SUPPLEMENTARY DATA

Adipose-Specific Deficiency of Fumarate Hydratase in Mice Protects against Obesity, Hepatic Steatosis and Insulin Resistance

By

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Supplementary Table 1. Primers used for genotyping.

Primer	Sequence (5'--3')
Cre-1	GATGGACATGTTCAGGGATC
Cre-2	AGCTTGCATGATCTCCGGT
Mfum12	AGCTAGGTGTAGTTGTTCTCA
Mfum26	GACCATACTCCTGGTTTACTTC
Neo-1	ATCCATCTTGTTCAATGGCCGATCC

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Supplementary Table 2. Primers used for real-time PCR.

Gene	Forward Sequence (5'--3')	Reverse Sequence (5'--3')
12s rRNA	ACCGCGGTCATACGATTAAC	CCCAGTTTGGGTCTTAGCTG
ACC1	ATTGGGCACCCCAGAGCTA	CCCGCTCCTTCAACTTGCT
ACLY	TGGATGCCACAGCTGACTAC	GGTTCAGCAAGGTCAGCTTC
ACSL1	CAACCCAGAACCATGGAAGT	GAGGGTGTTGGTTGGAAG
ARβ3	CCTTCCGTCGTCTTCTGTGT	ACTGTTGAGCGGTGGACTCT
ATP5α1	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
CD36	CCTTAAAGGAATCCCCGTGT	TGCATTTGCCAATGTCTAGC
ChREBP	CCAGCCTCAAGGTGAGCAAA	CATGTCCCGCATCTGGTCA
COX I	CTTTTATCCTCCCAGGATTTGG	GCTAAATACTTTGACACCGG
CPT1β	CCCATGTGCTCCTACCAGAT	CCTTGAAGAAGCGACCTTTG
CS	CAGCTACAGAAGGAAGTTGG	AGGAATAGCGAGGGTCAGTC
Cyclophilin	GCATACAGGTCCTGGCATC	CACCTTCCCAAAGACCACA
Cyt C	GGAGGCAAGCATAAGACTGG	TCCATCAGGGTATCCTCTCC
DGAT1	CCGTGTTTGCTCTGGCATC	TGACCTTCTTCCCTGTAGAG
DGAT2	CCTTCCTGGTGCTAGGAGTG	CCAGTCAAATGCCAGCCA
Dio2	GCACGTCTCCAATCCTGAAT	TGAACCAAAGTTGACCACCA
FAS	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
FH	GCTGAAGTAAACCAGGAGT	CAGTCTGCCAAACCACCA
GAPDH	CACTCTTCCACCTTCGATGC	CCCTGTTGCTGTAGCCGTAT
GCG	CAGGGCCATCTCAGAACC	GCTATTGGAAAGCCTCTTGC
GLUT1	CCATCCACCACACTCACCAC	GCCCAGGATCAGCATCTCAA
GLUT4	TGGCCCTAAGTATTCAAGTTCTG	TTCCTTCTATTTGCCGTCCTC
GPAM	CAGTAACGAGTCCAGAAACCC	CGCCATTGCTGTAGAAGTTGAG
IDH3α	GAGGTTTTGCTGGTGGTGTT	TCCTCCTGGTCCTTGAATTG
LDHA	TGTGTGGAGTGGTGTGAATG	ACCTGCTTGTGAACCTCCTT
LXRα	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
mtND1	GGGATAACAGCGCAATCCTA	ATCGTTGAACAAACGAACCA
PC	GATGACCTCACAGCCAAGCA	GGGTACCTCTGTGTCCAAAGGA
PGC1α	ACTGAGCTACCCTTGGGATG	TAAGGATTTCGGTGGTGACA
PGC1β	GGCAGGTTCAACCCCGA	CTTGCTAACATCACAGAGGATATCTTG
PPARα	TCTGGAAGCTTTGGTTTTGC	GACTGAGGAAGGGCTGGAAG
PPARγ	ACCACTCGCATTCCTTTGAC	TGGGTCAGCTCTTGTGAATG
PRDM16	TGGCCTTCATCACCTCTCAGAA	TTTCTGATCCATGGCTCCTGTGA
SDHB	ACCCCTTCTCTGTCTACCG	AATGCTCGCTTCTCCTTGTAG
SREBP1c	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGGCCAG
TFAM	CAGGAGGCAAAGGATGATTC	CCAAGACTTCATTTCATTGTCG
UCP1	CAGGATTGGCCTCTACGACTCA	CACTGCCACACCTCCAGTCA
LCAD	TCTTTTCCTCGGAGCATGACA	GACCTCTCTACTCACTTCTCCAG
MCAD	GATCCATCACCCTCGTGTAAC	AAGCCCTTTTCCCCTGAAG
SCAD	TCGCTGGTCCCTTCGTAGAT	TGGGATGGGCTTCAAAATAG

