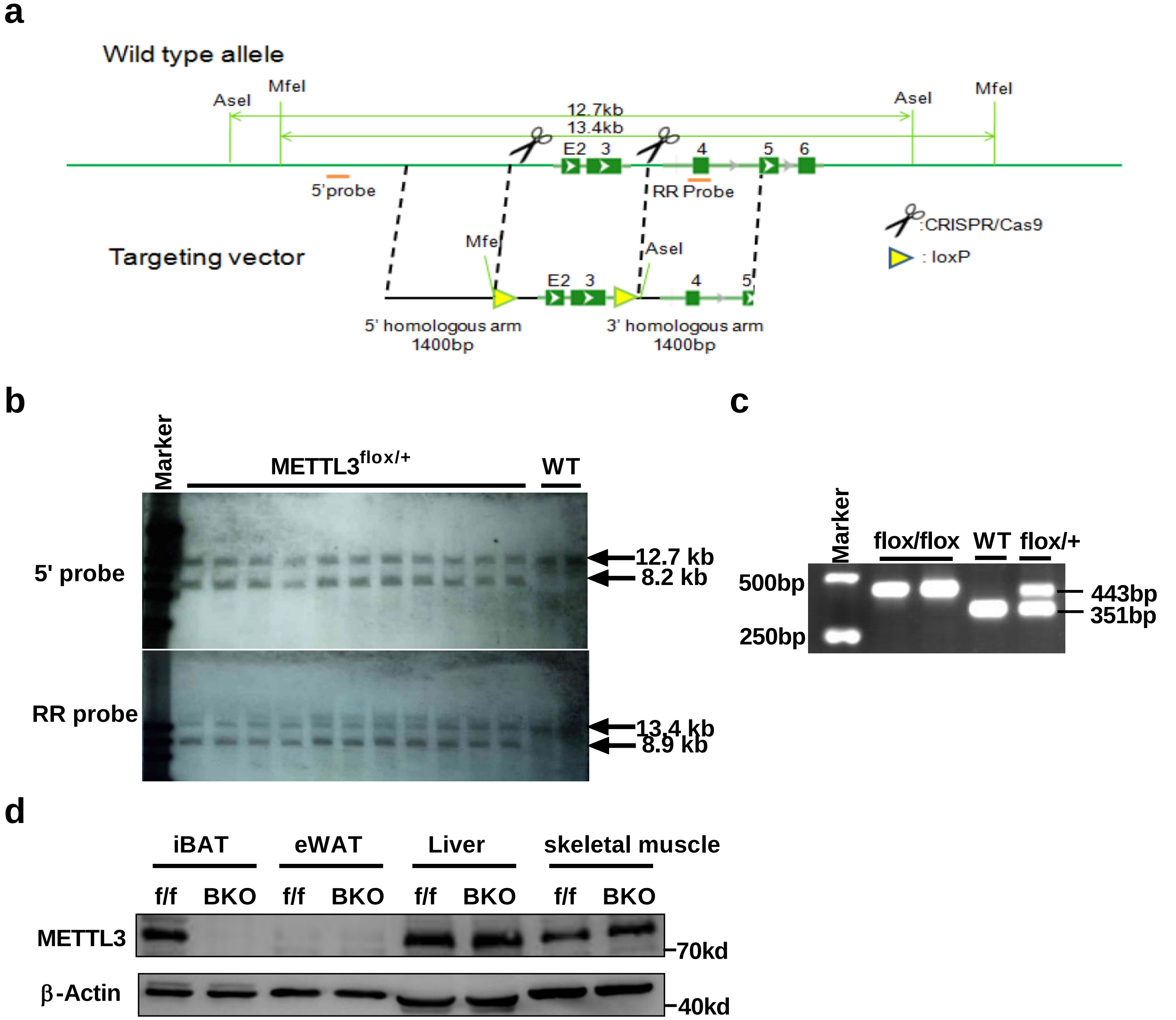
METTL3 is essential for postnatal development of brown adipose tissue and energy expenditure in mice

Wang et al.

