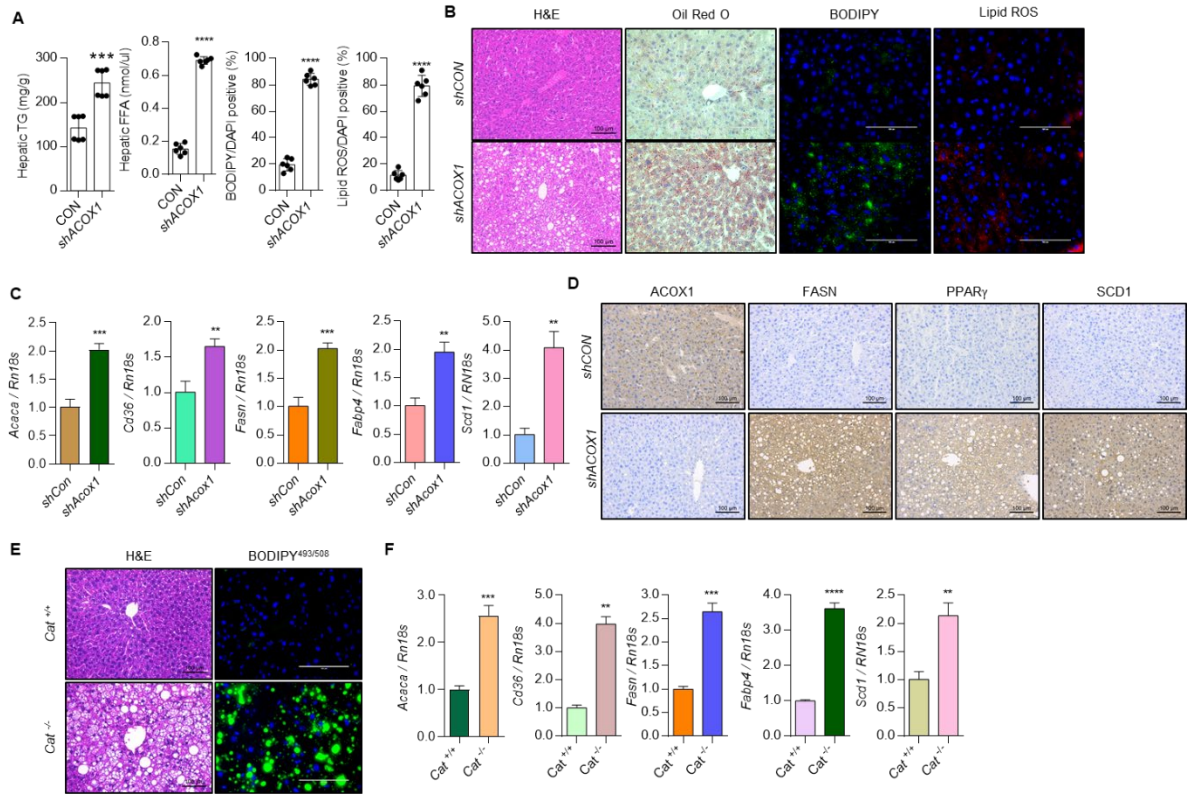

Figure S1. Peroxisome dysfunction induces hepatic lipid accumulation, related to Figure 1.

(A) Positive percentage of TG, FFA, BODIPY and Lipid ROS in *shCON* and *shAcox1* mouse liver (n = 6). (B) Representative images of H&E, Oil Red , BODIPY, and Lipid ROS staining in *shCON* and *shAcox1* mouse liver (n = 5; Scale bars, 100 μm). (C) Expression levels of *Acaca*, *Cd36*, *Fasn*, *Fabp4*, and *Scd1* of *shCon* and *shAcox1* mouse liver (n = 3). (D) Representative images of ACOX1, FASN, PPARγ, and SCD1 staining (n = 3; Sale bars, 100 μm). (E) Representative images of H&E and BODIPY staining in $Cat^{+/+}$ and $Cat^{-/-}$ mouse liver (n = 3; Scale bars, 100 μm). (F) Expression levels of *Acaca, CD36, Fasn, Fabp4* and *Scd1* in $Cat^{+/+}$ and $Cat^{-/-}$ mouse liver (n = 3). Values were expressed as means + s.d. An unpaired *t*-test was used for statistical analysis. **P < 0.01, ***P < 0.001, ****P < 0.0001.