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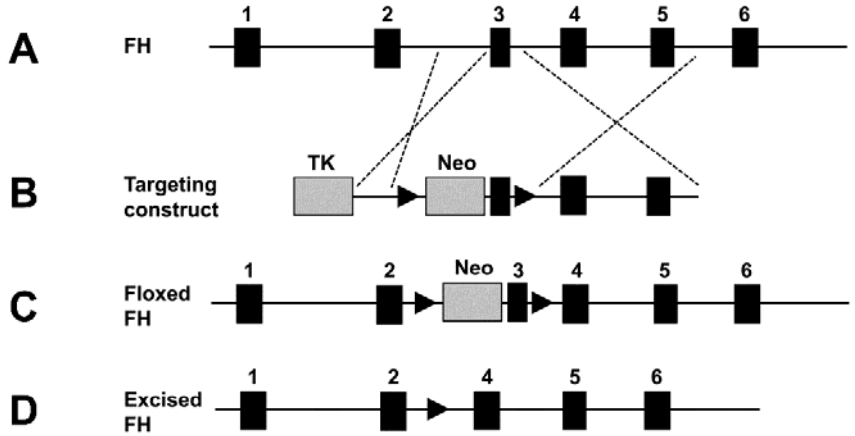
Supplementary Figure 1. Cre-mediated Gene Targeting of the *FH* Gene
(A) Genomic organization of the *FH* locus. Exons are shown as filled rectangles. (B) The targeting vector. The potential regions of recombination between the gene and the targeting vector are shown. (C) The targeted allele. (D) The excised FH allele. Neo, neomycin resistance cassette; TK, thymidine kinase; arrowheads, LoxP sites. The adenine of the initiation methionine codon is assigned position +1. An amplicon containing exon 3, 63 nt of intron 2 and 59 nt of intron 3 was cloned into a *BamHI* site engineered downstream of the neomycin resistance (Neo) gene of pMC1neo PolyA (Stratagene, La Jolla, CA). The normal *HindIII* site in exon 3 was removed without changing the peptide sequence by replacing the T8950 residue with A. Oligonucleotides containing LoxP sites were cloned into the 5' and 3' extremities of this insert. Then a 971 bp amplicon from intron 2 of mouse *FH* was cloned upstream of the 5' loxP site. Fourth, the long arm, a 4995 nt amplicon including FH exons 4 and 5, was cloned downstream of the 3' loxP site. Fifth, the thymidine kinase gene with a PGK promoter was inserted.



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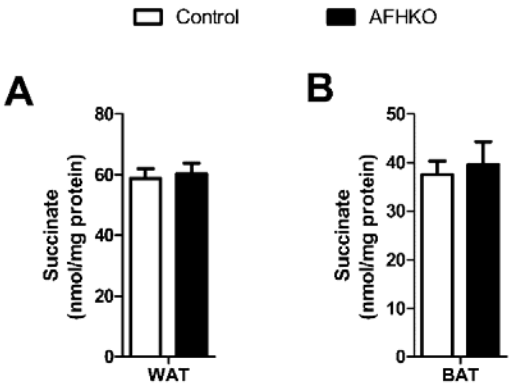
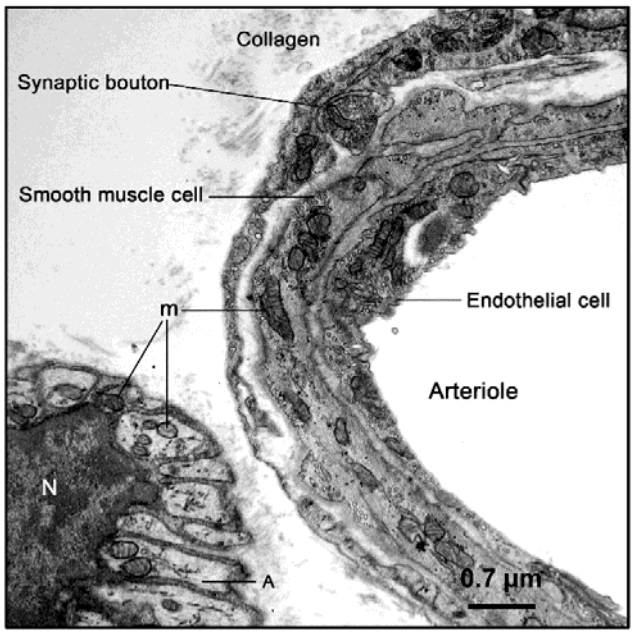
Supplementary Figure 2. Succinate levels in AFHKO adipose tissue.
Succinate levels in AFHKO WAT (A) and BAT (B). (5-month-old males, n=8).