Supplementary Figure 1



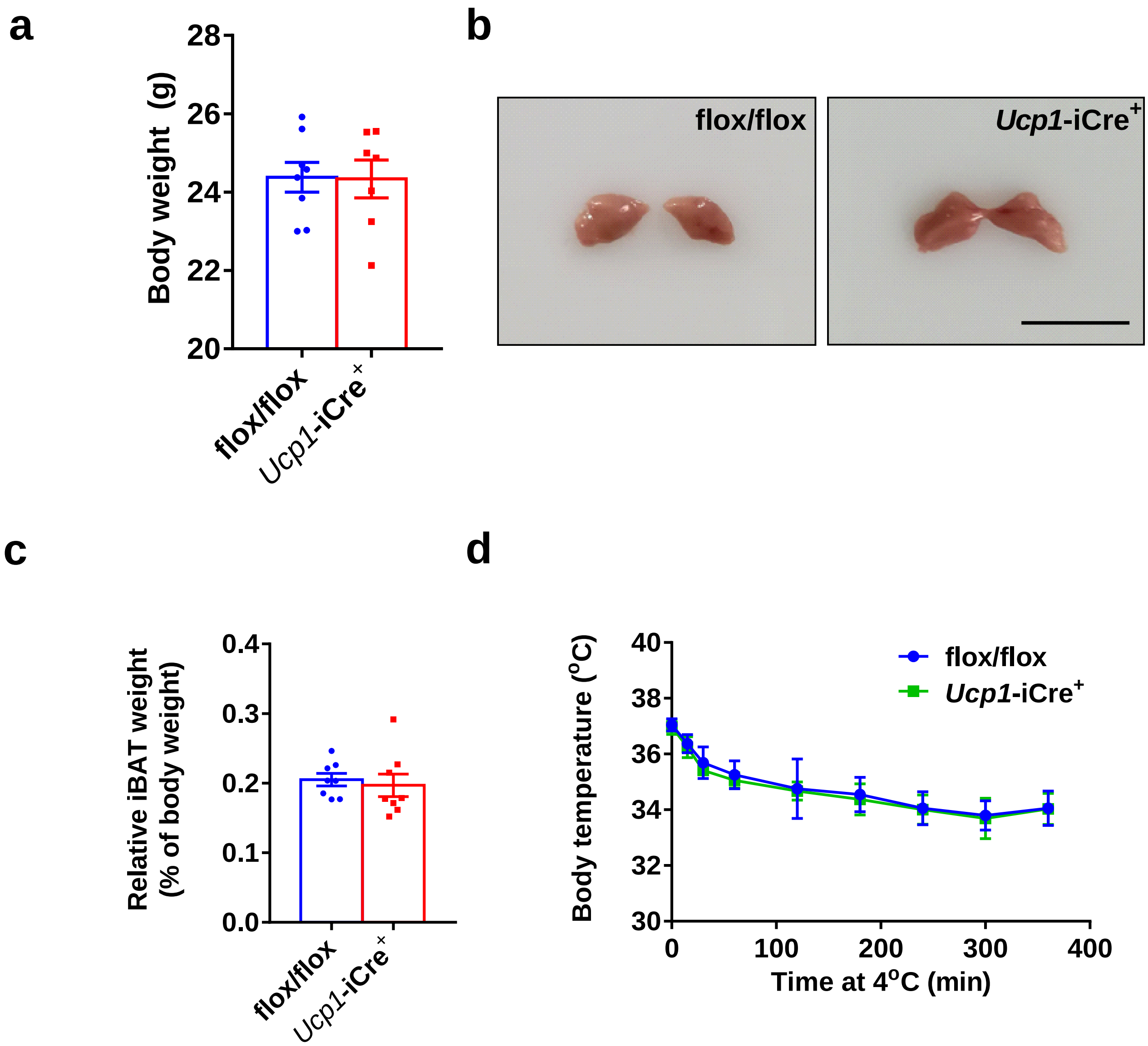
Supplementary Figure 1. Generation of *Mettl3*$^{flox/flox}$ and *Mettl3* BKO mice.

(a) *Mettl3* alleles and targeting construct. Top: wildtype *Mettl3* allele (exons indicated as solid green rectangles). Two flanking DNA fragments used for Southern hybridization are shown in red. Middle: targeting vector containing two loxP sites (yellow triangles). Insertion of the targeting vector by CRISPR/Cas9 technique yielded the *Mettl3* lox allele. (b) Southern blot of tail genome DNA from *Mettl3*$^{flox/+}$ (heterozygous) and WT mice. (c) Genotyping of PCR products of *Mettl3*$^{flox/flox}$ (homozygous), *Mettl3*$^{flox/+}$ (heterozygous) and WT mice. (d)

METTL3 protein levels in iBAT, eWAT, liver and skeletal muscle of 8-week-old *Mettl3*$^{flox/flox}$ and BKO mice. These experiments were repeated at least three times independently with similar results.

Source data are provided as a Source Data file.