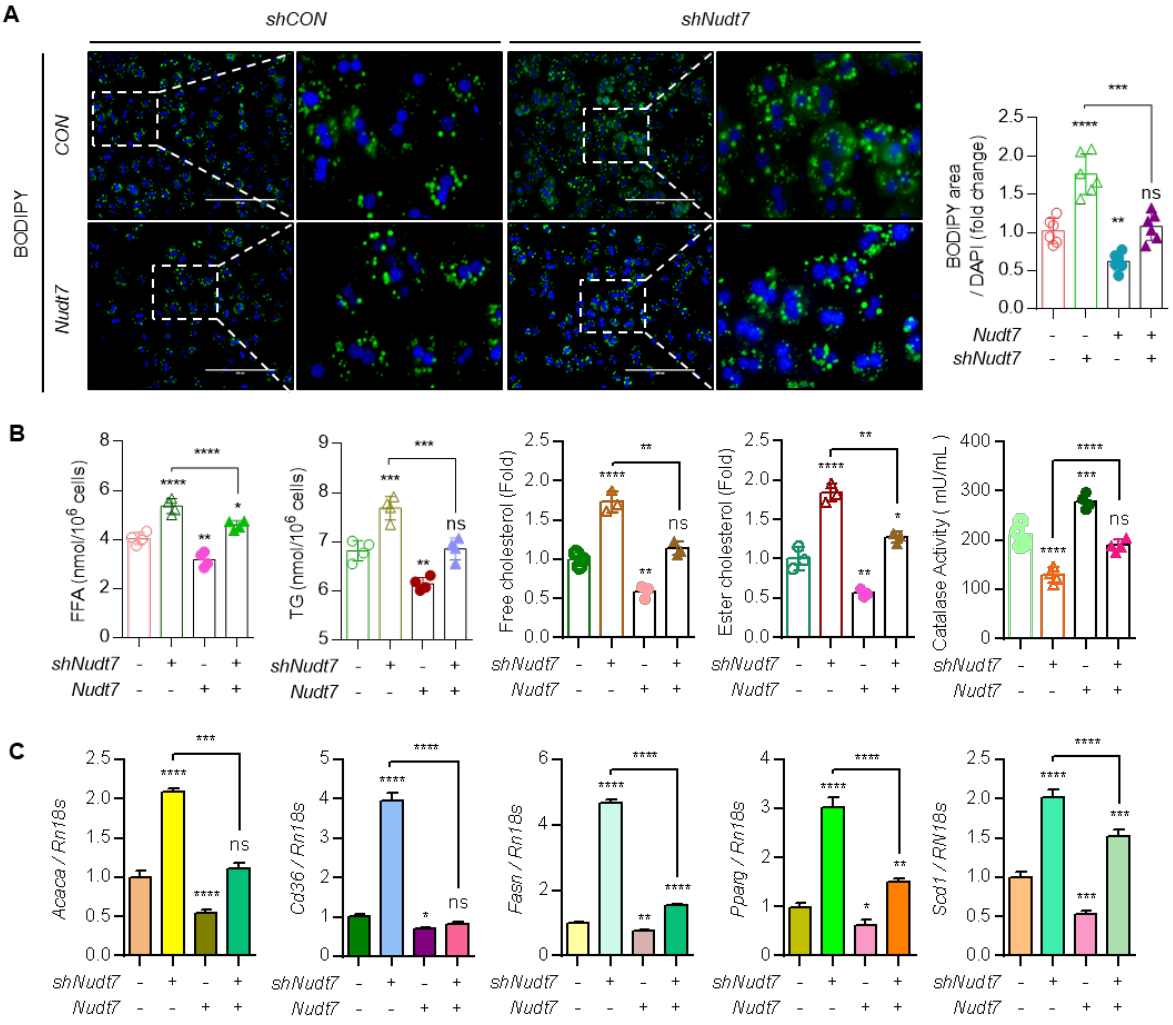

Figure S2. In-vitro modulation of Nudt7 alters lipid accumulation, related to Figure 2.

(A) Representative image of BODIPY staining in *shNudt7* or *Nudt7* hepatocytes and bar graph of BODIPY positive percentage was represented as the fold of CON (n=5). (B) Analysis of FFA (n = 4), TG (n = 4), free cholesterol (n = 3), ester cholesterol (n = 3) levels, and catalase activity (n = 4). (C) Expression levels of *Acaca, Cd36, Fasn, Pparg,* and *Scd1* were analyzed using qRT-PCR (n = 3). Values were expressed as means + s.d. An unpaired Student's t-test and one-way ANOVA multiple test were used for statistical analysis. ns = non-significant, *P ≤ 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