SUPPLEMENTARY DATA

Supplementary Figure 3. Normal ultrastructure of mitochondria of non-adipose cells from AFHKO BAT. N, nucleus of a Schwann cell; m, mitochondria; A, axon.



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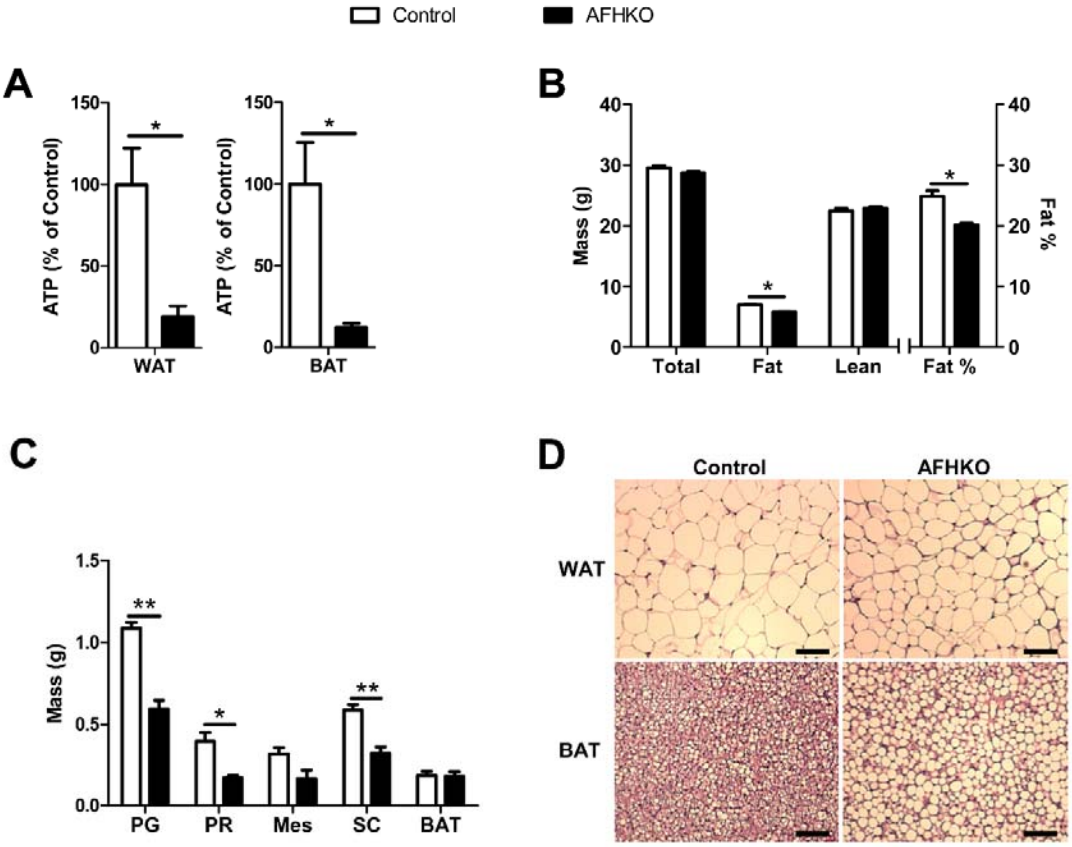
Supplementary Figure 4. 9-week-old AFHKO mice have low ATP and WAT mass.
(A) ATP levels in 9-week-old AFHKO WAT and BAT. (B) Body composition of 9-week-old mice. (C) WAT depot masses and BAT mass of control and AFHKO males at 9 weeks of age. PG, perigonadal; PR, perirenal; Mes, mesenteric; SC, subcutaneous; BAT, interscapular BAT. (D) Representative hematoxylin-eosin stained sections of WAT and BAT from 9-week-old control and AFHKO mice receiving a normal chow diet. Scale bar = 100 μm. (n=3). *, $P<0.05$; **, $P<0.01$.