Supplementary Figure 2



Supplementary Figure 2. ***Mettl3*$^{flox/flox}$ mice display similar body weight, similar size of iBAT and similar cold tolerance with *Ucp1*-iCre mice.**

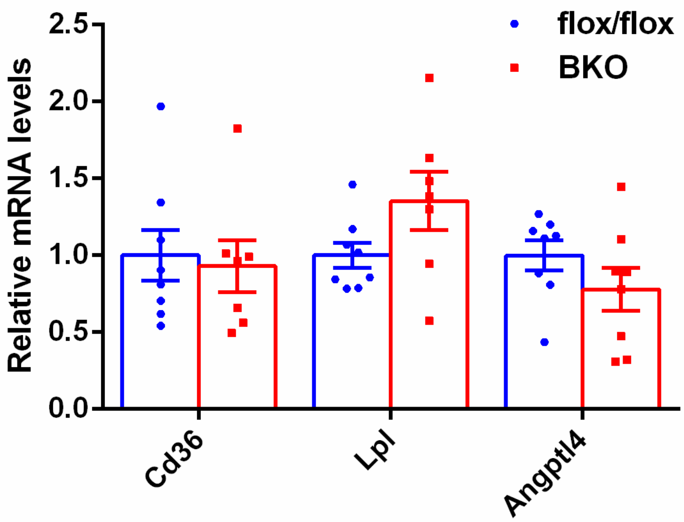
(a)The body weight of 8-week-old *Mettl3*$^{flox/flox}$ and *Ucp1*-iCre mice (*Mettl3*$^{flox/flox}$, n = 8; *Ucp1*-iCre, n = 7)**.**

(b, c) Gross appearance and relative weight of iBAT in *Mettl3*$^{flox/flox}$ and *Ucp1*-iCre mice at 8-week old (n=8 for each group). The scale bar represents 1 cm.

(d) The rectal temperature of 8-week-old *Mettl3*$^{flox/flox}$ and *Ucp1*-iCre mice during acute cold exposure (4℃) (n=7 for each group).

Data represent the mean ± SEM. Source data are provided as a Source Data file.