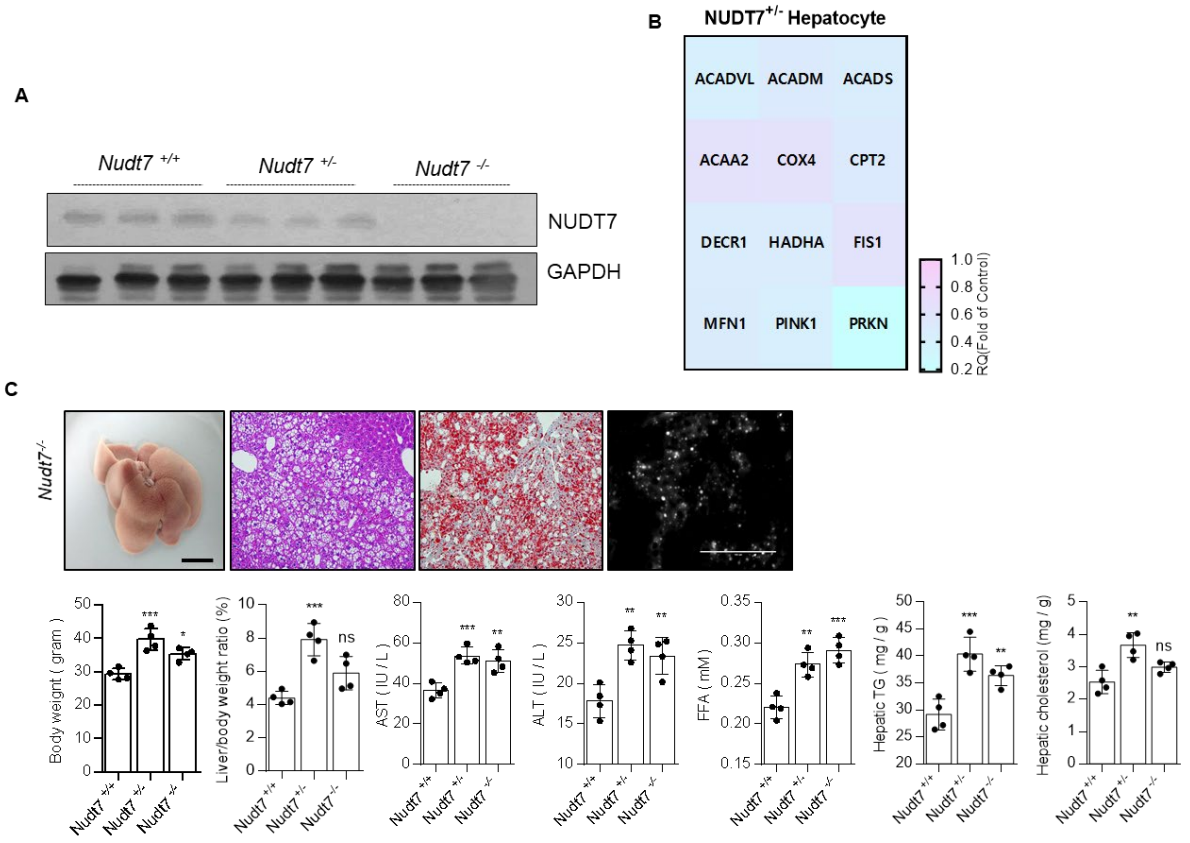

Figure S3. *Nudt7*$^{-/-}$ mice induces hepatic lipid accumulation, related to Figure 3.

(A) The expression level of NUDT7 was analyzed by immunoblotting in *Nudt7*$^{+/+}$, *Nudt7*$^{+/-}$, and *Nudt7*$^{-/-}$ liver (n = 3; GAPDH was used for loading control). (B) The expression level of mitochondria related genes was analyzed by qRT-PCR (n = 3). (C) Representative images of liver, H&E, Oil Red O, and Filipin staining of *Nudt7*$^{-/-}$ NCD liver (n = 4). The weight of body and liver, and the level of AST, ALT, FFA, TG, and cholesterol were analyzed in *Nudt7*$^{+/+}$, *Nudt7*$^{+/-}$, and *Nudt7*$^{-/-}$ mouse (n = 4). Values were expressed as means + s.d. An unpaired Student's t-test and one-way ANOVA multiple test were used for statistical analysis. ns = non-significant, *P ≤ 0.05, **P < 0.01, ***P < 0.001.

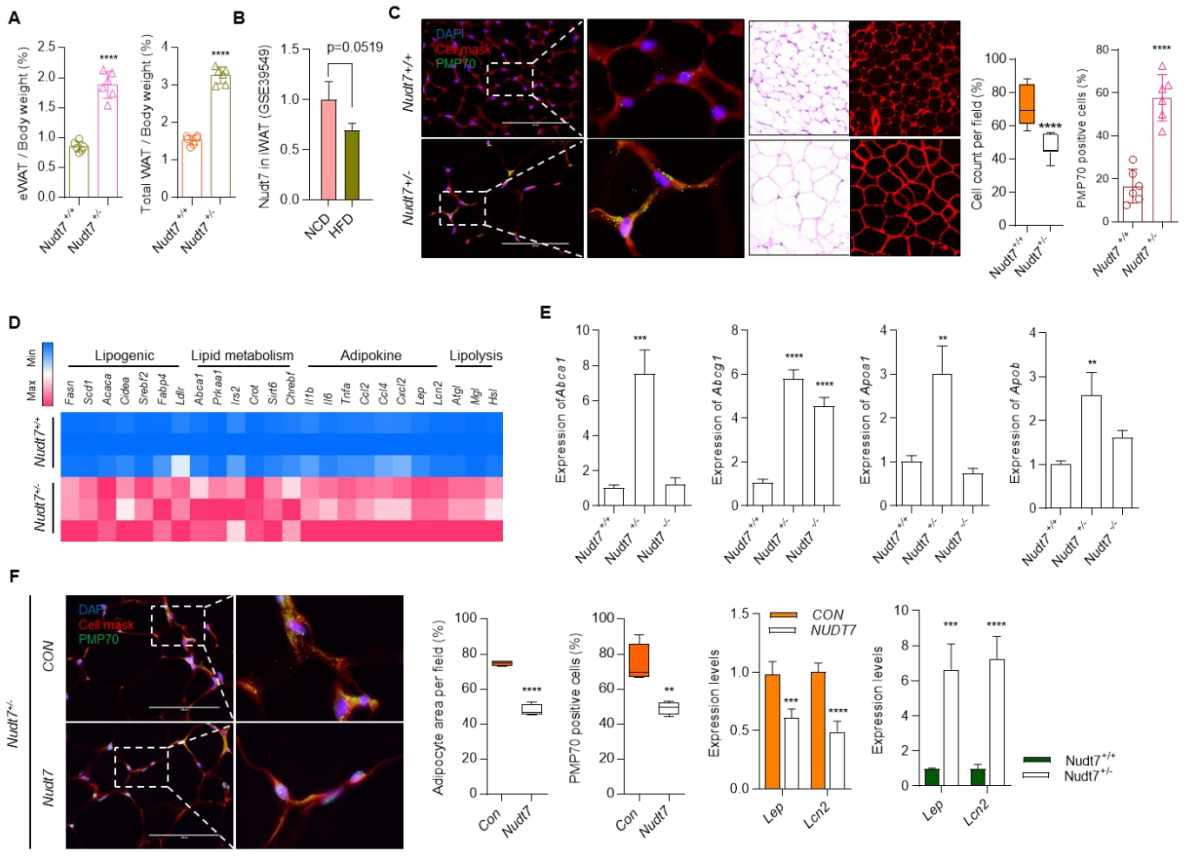

Figure S4. *Nudt7*$^{+/-}$ mice displays adipocyte enlargement and adipose expansion, related to Figure 3.