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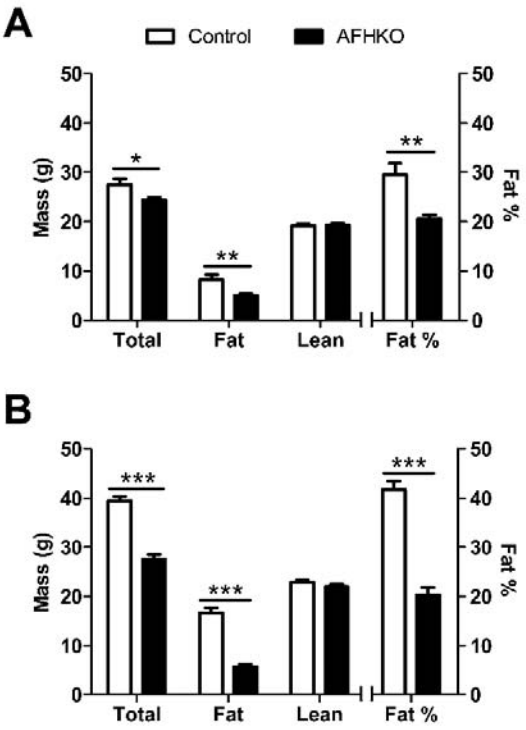
Supplementary Figure 5. Female AFHKO mice are lean.
Body composition of 5-month-old (A) and 10-month-old (B) AFHKO female mice (n=8). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.



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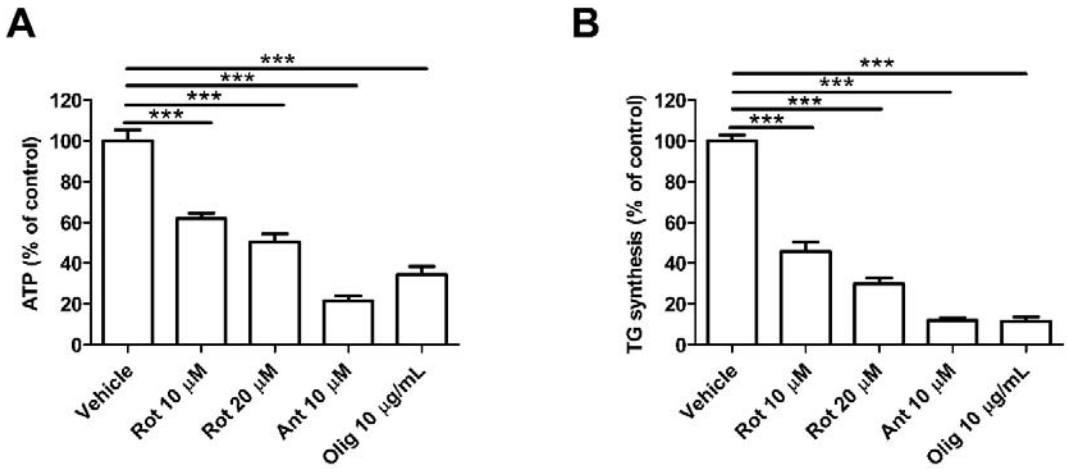
Supplementary Figure 6. ATP content and TG synthesis are reduced by respiratory chain inhibitors.

(A) ATP levels were measured in isolated perigonadal adipocytes treated with mitochondrial respiratory chain inhibitors for 3 hours (n=6). (B) TG synthesis in isolated normal perigonadal white adipocytes treated with the inhibitors for 3 hours (n=6). Rot, rotenone; Ant, antimycin A; Olig, oligomycin. ***, $P<0.001$.



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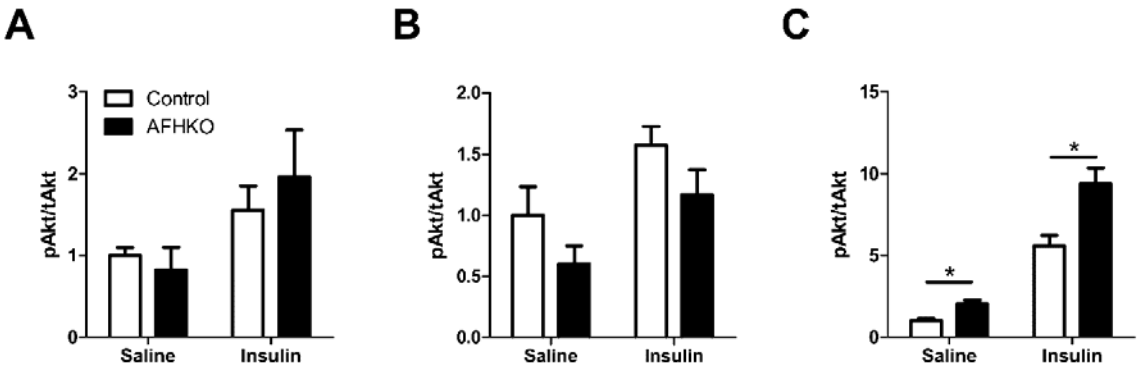
Supplementary Figure 7. Quantification of Akt Ser473 phosphorylation.
The level of Akt phosphorylation at Ser473 (pAkt) was quantified by Image J software and normalized to that of total Akt (tAkt). The pAkt/tAkt ratio of an estimated control tissue was assigned a value of 1.0. (A) WAT, (B) Liver and (C) Skeletal muscle. *, $P<0.05$.