Supplementary Figure 3

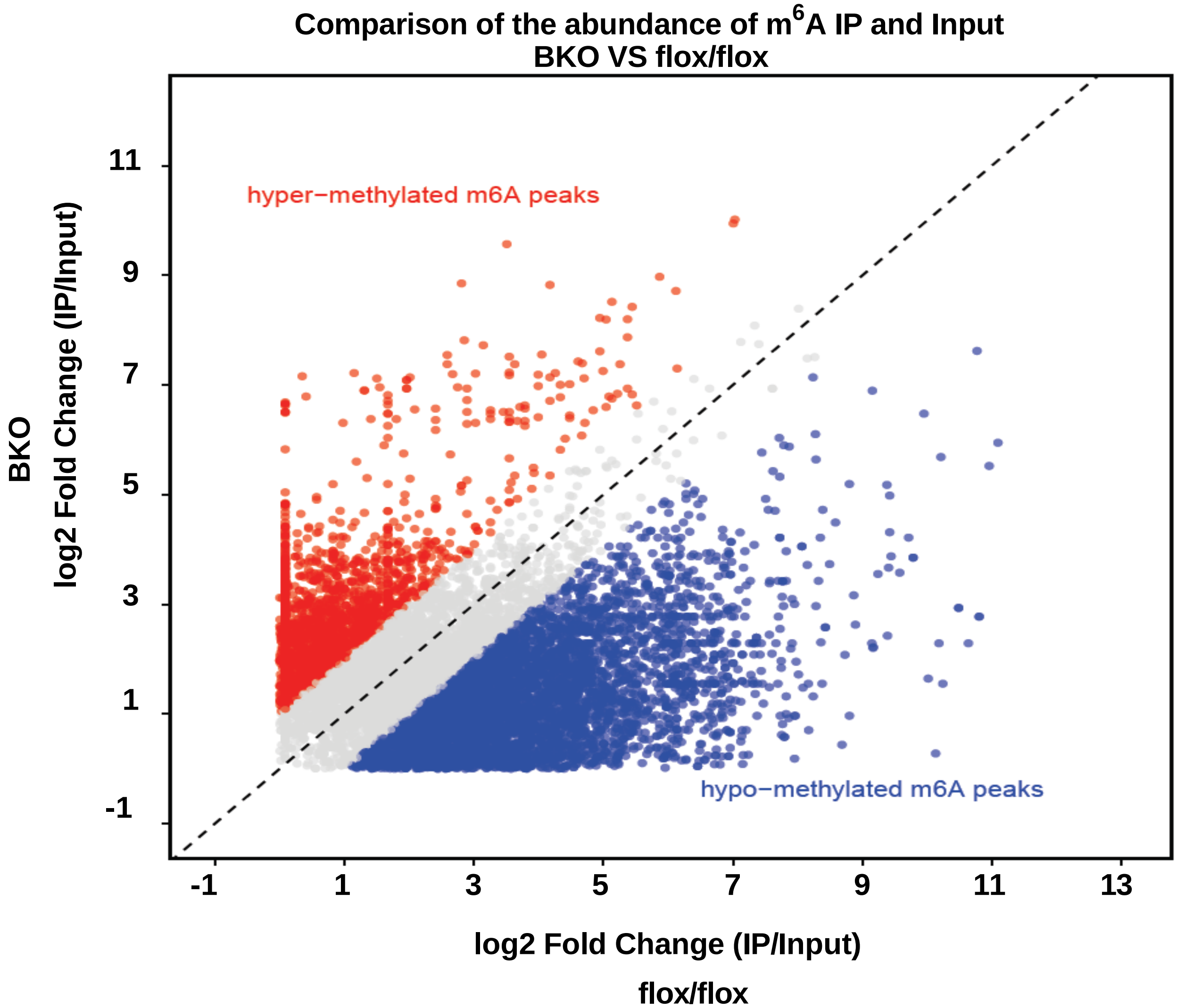


Supplementary Figure 3. BAT-specific deletion of *Mettl3* does not affect the expression of lipid uptake associated genes in iBAT.

Relative *Cd36, Lpl* and *Angptl4* mRNA levels were measure by qPCR assays in iBAT of 8-week-old *Mettl3*$^{flox/flox}$ and BKO mice (Cd36, Lpl in BKO, n = 7; Others, n=8 for each group)**.**

Data represent the mean ± SEM. Source data are provided as a Source Data file.

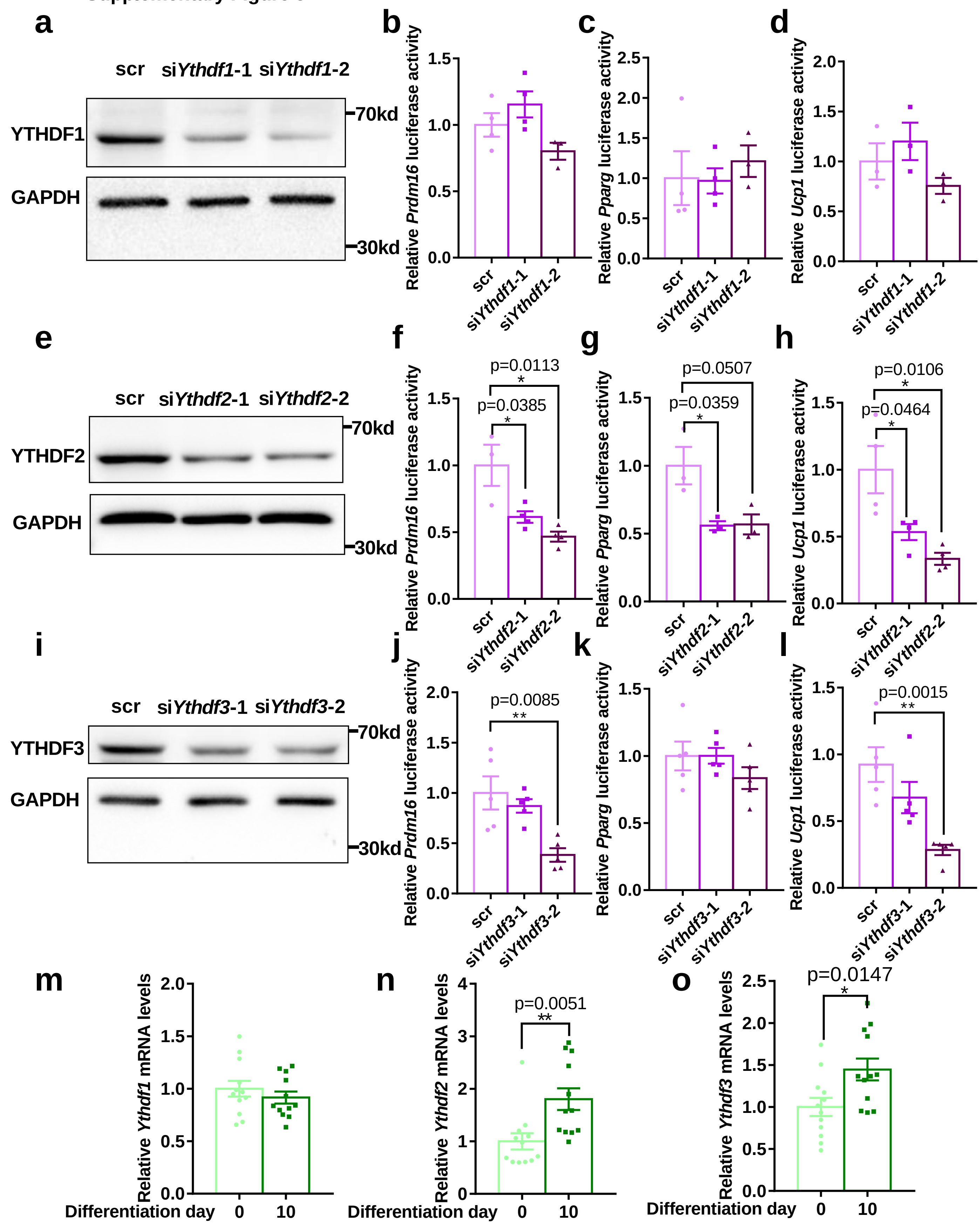
Supplementary Figure 4



Supplementary Figure 4. METTL3 is essential for mRNA m^{6}A modification in iBAT.

