Adipocyte YTH N(6)-methyladenosine RNA-binding protein 1 protects against obesity by promoting white adipose tissue beiging in male mice

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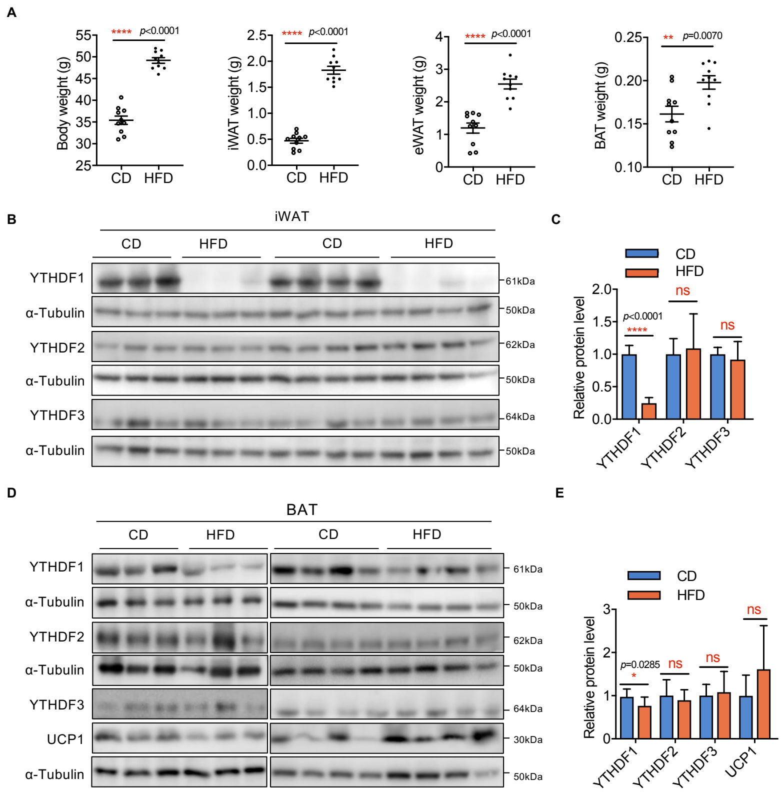
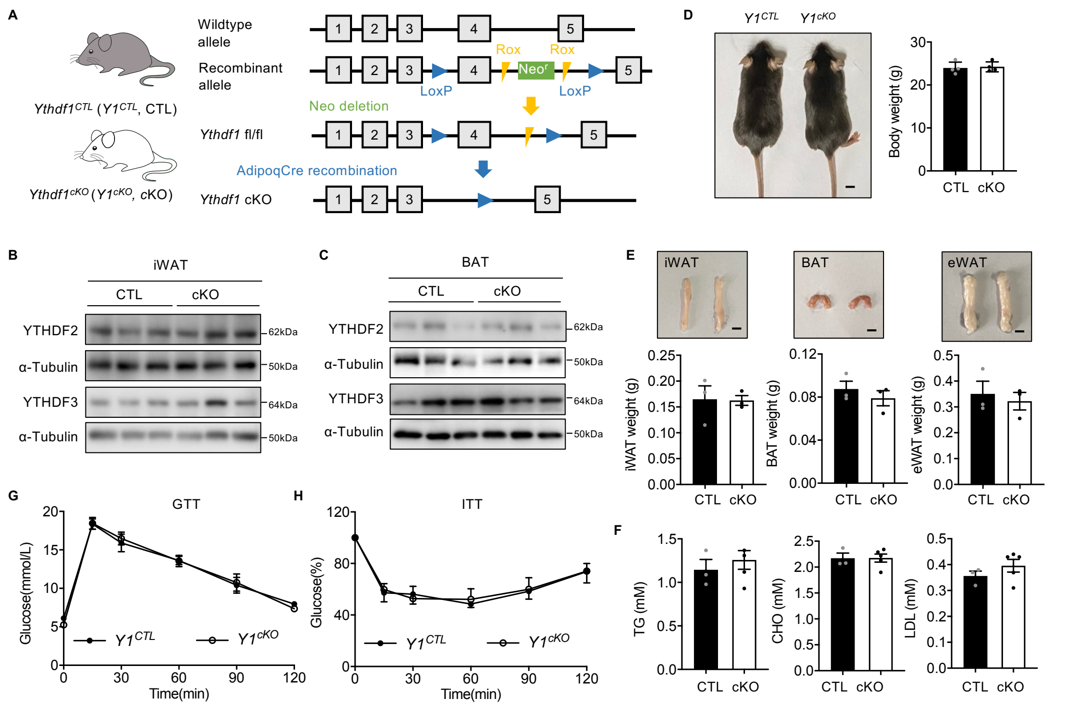
SUPPLEMENTARY INFORMATION