(A) The weight of eWAT and total WAT to body weight (n = 4). (B) The expression level of Nudt7 in GSE39549. (C) Representative images of cell mask orange and PMP70 staining and graphs with adipocyte area per filed and percentage of PMP70-positive cell counting (n = 3; Scale bars, 100 μm). (D) The expression levels of genes in lipid metabolism were analyzed by qRT-PCR (n = 3). (E) The expression levels of Aba1, Abcg1, Apoa1, and Apob in *Nudt7*$^{+/+}$, *Nudt7*$^{+/-}$, and *Nudt7*$^{-/-}$ adipocytes (n = 3). (F) Representative images of cell mask orange and PMP70 staining and graphs with adipocyte area per filed and percentage of PMP70-positive cell counting (n = 4; Scale bars, 100 μm), and expression levels of *Lep* and *Lcn2* in *Nudt7*$^{+/-}$, adipocytes with NUDT7 restoration. Values were expressed as means + s.d. An unpaired

Student's t-test and one-way ANOVA multiple test were used for statistical analysis. **P < 0.01, ***P < 0.001, ****P < 0.0001.

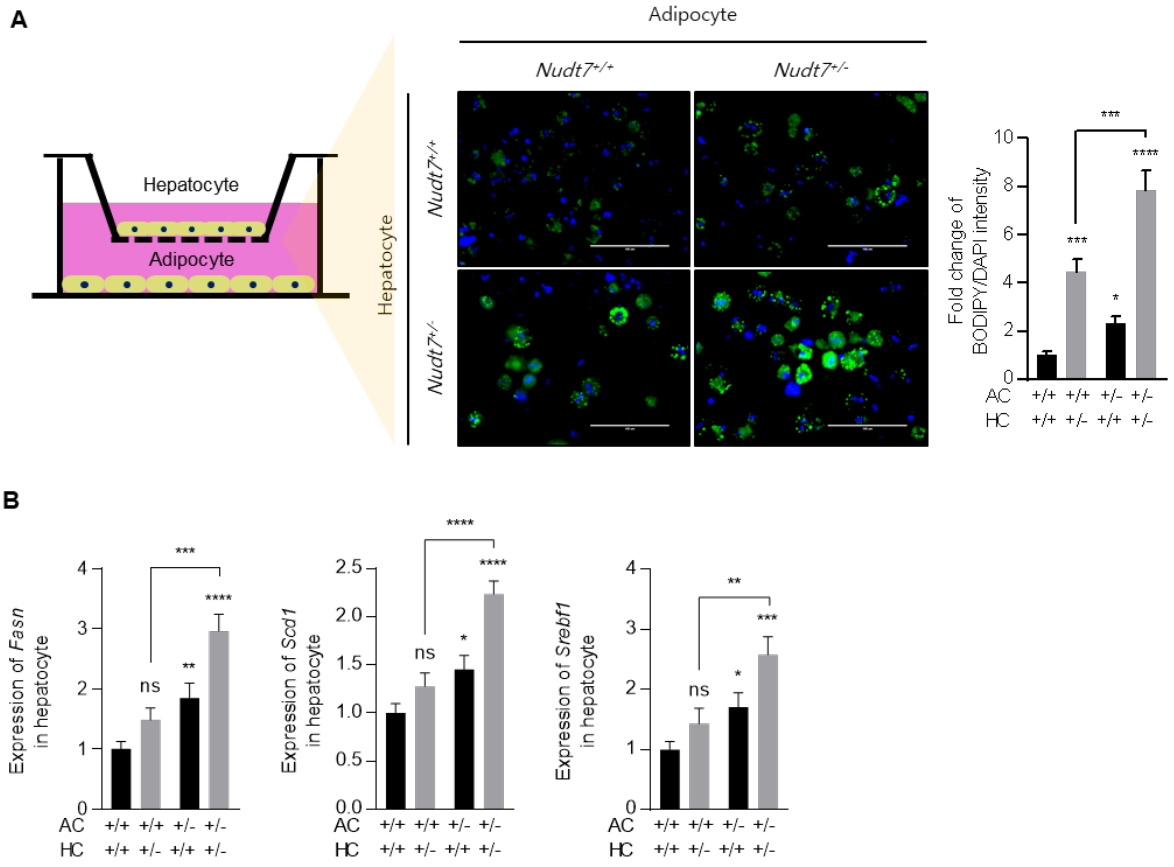

Figure S5. Increased lipolysis of adipose tissue affects hepatic lipid accumulation in *Nudt7*$^{-/-}$ mice, related to Figure 3.