The m^{6}ARIP-seq analysis of iBATs was performed in 8-week-old *Mettl3*$^{flox/flox}$ and BKO mice. Comparison of the abundance of m^{6}A IP and Input mRNAs (BKO VS flox/flox).

Supplementary Figure 5



Supplementary Figure 5. m^{6}A reader proteins regulate *Prdm16, Pparg* and *Ucp1* luciferase activity.

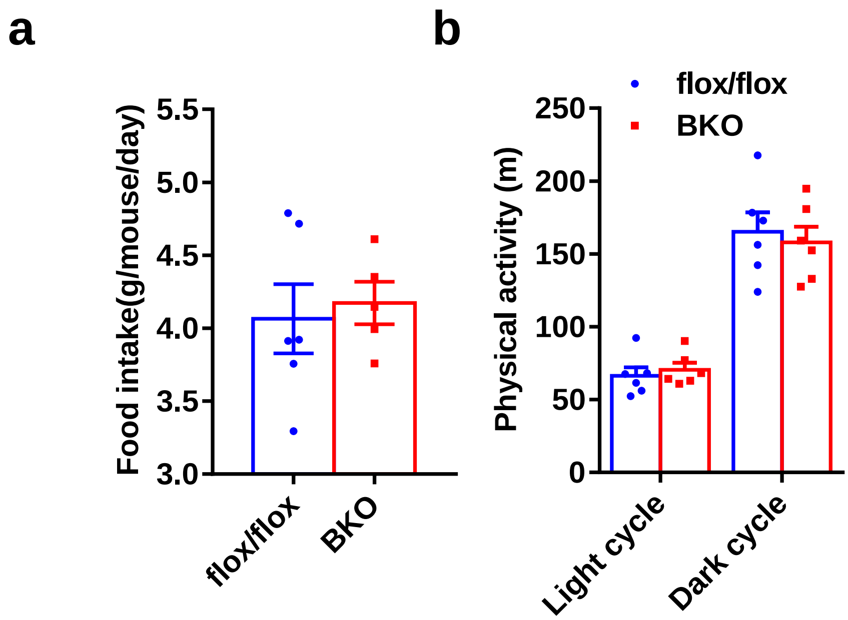
Primary brown preadipocytes seeded in 24-well plates were co-transfected with pMIR-REPORT Luciferase vectors (*Prdm16*, *Pparg*, *Ucp1*), siRNAs (Scramble siRNA, si*Ythdf1*-1, si*Ythdf1*-2; Scramble siRNA, si*Ythdf2*-1, si*Ythdf2*-2; or Scramble siRNA, si*Ythdf3*-1, si*Ythdf3*-2) and β-galactosidase (β-Gal) reporter plasmid by X-tremeGENE siRNA Transfection Reagent for 24 hours. Cells were then induced to differentiate for 48 hours. (a, e, i) YTHDF1-3 and GAPDH protein levels were measured by immunoblotting. (b-d, f-h, j-l) Relative *Prdm16*, *Pparg* and *Ucp1* luciferase activity were measured and normalized to β-Gal levels (n=3-5 for each group). (m-o) The relative *Ythdf1-3* mRNA levels in mature primary brown adipocytes (10 days after differentiation) and preadipocytes (before differentiation) (n=12 for each group).

These cell culture experiments were repeated three times independently with similar results.

Data represent the mean ± SEM. Significance was determined by unpaired two-tailed Student's *t* test analysis.

*, $p < 0.05$. **, $p < 0.01$. Source data are provided as a Source Data file.