Supplementary Figures 1-7

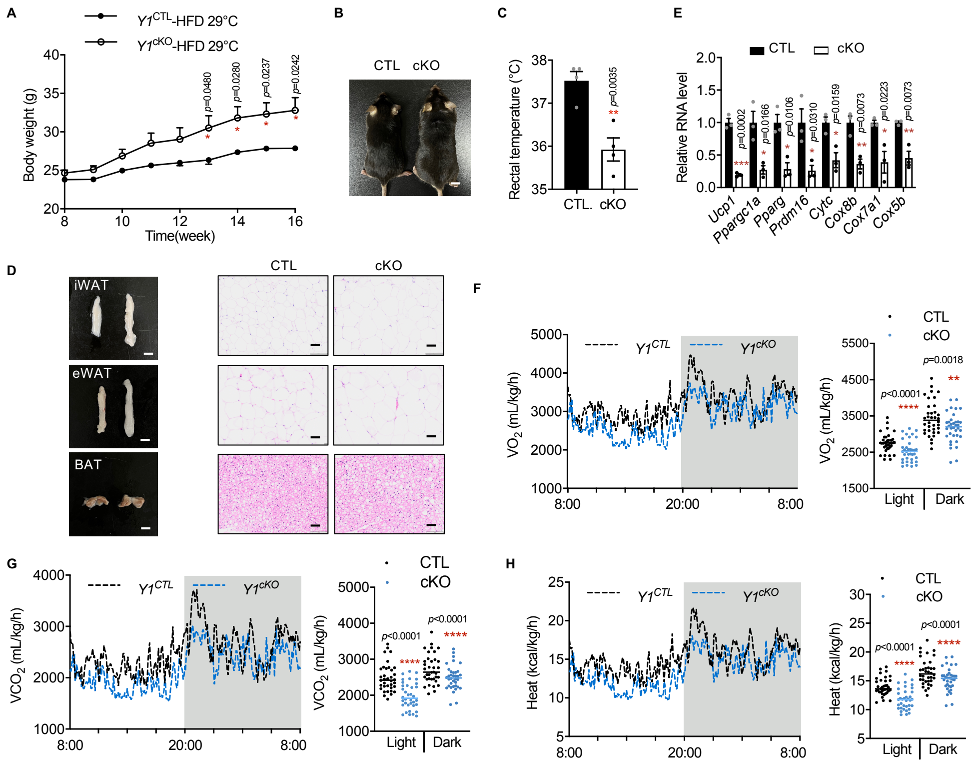
Supplementary Tables 1-2



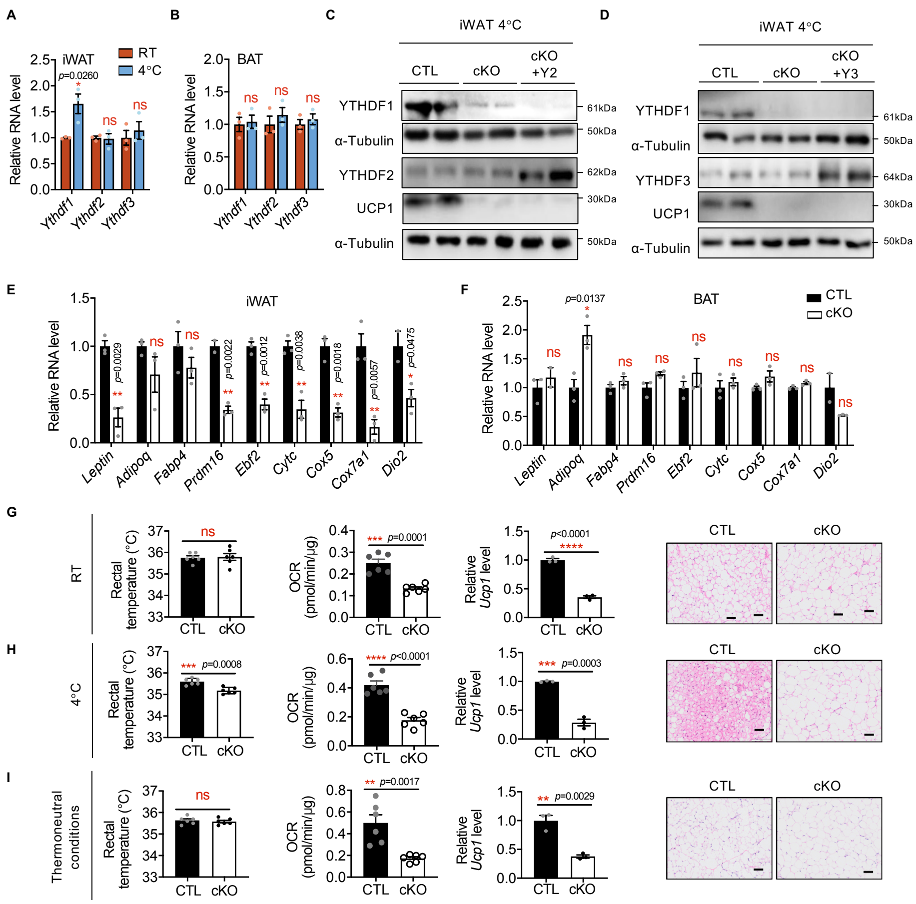
Supplementary Fig. 1 Adipose YTHDF1 expression is reduced in obesity. **A** Weights of Body, iWAT, eWAT, and BAT of CD- and HFD-fed mice (n = 10). Individual values and mean ± SEM were shown. **B, D** Immunoblot analysis of YTH family proteins in iWAT (**B**) and BAT (**D**) of CD- and HFD-fed mice. **C, E** The quantification of protein bands. Data in **A,C,E** were presented as mean ± SEM (n = 10). ns, not significant * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, two-sided t-test. Source data are provided as a Source Data file.