(A) Co-culture of hepatocytes and adipocytes isolate from *Nudt7*$^{+/+}$ or *Nudt7*$^{+/-}$ mice. Representative image of BODIPY staining and bar graph of BODIPY intensity in hepatocytes was represented as fold of control. (B) The expression levels of *Fasn*, *Scd1*, and *Srebf1* in hepatocyte were analyzed by qRT-PCR (n = 3). Values were expressed as means + s.d. An unpaired t-test was used for statistical analysis. ns = non-significant, *P ≤ 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

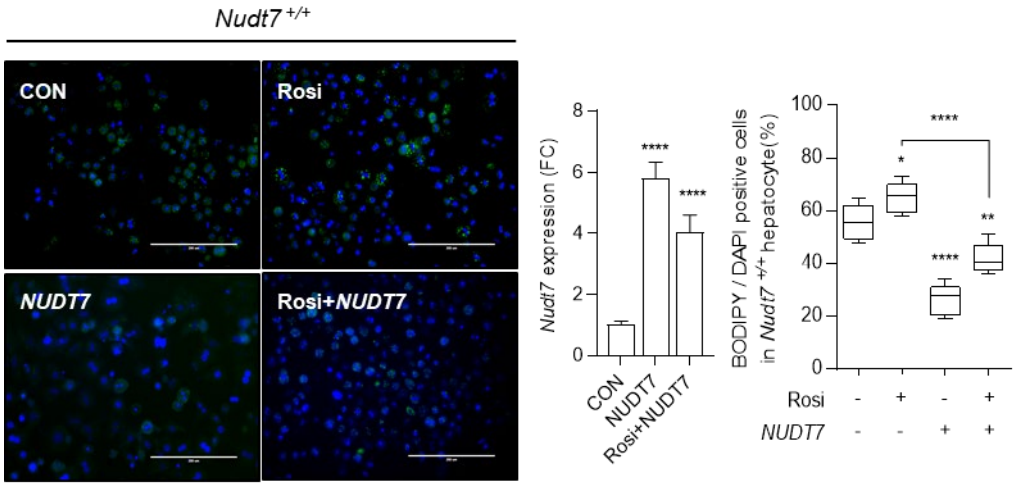

Figure S6. Exposure of PPARγ agonist induces lipid accumulation in *Nudt7*$^{+/+}$ hepatocytes, related to Figure 4.

Representative image of BODIPY staining, positive cell counting (n = 3), and *Nudt7* expression level in *Nudt7*$^{+/-}$ hepatocytes in the presence of 10 μM rosiglitazone (Rosi) with/without introduction of pcDNA-Nudt7. Scale bars, 200 μm. Values were expressed as means + s.d. An unpaired t-test or one-way ANOVA were used for statistical analysis. *P ≤ 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

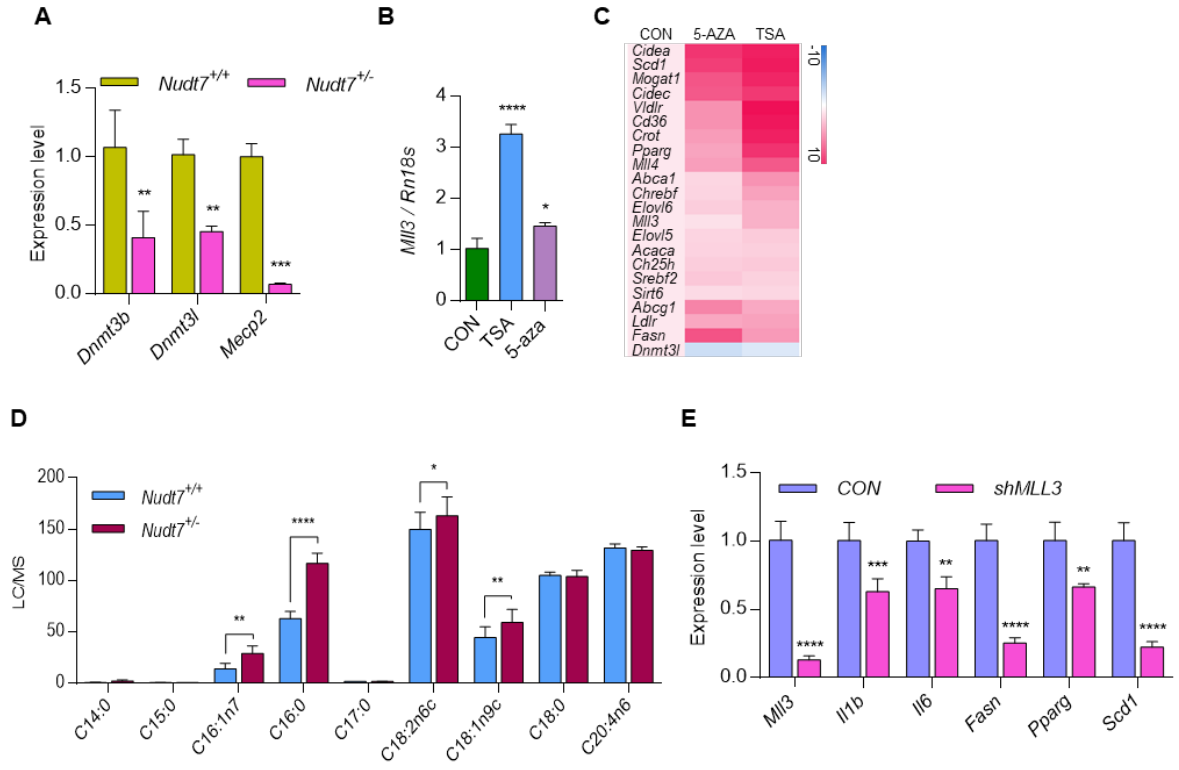

Figure S7. The possible involvement of *Mll3* in hepatic lipogenesis, related to Figure 5.