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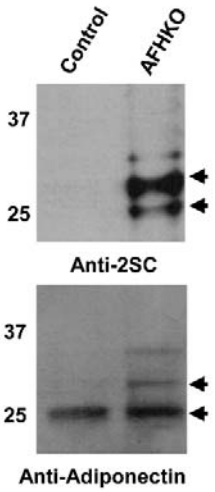
Supplementary Figure 8. Succination of adiponectin is increased in AFHKO Adipose Tissue.
Top, total adipose tissue lysates (0.45 mg of protein) were immunoprecipitated with anti-adiponectin antibody and analyzed by SDS-PAGE, and then probed with anti-2SC antibody to detect succination; bottom, the same amount of precipitated protein was probed with anti-adiponectin antibody. The numbers indicate molecular weights of marker proteins (Kilodaltons). Arrows indicate adiponectin monomers.



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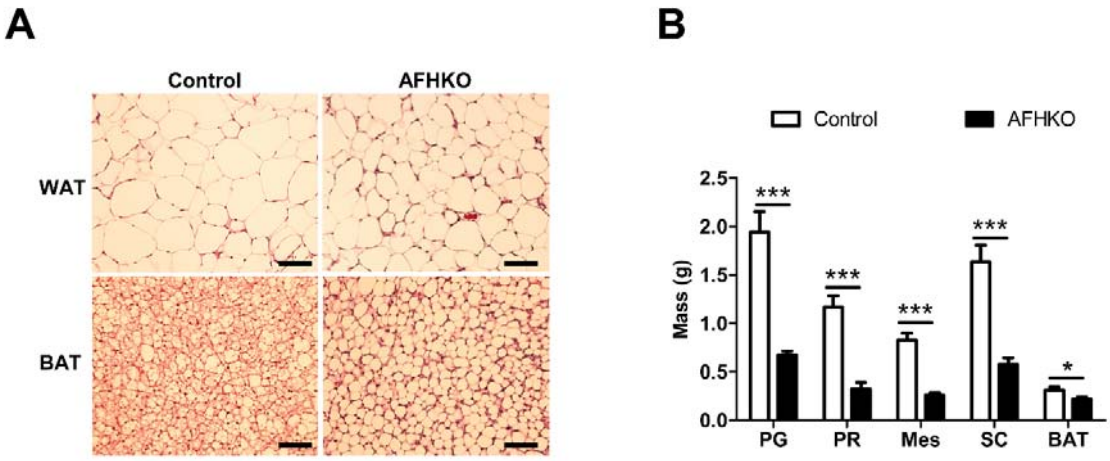
Supplementary Figure 9. AFHKO mice are resistant to HFD-induced obesity.
(A) Representative hematoxylin-eosin staining of perigonadal WAT and BAT (n=3). Scale bar, 100 μm. (B) Masses of different adipose tissue depots (n=8). PG, perigonadal; PR, perirenal; Mes, mesenteric; SC, subcutaneous; BAT, interscapular BAT. *, $P<0.05$; ***, $P<0.001$.