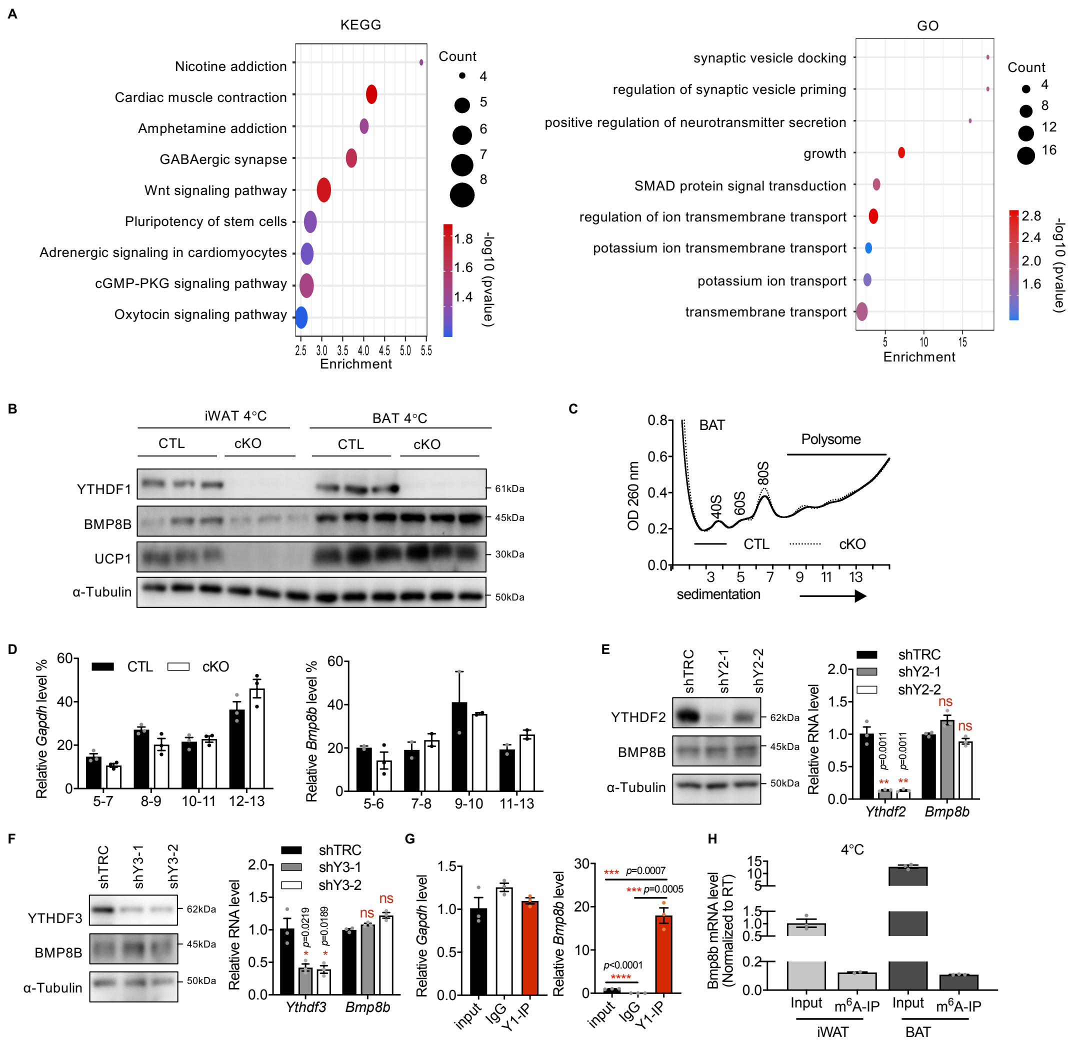
Supplementary Fig. 2 Adipocyte-specific knockout of *Ythdf1* does not affect weight gain under normal condition. **A** Schematic of the *Ythdf1* deletion mouse. **B, C** Expression of YTHDF2/3 in iWAT (**B**) and BAT (**C**) of $Y1^{CTL}$ and $Y1^{cKO}$ mice. **D** Gross view of mice ($Y1^{CTL}$ and $Y1^{cKO}$ mice) under normal condition. Body weights of $Y1^{CTL}$ and $Y1^{cKO}$ littermates at 8 weeks of age (n = 4). **E** Weights and gross view of iWAT, BAT, and eWAT from $Y1^{CTL}$ and $Y1^{cKO}$ mice at 8 weeks of age (n = 3). **F** Serum concentrations of TG, CHO, and LDL in $Y1^{CTL}$ and $Y1^{cKO}$ mice (n = 3). **G, H** Glucose tolerance (**G**) and insulin tolerance (**H**) at 8 weeks of age (n = 4). Data in **D-H** were presented as mean ± SEM. Source data are provided as a Source Data file.