(A) The expression levels of Dnmt3b, Dnmt3l, and Mecp2 were analyzed by qRT-PCR (n = 3). (B) Primary cultured hepatocyte was treated with TSA or 5-aza and the expression level of Mll3 was analyzed by qRT-PCR (n = 3). (C) The expression level of lipogenic genes (n = 3) was analyzed by qRT-PCR. (D) Lipidomic analysis of *Nudt7*$^{+/+}$ and *Nudt7*$^{+/-}$ liver (n = 3). (E) The expression levels of *Mll3*, lipogenic genes, and inflammatory cytokine (n = 3). Values were expressed as means + s.d. An unpaired *t*-test or one-way ANOVA multiple comparisons were used for statistical analysis. *P ≤ 0.05, **P < 0.01, **P < 0.01, ***P < 0.001, ****P < 0.0001.

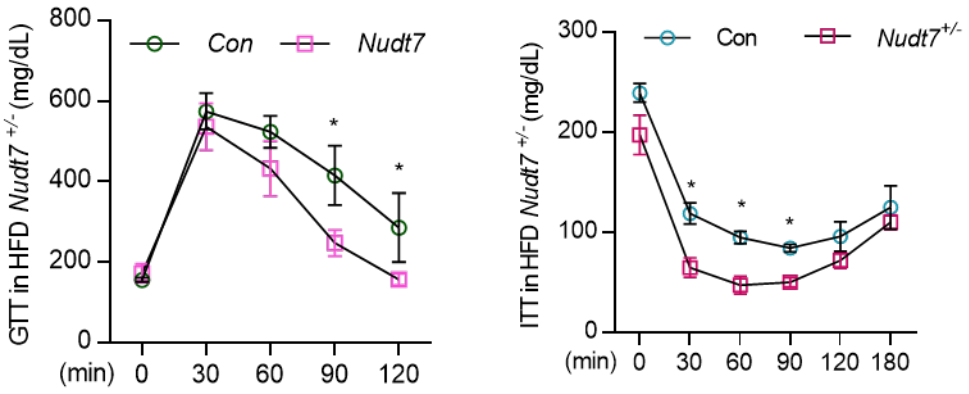

Figure S8. Impaired glucose and insulin tolerance by *Nudt7* deficiency could rescue by the restoration of *Nudt7*, related to Figure 6. Analysis of glucose and insulin tolerance levels in HFD *Nudt7*$^{+/+}$ and *Nudt7*$^{+/-}$ mice. An unpaired Student's *t*-test was used for statistical analysis. *$P \leq 0.05$.

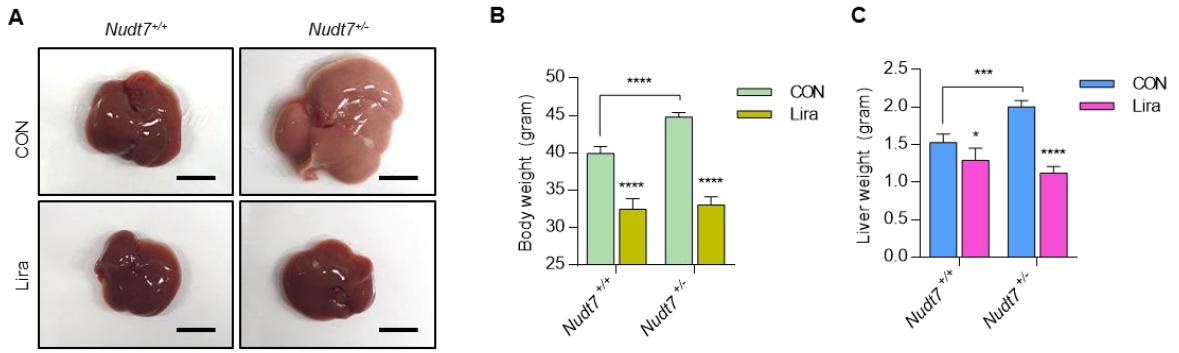

Figure S9. The effect of liraglutide in *Nudt7*$^{+/-}$ liver, related to Figure 7.

(A) Representative images of mouse liver of HFD fed *Nudt7*$^{+/-}$ mice with liraglutide or equal volume of sterile saline i.p. injected mouse. (B) Body weight and (C) liver weight were analyzed (n = 4).

Table S1. Clinical characteristics of enrolled patients, related to Figure 2.