Supplementary Figure 6

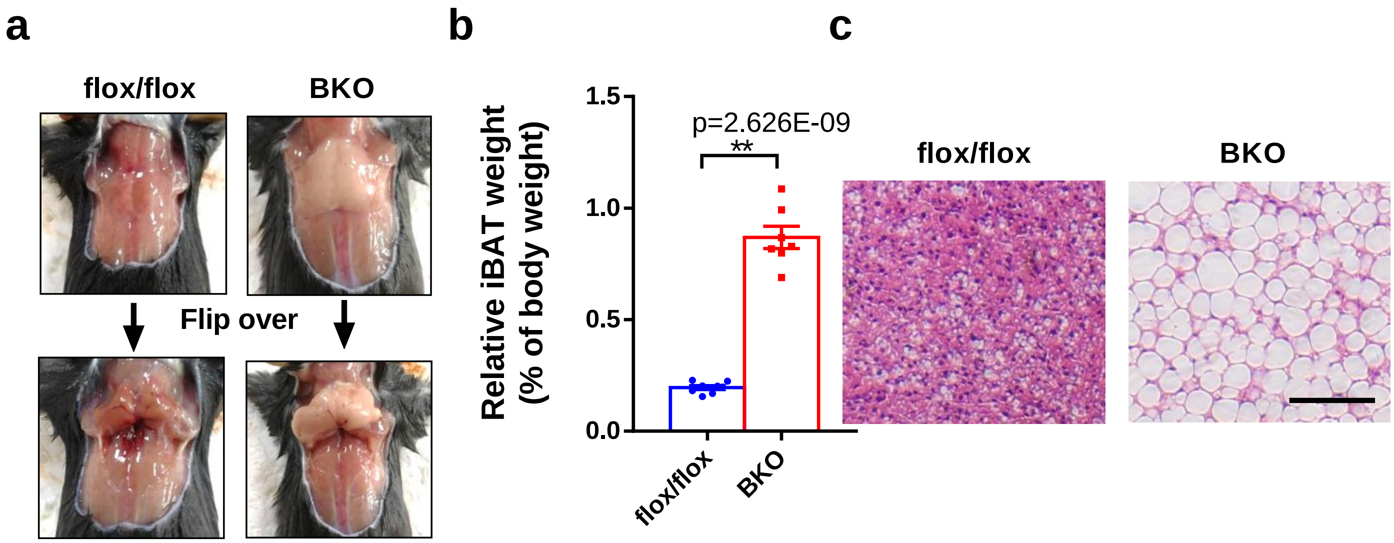


Supplementary Figure 6. *Mettl3* deficiency in BAT does not affect food intake or physical activity.

(a) The food intake of 8-week-old *Mettl3*$^{flox/flox}$ and BKO mice (*Mettl3*$^{flox/flox}$, n = 6; BKO, n = 5).

(b) The physical activity of 8-week-old *Mettl3*$^{flox/flox}$ and BKO mice (n = 6 for each group).

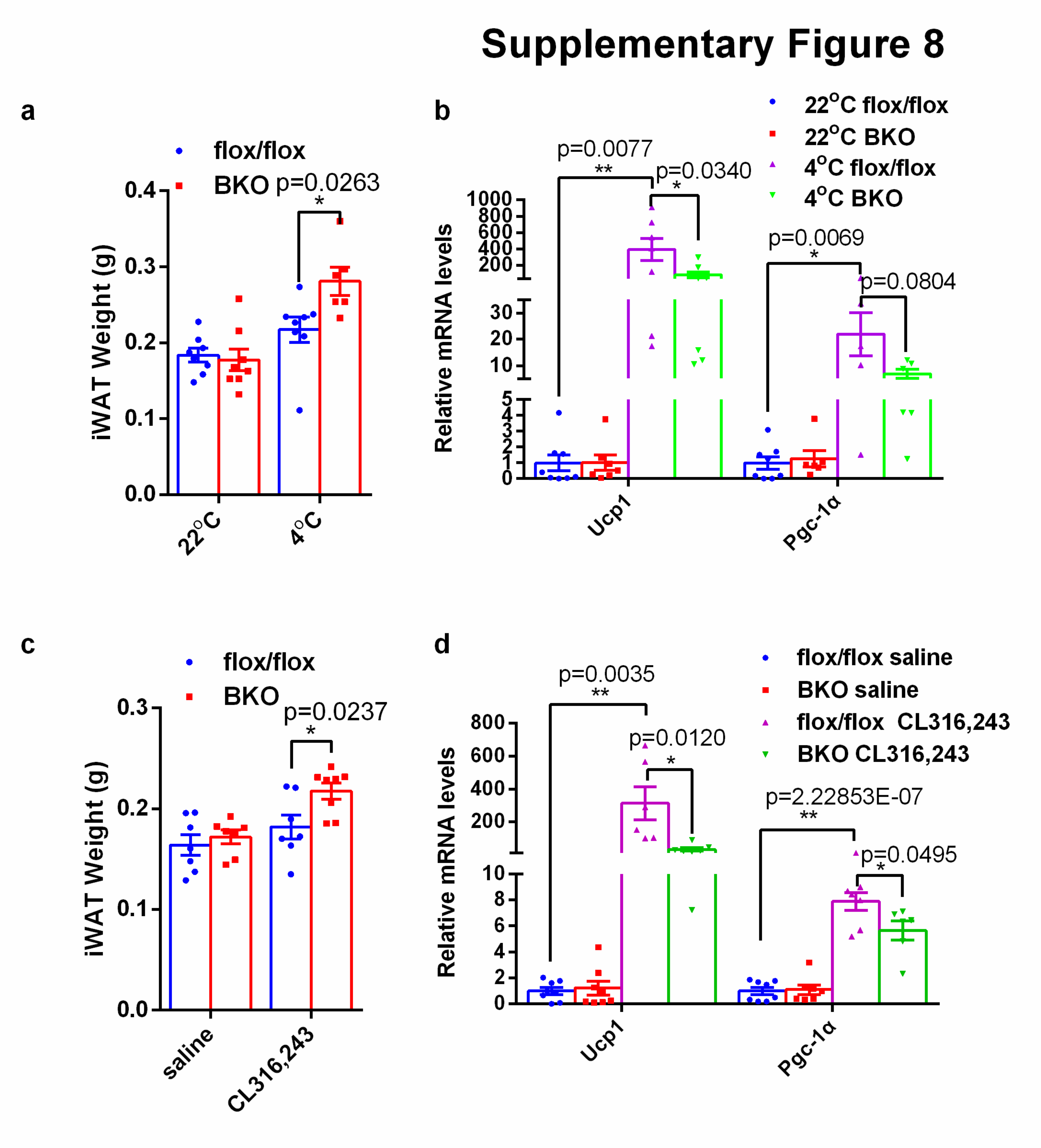
Data represent the mean ± SEM. Source data are provided as a Source Data file.