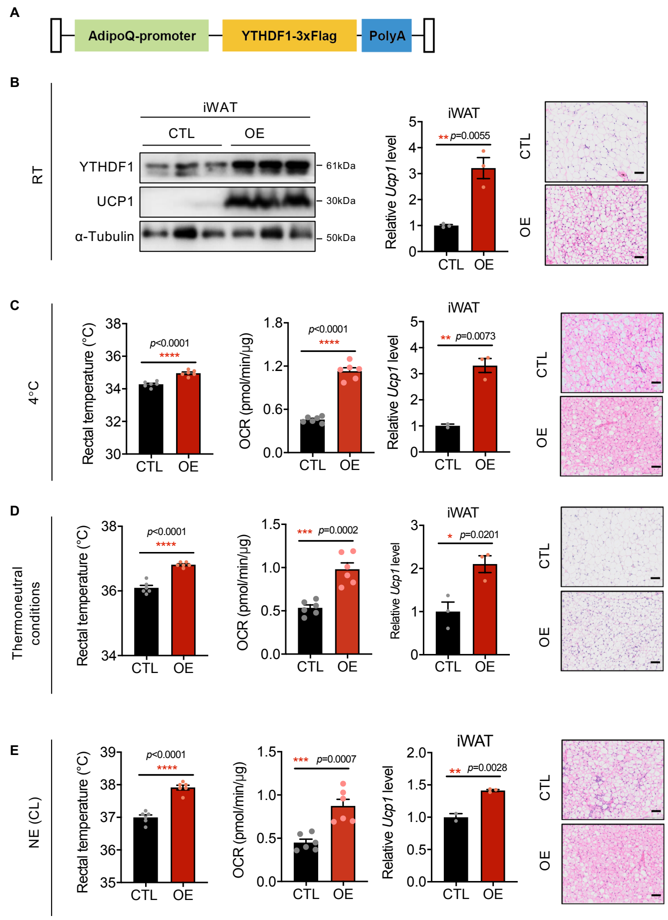
Supplementary Fig. 3 Deletion of *Ythdf1* exacerbates obesity and reduces thermogenesis under thermoneutral condition. **A-H** *Y1*^{CTL} and *Y1*^{cKO} littermates were fed under thermoneutral condition (29°C). **A** Body weights of *Y1*^{CTL} and *Y1*^{cKO} mice. **B** Gross view of *Y1*^{CTL} and *Y1*^{cKO} mice. Scale bar, 0.5 cm. **C** Rectal temperature in *Y1*^{CTL} and *Y1*^{cKO} mice. **D** Gross view and H&E staining of iWAT, eWAT, and BAT from *Y1*^{CTL} and *Y1*^{cKO} mice. Scale bar, 0.5 cm and 50 µm. **E** Thermogenesis genes level in *Y1*^{CTL} and *Y1*^{cKO} mice. **F, G, H** O_2 consumption (**F**), CO_2 generation (**G**), and energy heat generation (**H**) of *Y1*^{CTL} and *Y1*^{cKO} mice fed with HFD. White and gray areas in the graphs indicate day and night, respectively. Data in **A,C,E-H** were presented as mean ± SEM (n = 4 biologically independent mice). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, two-sided t-test. Source data are provided as a Source Data file.



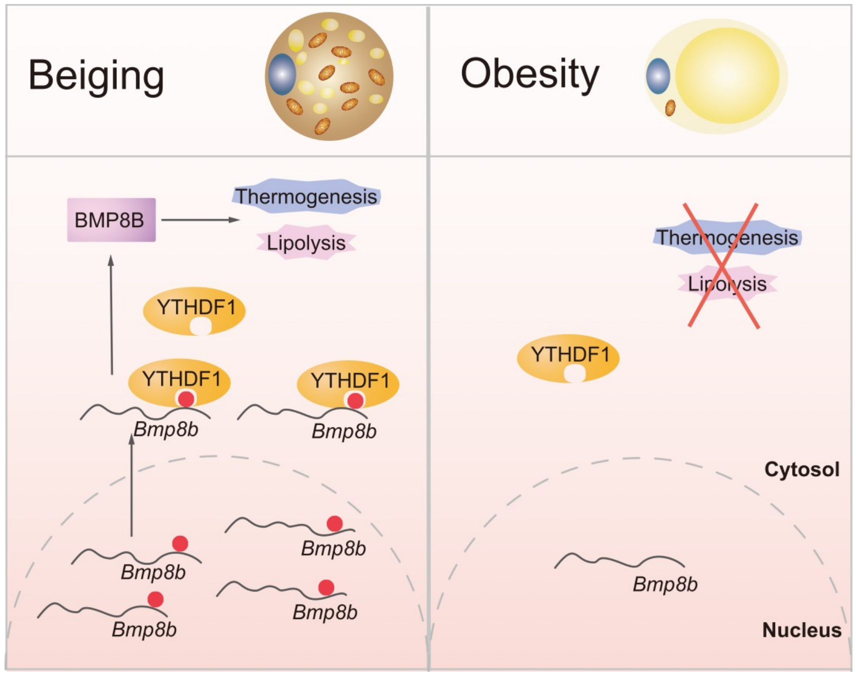