Variables	Median (IQR) or N (%)
Male : Female (%)	10: 12 (4.5 :54.5)
Age (year)	39.00 (33.00-47.75)
Hypertension, N (%)	10 (45.5)
Type 2 Diabetes, N (%)	10 (45.5)
Dyslipidemia, N (%)	10 (45.5)
Body mass index, kg/m^2	37.67 (33.95-44.02)
Total body fat (%)	47.15 (42.45-51.68)
Systolic BP, mmHg	140.00 (12.6.25-150.00)
Diastolic BP mmHg	80.00 (70.00-90.00)
eGFR mL/min/1.73 m^2	113.60 (79.45-121.25)
ALP, U/L	64.00 (50.75-79.00)
AST, IU/L	34.00 (20.75-43.00)
ALT, IU/L	33.50 (19.50-44.50)
γ-GT, U/L	31.00 (18.50-47.00)
Total cholesterol (mg/dL)	178.00 (147.50-201.00)
LDL cholesterol (mg/dL)	124.00 (92.25-142.00)
HDL- cholesterol (mg/dL)	45.50 (37.00-51.25)
Triglyceride (mg/dL)	124.50 (79.25-203.75)
HbA1C (%)	5.80 (5.60-6.30)
Fasting glucose (mg/dL)	105.00 (89.75-114.25)
HOMA_IR	4.56 (2.49-7.44)
Fibrotic Stage	
F1	4 (18.2)
F2	7 (77.3)
F3	1 (4.5)
NAS	
1	0 (0.0)
2	6 (27.3)
3	11 (50.0)
4	3 (13.6)
5	2 (9.1)

BP, Blood pressure; γ-GT, γ-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; HbA1c, glycated hemoglobin A1c; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDL, low-density lipoprotein, NAS; Nonalcholic fatty liver disease Activity Score.

Table S2. Primers used for qRT-PCR analysis, related to STAR Methods.

Description	Symbol	Forward (5' to 3')	Reverse (5' to 3')
Peroxisome profiling	*Abcd2*	GACCATGCCTATGAGACCTATTT	GTAAGAGACTGGTCAGGGTTTG
	Acad11	CCCAAAGAGTGTCGGGTTATAG	GTGTCCTGGGCCAATAGTAAA
	Acot1	CGTCATGGCTCTGGCTTATTA	CAGGTAGTTCACGGCTTCTT
	Acot2	CGTGATGGCTCTGGCTTATTA	CAGGTAGTTCACGGCTTCTT
	Acot8	ACTGGAGCCCAAACAGATG	AGGAAGGCGTAGTCAGAGATA
	Acox1	CGCACATCTTGGATGGTAGT	GGCTTCGAGTGAGGAAGTTATAG
	Acox3	ACCCACGGATAAGGAAGAGA	GAGAAGTGGTCCAAGGCATAG
	Acsf3	GAGTGGAAGTACGCATCATCTC	GAACCCTGGAGTCACCTTTG
	Acsl1	GCTTGTGGATGTGGAAGAAATG	TCTTGCTGGGTCTTTCAAGTAG
	Acsl3	GGCTGTGCACTGGAGATATT	GTACTCTCCTGCCTGTAGTTTC
	Aldh3a2	GGCTTCTCCTGACTATGAAAGG	CATCCATCTCTCCACCGAAAG
	Crat	GGAGGCCCATATCAACTTCTC	ATGTCCAGCAGAGCTTTCTC
	Crot	TGCTGTGATCATGCTCCTTAC	CTGAACCCTTCCATCTTCCTTC
	Ephx2	GTAAAGGGTTGGGACGAAAGA	GACATTTCAGGACGGAGTACAA
	Far1	CAAGAGCGAGTGGAGGAAAT	AGCCAGTTTAGGTTGGGTAAG
	Hacl1	TTTGGACTGCCTCCAAGATAC	TCACAGAAGGCCTGACATTATT
	Idh1	GTGGAGATGCAAGGAGATGAA	ATGCAGATCCAGTTCCACATAG
	Idi1	ACAGCAGAGATCAGATGCTAAA	GCTCGCCTGGGTTACTTAAT
	Idi2	CCCAGGACCAGATTTCCATAAA	ACCAACAGAAGGTAGCCAATC
	Mlycd	CTGCCATCTTCTACTCCATCAG	GCTCCTTGACCACTCTCTTTAT
	Mpv17	GCCCTCATCACCAACTACTATC	CAATAGCAACACACTGGACAAC
	Mvk	GGAGCAACTGGAGAAGCTAAA	TGCCAGGTACAGGTAGAGAA
	Nudt12	GGAAGAGGAAAGTGGAGTCAAA	GTAGACACTGCCACAGCTAAA
	Nudt7	CAGTTTGCTAGATGATGCTAAGG	AAAGGACGGAGTATTTGTTATATGG
	Peci	CCTCTGGTTGCGGTAGTAAAT	GAGTTGGCTGAATGGAGTATGA
	Pecr	GCGAAATGGGACAAACCTTATT	ACAGCAGGAAACATACCAGAG
	Pex1	GGAGATGGAGAATGTGGCTTAG	CCAAAGTAGAGCCGGTACATATT
	Pex11a	TCCTTCCTCCTCCTCCTATTC	GCCGAGATTGGACTTGTAGAT
	Pex11b	ATCTGAGCCGTGATGCTTATG	CCTGAGACTCCTACTCCAGAAA
	Pex11g	AGTATGGCCTGGGAACAAAG	GCGATATGCTCACAAGGATAGT
	Pex12	CAGAAGCGTTGGTGAGAAGATA	CAGGAAGAATACACCCACAGAG
	Pex19	GCTAGATCTCATGCAGCAGTTA	TTGAGAGCATCCAGGTCAAAG
	pex26	CTGGAGCTGTGCATCCTTT	AGGAAGGCCCTGGTTATCT
	Pex6	CTGACTGGTGCAGATCTCTATTC	CAGCTCTAGCCCTTCCTCTA
	pex7	CATGTCCTTGTCACCTGTAGTG	CTCCTGCGTGTGCTCTTTAT
	Pipox	CAAGGGATCGACCATGAGTATC	ATAGAGGACACCTCCAGTCTT
	Prdx1	CCGCTCTGTGGATGAGATTATAC	GTATCACTGCCAGGTTTCCA
	Rhoc	TCCGAAAGAAGCTGGTGATTG	AAAGACGGTAGGCACGTAGA
	Scp2	CGGACCACTCCAACTGATAAA	TCCCAGCATACCCAAACATC
	Slc22a5	ATAATCCTGTGGCTGACCATATC	CCAGTAGGAAGCAGTTCACATAG
	Sod1	CTCAGGAGAGCATTCCATCATT	CTCCCAGCATTTCCAGTCTT
	Xdh	CCACATGGACAACGCCTATAA	GCTGTGTTAGAGGGCAGATTAG
	Rn18s	CCAGTAAGTGCGGGTCATAAG	GGCCTCACTAAACCATCCAA
Lipogenic genes	*Acaca*	AGCCAGAAGGGACAGTAGAA	CTCAGCCAAGCGGATGTAAA
	Cd36	CTGGGACCATTGGTGATGAAA	CACCACTCCAATCCCAAGTAAG
	Cidea	GCAACCAAAGAAATCGGGAATAG	CTCGTACATCGTGGCTTTGA
	Elovl5	GGTGTGTGGGAAGGCAAATAC	TGGAGAAGTAGTACCACCAGAG
	Fabp4	GGATGGAAAGTCGACCACAATA	TGGCTCATGCCCTTTCATAA
	Fasn	AGACCCGAACTCCAAGTTATTC	GCAGCTCCTTGTATACTTCTCC