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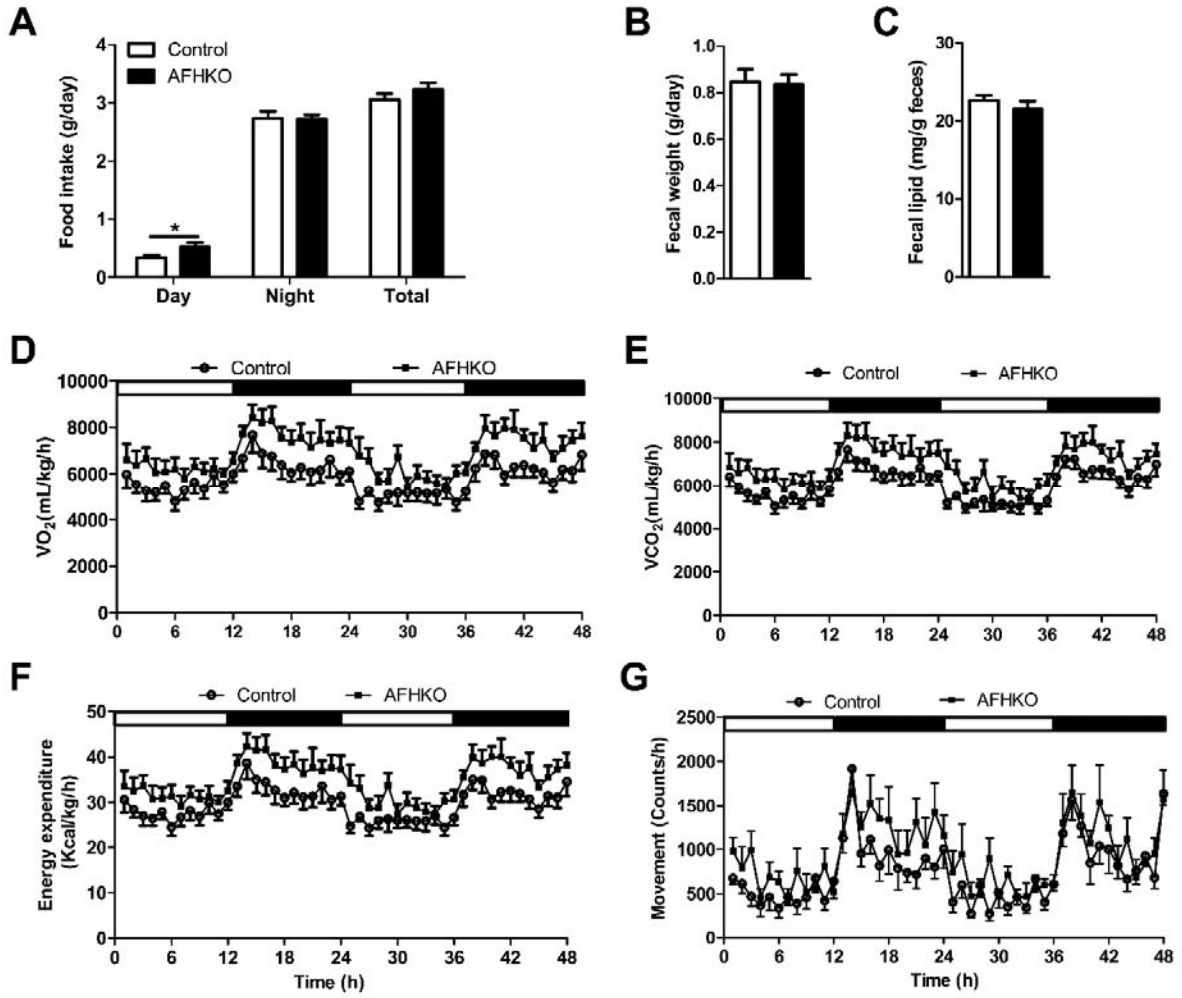
Supplementary Figure 10. Energy balance in AFHKO mice.
(A) Food intake was measured on chow diet. (B) Fecal weight and (C) fecal lipid content. (D-G) After acclimatization to the metabolic chamber for 48 h, calorimetry and other measurements were performed for 48 h with free access to food and water. (D) Oxygen consumption. (E) Carbon dioxide production. (F) Energy expenditure. (G) Physical activity. VO_2, VCO_2 and energy expenditure values were adjusted to lean mass. (5-month-old males, n=6). *, $P<0.05$.



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Supplementary Figure 11. Effects of thermoneutrality on energy metabolism in AFHKO mice. Eight pairs of AFHKO and control mice were raised at 21° C on chow diet for 9 months, then were housed individually at thermoneutrality (30° C) for 1 month. Food intake measurement (A) and Dexa scanning (B) were performed before placing at 30° C and after 1 month at 30° C. (C and D) Representative histological sections of perigonadal WAT (C) and BAT (D) (hematoxylin-eosin, n=3). Scale bar = 100 µm. (E) Fat mass increase of control and AFHKO mice after 1 month at 30° C. (F) Skeletal muscle TG content (quadriceps, n=8). *, $P<0.05$; ***, $P<0.001$.