Supplementary Fig. 4 Deletion of *Ythdf1* impairs WAT beiging. **A, B** mRNA levels of YTH family proteins expression in iWAT (**A**) and BAT (**B**) from mice housed at room temperature (RT) or 4°C (n=3 biologically independent mice). **C, D** UCP1 expression in iWAT of $Y1^{CTL}$ and $Y1^{cKO}$ mice with or without YTHDF2/3 overexpression after cold exposure. **E, F** mRNA levels of adipogenic genes (*Lept, adipoq, Fabp4*), BAT-specific genes (*Prdm16, Ebf2*), and mitochondrial specific genes (*Cytc, Cox5, Cox7, Dio2*) in iWAT (**E**) and BAT (**F**) from $Y1^{\mathrm{CTL}}$ and $Y1^{\mathrm{cKO}}$ mice (n=3 biologically independent mice). **G-I** Rectal temperature, the oxygen consumption rates (OCR) of iWAT, and the *Ucp1* expression were measured under RT (**G**), cold (**H**), and thermoneutral conditions (29°C) (**I**) in $Y1^{CTL}$ and $Y1^{cKO}$ mice (n = 6). H&E staining of iWAT was shown in right panel. Scale bar, 50 μm. All the data were presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, two-sided t-test. Source data are provided as a Source Data file.