	Ldlr	ATCCACCGCAACATCTACTG	GGAACAGTGTCCTCCTCTTTAC
	Scd1	CAACTTCACCACGTTCTTCATC	CCCGTCTCCAGTTCTCTTAATC
	Srebf1	ACTTCCCTGGCCTATTTGACC	GGCATGGACGGGTACATCTT
Lipid metabolic genes	*Abca1*	GTTTCCGGGAAGTGTCCTAAA	CTGGGAGAGGATGCTGAATATC
	Cat	GATGGTAACTGGGATCTTGTGG	GTGGGTTTCTCTTCTGGCTATG
	Chrebf	CAGCTGCGGGATGAAATAGA	CAAAGCGCTGATGTGTGATG
	Crot	TGCTGTGATCATGCTCCT	CTGAACCCTTCCATCTTCCTT
	Irs2	ATGGGTACATGAGCATGGATAG	CAGGCGTGGTTAGGGAATAA
	Prkaa1	GATCCTTTCCGGTGTGGATTAT	GAAAGACCAAAGTCGGCTATCT
	Ppara	CGGTGTGTATGAAGCCATCT	TAAGGAACTCGCGTGTGATAAA
	Pparg	CTGGCCTCCCTGATGAATAAAG	AGGCTCCATAAAGTCACCAAAG
	Sirt6	CGTCTGGTCATTGTCAACCT	GAGTCTGCACATCACCTCATC
Adipokine genes	*Ccl2*	CTCACCTGCTGCTACTCATTC	ACTACAGCTTCTTTGGGACAC
	Ccl4	CCACTTCCTGCTGTTTCTCTTA	CAAAGACTGCTGGTCTCATAGT
	Ccl5	GCCCACGTCAAGGAGTATTT	CTTGAACCCACTTCTTCTCTGG
	Cxcl2	GACAGAAGTCATAGCCACTCTC	GCCTTGCCTTTGTTCAGTATC
	Il1b	CCACCTCAATGGACAGAATATCA	CCCAAGGCCACAGGTATTT
	Il6	CCAGAGTCCTTCAGAGAGATACA	CCTTCTGTGACTCCAGCTTATC
	Lep	GACTTCATTCCTGGGCTTCA	ATTCTCCAGGTCATTGGCTATC
	Lcn2	TGGCCCTGAGTGTCATGTG	CTCTTGTAGCTCATAGATGGTGC
	Tnfa	TTGTCTACTCCCAGGTTCTCT	GAGGTTGACTTTCTCCTGGTATG

Lipolysis genes	*Atgl*	CATCCGTGGCTGTCTACTAAAG	GACGTTCTCTCCGTCTGAAAC
	Hsl	CATCAACCACTGTGAGGGTAAG	AAGGGAGGTGAGATGGTAACT
	Mgl	AAGAGTGGAGCGAGCAATG	GATGATTCCATGAGCAGGTAGG
Histone modification genes	*Dnmt3b*	GGTCTCCAGCCTTCTGAATTAC	CAGAGCCATTCCCATCATCTAC
	Dnmt3l	GCAGAGACTACCAGAATGCTATG	TGACTTGGGCTTGCAGATAC
	Mecp2	AGGCAGGCAAAGCAGAAACATCAG	TCATACTTTCCAGCAGATCGGCCA
	Mll1	CGAGGAAAGAGATCAGCAGAAG	AGTCCGCGTGAAGTTGTAATAG
	Mll2	AGTACCTGAAAGGCGAAGAAC	CGCCCGTAGCGGAATAAATA
	Mll3	CGACTCTCTGTTGACCCTTATG	ATTGCTGGGTGTGGAGTTAG
	Mll4	GTTCGGATAGGAAGGAGCTTATG	CATCCTCAAGCCCTGGTAAAT