Supplementary Figure 7. The morphology of iBAT in BKO mice under the cold exposure condition still appears abnormal, enlarged and “whitening”.

(a, b, c) Gross appearance, relative weight, and H&E staining of iBATs in *Mettl3*$^{\text{flox/flox}}$ and BKO mice (8 weeks) after acute cold exposure (4℃) for 6 h (b: *Mettl3*$^{\text{flox/flox}}$, n = 8; BKO, n = 7). The scale bar represents 100 μm. Data represent the mean ± SEM. Significance was determined by unpaired two-tailed Student's *t* test analysis. **, p< 0.01. Source data are provided as a Source Data file.

Supplementary Figure 8



Supplementary Figure 8. BAT-specific deletion of *Mettl3* impairs the browning of WAT in response to chronic cold exposure or the β-adrenergic agonist.

BKO mice and flox/flox controls were exposed for chronic cold challenge (4℃ 7d) or injected intraperitoneally with CL316,243 for 4 days to induce browning of WAT. (a, c) The weight of iWAT was measured in BKO and flox/flox mice (n=6-8 for each group). (b, d) The relative *Ucp1* and *Pgc-1α* mRNA levels were measured by qPCR analysis in iWAT of BKO and flox/flox mice (n=5-8 for each group).

Data represent the mean ± SEM. Significance was determined by unpaired two-tailed Student's *t* test analysis.

*, $p < 0.05$.**, $p < 0.01$. Source data are provided as a Source Data file.