Supplementary Fig. 5 Identification of YTHDF1-regulated mRNAs. **A** Top KEGG and GO analysis terms enriched for translational upregulated transcripts. **B** The protein level of BMP8B in iWAT and BAT from $Y1^{CTL}$ and $Y1^{cKO}$ mice after cold exposure. **C** Polysome profiles of BAT from $Y1^{CTL}$ and $Y1^{cKO}$ mice. **D** The distributions of *Gapdh* and *Bmp8b* in polysome fractions. Data were presented as mean ± SEM (n = 3). **E, F** The expression of YTHDF2/3 and BMP8B in 3T3-L1 cells with or without *Ythdf2/3* knockdown (n=3 biologically independent samples). **G** RIP-qPCR analysis of the association of *Bmp8b* with YTHDF1 in iWAT of cold-treated mice. **H** Methylated RNA immunoprecipitation (MeRIP)-qPCR analysis of m^{6}A levels of *Bmp8b* mRNA in iWAT and BAT from cold-treated mice. **G, H** n=3 biologically independent mice. Data in **E-H** were presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$. All of the *P*-values are determined by unpaired two-sided t-test. Source data are provided as a Source Data file.



Supplementary Fig. 6 Overexpression YTHDF1 enhances thermogenic capacity. **A** Schematic of AdipoQ-promoter driven YTHDF1 expression. **B-E** Mice expressing YFP (CTL) or YTHDF1 (OE) were treated under RT (**B**), 4°C (**C**), thermoneutral condition (29°C) (**D**), or CL stimulation (**E**). The UCP1 expression, rectal temperature, OCR, and H&E staining of iWAT were determined. Scale bar, 50 µm. All the data were presented as mean ± SEM (n = 6 biologically independent mice). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, two-sided t-test. Source data are provided as a Source Data file.



Supplementary Fig. 7 A proposed model of YTHDF1-promoted WAT beiging.

Supplementary Table 1. Sequences of primers used in this study

Primers for plamids construction	
Bmp8b-CDS-F	5’-AGATCGCCGTGTAATTCTAGAGTGGTCCAAGAGCAC-3’
Bmp8b-CDS-R	5’-GCCGGCCGCCCCGACTCTAGAACAGCACACCTTGGG-3’
Bmp8b-3’UTR-1-F	5’-AGATCGCCGTGTAATTCTAGAGTCCCTGCCCAACAG-3’
Bmp8b-3’UTR-1-R	5’-GCCGGCCGCCCCGACTCTAGATTTTTTAAACTCTTCTCTAGAAATCCCA-3’
Bmp8b-3’UTR-2-F	5’-AGATCGCCGTGTAATTCTAGACCCAGAACAGCAGCC-3’
Bmp8b-3’UTR-2-R	5’-GCCGGCCGCCCCGACTCTAGACTACTGGAGGGTCCC-3’
YTHDF1-F	5’-TGGCAAAGAATTGGATCCGCCACCATGTCGGCCACCAGCGTG-3’
YTHDF1-R	5’-TTGTTTGTTTCGACTCTGCCGT-3’
Primers for shRNA plamids construction	
shTRC	5’-CAACAAGATGAAGAGCACCAA-3’
sh*Ythdf1*	5’-GCTGAAGATTATCGCTTCCTA-3’
Pimers for genotyping	
Ythdf1-F	GCCAGTAGTGTGGTGTTTTGAG
Ythdf1-R	AGAGCTTCCAACTGCTATGTGG
Adipoq-Cre-F	ACGGACAGAAGCATTTTCCA
Adipoq-Cre-R	ACGGACAGAAGCATTTTCCA