Supplementary Table 1 Primers for qPCR

Genes	Forward	Reverse
Pparg2	5'-TGGGTGAAACTCTGGGAGATTC-3'	5'-GAGAGGTCCACAGAGCTGATTCC-3'
Pgc-1α	5'-TGGACGGAAGCAATTTTTCA-3'	5'-TTACCTGCGCAAGCTTCTCT-3'
Adiponectin	5'-GCACTGGCAAGTTCTACTGCAA-3'	5'-GTAGGTGAAGAGAACGGCCTTGT-3'
Prdm16	5'-CAGCACGGTGAAGCCATTC-3'	5'-GCGTGCATCCGCTTGTG-3'
Cebpb	5'-TTATAAACCTCCCGCTCGGC-3'	5'-TTCCATGGGTCTAAAGGCGG-3'
Ucp1	5'-TGGAAAGGGACGACCCCTAA-3'	5'-CAGGAGTGTGGTGCAAAACC-3'
Adrb3	5'-CTATGCCAACTCCGCCTTCA-3'	5'-GCCATCAAACCTGTTGAGCG-3'
Ndufa8	5'-GAGTTTATGCTGTGCCGCTG -3'	5'-TACTCTGTGAAAGGCTCCGC-3'
Uqcrq	5'-TTCAGCAAAGGCATCCCCAA-3'	5'-CGACTGCTCAAACTCCTGGT-3'
Sdhb	5'-GACGTCAGGAGCCAAAATGG-3'	5'-CTCGACAGGCCTGAAACTGC-3'
Glut4	5'-TCTCCAACTGGACCTGTAAC-3'	5'-TCTGTACTGGGTTTCACCTC-3'
Cox5b	5'-AGAAGGGACTGGACCCATACA -3'	5'-CCTTTGTGCAGCCAAAACCA-3'
Cebpa	5'-CAAGAACAGCAACGAGTACCG-3'	5'-GTCACTGGTCAACTCCAGCAC-3'
Cox6b1	5'-AACTACCTGGACTTCCACCG-3'	5'-GGTACCACTCACACACGGAG-3'
Acta1	5'-CGACGGGCAGGTCATCA-3'	5'-ACCGATAAAGGAAGGCTGGAA-3'
Myoglobin	5'-CATGGACAGGAAGTCCTCATCG-3'	5'-CTGTGAGCACGGTGCAACCATG-3'
MHC-1β	5'-GAGGAAGAGTGAGCGGCG-3'	5'-GCCGCAGTAGGTTCTTCCTGT-3'
MHC-IIa	5'-TACAACCTCAAAGAGCGTTATGCA-3'	5'-AAGGGTTGACGGTGACACAGA-3'
MEF2c	5'-GAGCGTGCTGTGCGACTGT-3'	5'-CGTGCGGCTCGTTGTACTC-3'
Casq2	5'-ACATCAAAGACCCACCCTACGT-3'	5'-CGATGTGGATCCCATTCAAGT-3'
Tnnc1	5'-CCTGTGGTGCCTCCTTTGATT-3'	5'-TGCGGTCTTTTAGTGCAATGAG-3'
ATGL	5'-GAGGAATGGCCTACTGAACCAA-3'	5'-AGGCTGCAATTGATCCTCCTC-3'
Fasn	5'-TTGACGGCTCACACACCTAC-3'	5'-CGATCTTCCAGGCTCTTCAG-3'
Srebp1	5'-AACGTCACTTCCAGCTAGAC-3'	5'-CCACTAAGGTGCCTACAGAGC-3'
Cidea	5'-ATCACAACTGGCCTGGTTACG-3'	5'-TACTACCCGGTGTCCATTTCT-3'
Cpt1b	5'-CATGTATCGCCGCAAACTGG-3'	5'-GGTGCTGTAGCAAGTCTGTCT-3'
Cox6a	5'-GAGGGTTCAGCTCGGATGTG-3'	5'-GGTCTCTCGTGCTCTTCGTG-3'
Cox6b1	5'-AACTACCTGGACTTCCACCG-3'	5'-GGTACCACTCACACACGGAG-3'
Cox7a1	5'-TCTTCCAGGCCGACAATGAC-3'	5'-GCCCAGCCCAAGCAGTATAA-3'
nLPL	5'-GGATGGACGGTAAGAGTGATTC-3'	5'-ATCCAAGGGTAGCAGACAGGT-3'
mtND1	5'-GTGGCTCATCTACTCCACTGA-3'	5'-TCGAGCGATCCATAACAATAA-3'
Pfkm	5'-GGCGGAGGAGAGCTAAAACT -3'	5'-CCCTGACGGCAGCATTCATA-3'
Cpt2	5'-CAAAAGACTCATCCGCTTTGTTC-3'	5'-CATCACGACTGGGTTTGGGTA-3'
MG2	5'-GCTGTGGCGGTAGTGGAA-3'	5'-ATGAGGGCCTTGGGTGTG -3'
HSL	5'-GTGGTGTGTAACTAGGATTGACTCT-3'	5'-GAACGCTGAGGCTTTGATCTTG-3'
aP2	5'-AAGGAAAGTGGCAGGCATGG-3'	5'-CACGCCCAGTTTGAAGGAAATC-3'

36B4	5'-AAGCGCGTCCTGGCATTGTCT-3'	5'-CCGCAGGGGCAGCAGTGGT-3'
Prdm16-m6A	5'-AAAAGGACCCAGGTAGCCCT-3'	5'-GGACCCGTGTCCCAACTATC-3'
PPARg-m6A	5'-GACAGACCTCAGGCAGATCG -3'	5'-AAGGAACACGTTGTCAGCGG-3'
UCP1-m6A	5'-CAGGACGGTGCCCTGTATTT-3'	5'-TCGTGGTCTCCCAGCATAGA-3'
Lpl	5'-AGAAGGGAAAGGACTCAGCAG-3'	5'-TCAAACACCCAAACAAGGGTA-3'
Angptl4	5'-ACTTCAGATGGAGGCTGGAC-3'	5'-TCCGAAGCCATCCTTGTAGG-3'
Cd36	5'-GGAGTGGTGATGTTTGTTGCT-3'	5'-GCACACACCACCATTTCTTCT-3'
Ythdf1	5'-CTGCAGTTAAGACGGTGGGT-3'	5'-TAGCAATGGCTGCCCATGAA-3'
Ythdf2	5'-AGCCAATGAGGAAAGGGCATT-3'	5'-CTCCCCAAACACAGAGACTCAA-3'
Ythdf3	5'-TGTTCTATCTTGATTTGACTTTGCT-3'	5'-ATAGCTGTTATTCTGATTTGTCTGG-3'