Supplementary Table 2. Sequences of primers used in this study

Pimers for RT-qPCR	
Ythdf1-F	5'-ACAGTTACCCCTCGATGAGTG-3'
Ythdf1-R	5'-GGTAGTGAGATACGGGATGGGA-3'
Ythdf2-F	5'-GAGCAGAGACCAAAAGGTCAAG-3'
Ythdf2-R	5'-CTGTGGGCTCAAGTAAGGTTC-3'
Ythdf3-F	5'-CATAGGGCAACAGAGGAAACAG-3'
Ythdf3-R	5'-ATCTCCAGCCGTGGACCAT-3'
Bmp8b-F	5'-TCCACCAACCACGCCACTAT-3'
Bmp8b-R	5'-CAGTAGGCACACAGCACACCT-3'
Ucp1-F	5'-GAGGTCGTGAAGGTCAGAAT-3'
Ucp1-R	5'-CTGTGGTGGCTATAACTCTGTAA-3'
Dio2-F	5'-GGTGGTCAACTTTGGTTCAGCC-3'
Dio2-R	5'-AAGTCAGCCACCGAGGAGAACT-3'
HSL-F	5'-GCTGGGCTGTCAAGCACTGT-3'
HSL-R	5'-GTAACTGGGTAGGCTGCCAT-3'
Cidea-F	5'-GGTGGACACAGAGGAGTTCTTTC-3'
Cidea-R	5'-CGAAGGTGACTCTGGCTATTCC-3'
Ppargc1a-F	5'-CCCTGCCATTGTTAAGACC-3'
Ppargc1a-R	5'-TGCTGCTGTTCCTGTTTTC-3'
Pparg-F	5'-GGAAGACCACTCGCATTCCTT-3'
Pparg-R	5'-GTAATCAGCAACCATTGGGTCA-3'
Prdm16-F	5'-CCACCAGCGAGGACTTCAC-3'
Prdm16-R	5'-GGAGGACTCTCGTAGCTCGAA-3'
Adrb3-F	5'-TCTCTGGCTTTGTGGTCGGA-3'
Adrb3-R	5'-GTTGGTTATGGTCTGTAGTCTCG-3'
Cox8b-F	5'-GAACCATGAAGCCAACGACT-3'
Cox8b -R	5'-GCGAAGTTCACAGTGGTTCC-3'
Leptin-F	5'-GGGCTTCACCCCATTCTGA-3'
Leptin-R	5'-TGGCTATCTGCAGCACATTTTG-3'

Adipoq-F	5’-GTTCCCAATGTACCCATTCGC-3’
Adipoq-R	5’-TGTTGCAGTAGAACTTGCCAG-3’
Fabp4-F	5’-GACGACAGGAAGGTGAAGAG-3’
Fabp4-R	5’-ACATTCCACCACCAGCTTGT-3’
Ebf2-F	5’-GCT GCG GGA ACC GGA ACG AGA-3’
Ebf2-R	5’-ACA CGA CCT GGA ACC GCC TCA-3’
Cytc-F	5’-CCAAATCTCCACGGTCTGTTC-3’
Cytc-R	5’-ATCAGGGTATCCTCTCCCCAG-3’
Cox5b-F	5’-GCTGCATCTGTGAAGAGGACAAC-3’
Cox5b-R	5’-CAGCTTGTAATGGGTTCCACAGT-3’
Cox7a1-F	5’-CAG CGT CAT GGT CAG TCT GT-3’
Cox7a1-R	5’-AGA AAA CCG TGT GGC AGA GA-3’
ATGL-F	5’-GAGGAATGGCCTACTGAACCAA-3’
ATGL-R	5’-AGGCTGCAATTGATCCTCCTC-3’
Pref1-F	5’-GACCCACCCTGTGACCCC-3’
Pref1-R	5’-CAGGCAGCTCGTGCACCCC-3’
Adipsin-F	5’-CGTACCATGACGGGGTAGTC-3’
Adipsin-R	5’-ATCCGGTAGGATGACACTCG-3’
Luciferase-F	5’-CTAAGGAAGTCGGGGAAGCG-3’
Luciferase-R	5’-ATCCCCCTCGGGTGTAATCA-3’