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Supplemental Information

Lipid Droplets in Brown Adipose Tissue Are Dispensable for Cold-Induced Thermogenesis

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Supplementary Figure 1

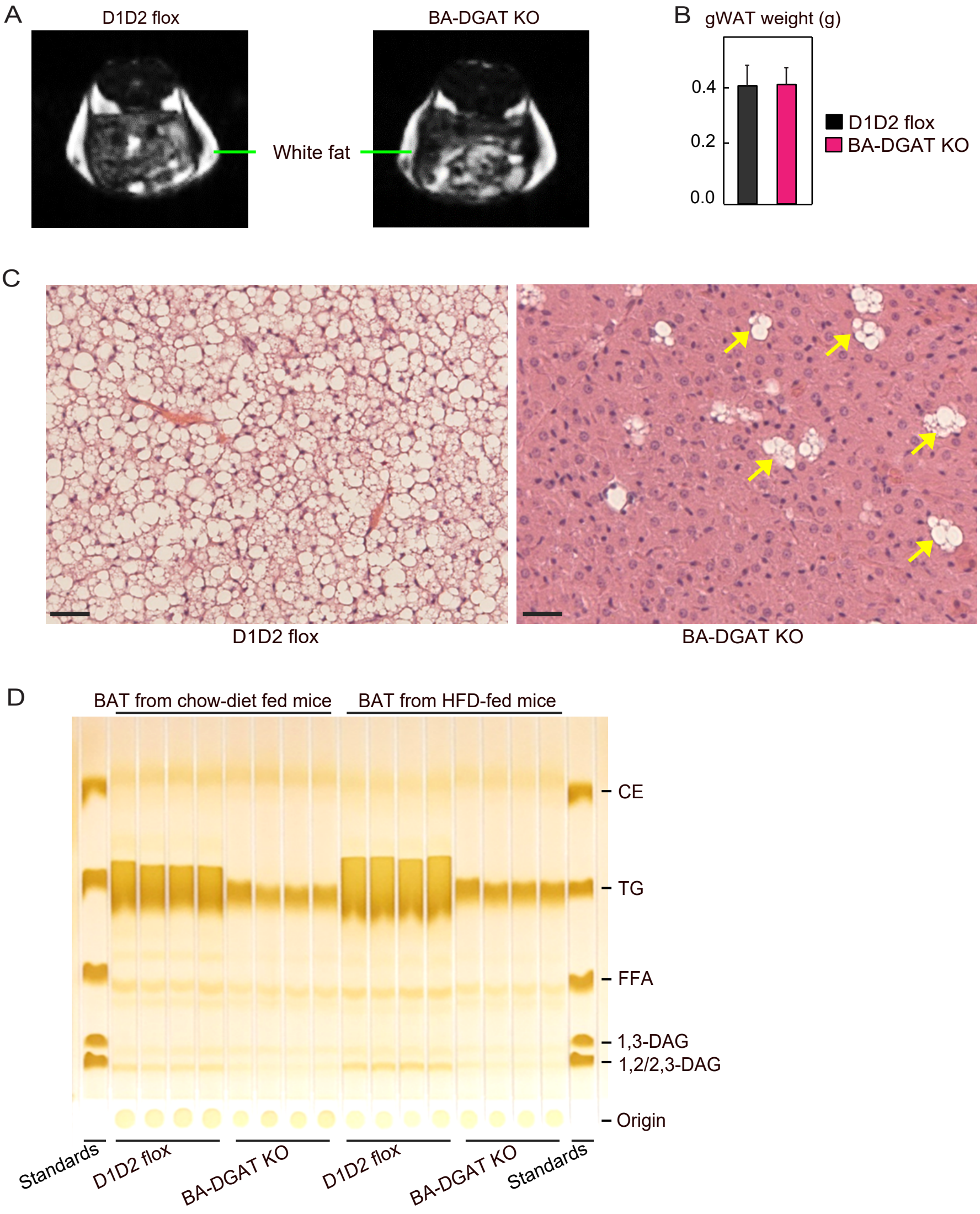
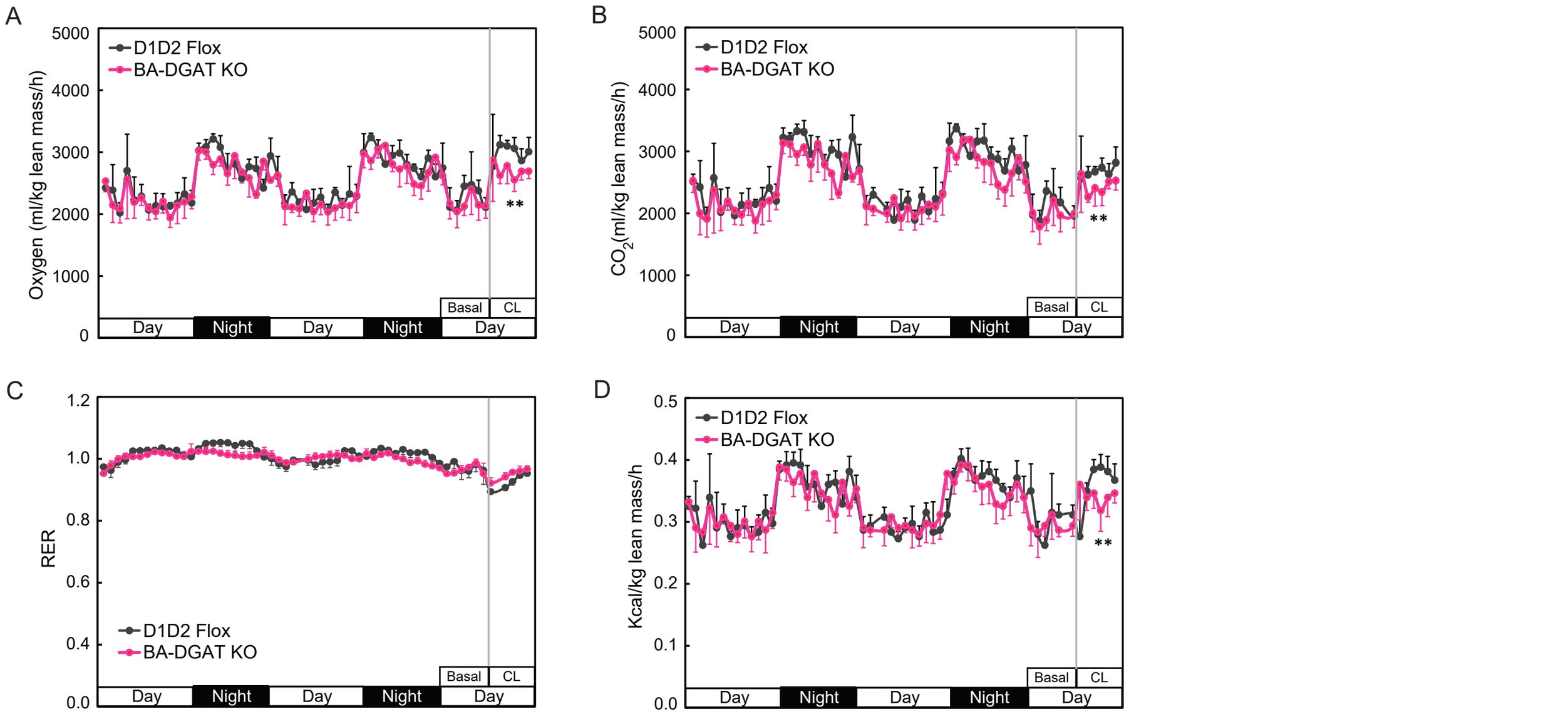


Figure S1. Related to Figure 1. Fat mass Analysis of BA-DGAT KO Mice and Thin Layer Chromatography Analysis of Neutral Lipids from BAT.
(A) Nuclear magnetic resonance imaging (MRI) scans of D1D2 flox and BA-DGAT KO mice (n=3).
(B) Weights of gonadal fat depots from chow-diet-fed mice (n=6).
(C) H&E-stained sections of BAT. LDs were absent in nearly all cells of BAT depots in BA-DGAT KO mice. A few cells of unknown identity (arrow heads) in BAT of BA-DGAT KO mice still had LDs. Scale bars, 50 μm.
(D) Thinlayer chromatography analysis of neutral lipids extracted from BAT. Triglycerides present in BAT of BA-DGAT KO mice account for few cells of unknown identity that still had LDs. CE, cholesterol esters; TG, triglycerides; FFA, free fatty acids; DAG, diacyl glycerol. (n=4). Lipids loaded onto TLC were normalized to proteins.
Data are presented as mean ± SD. ***p<0.001 by t-test.

Supplementary Figure 2



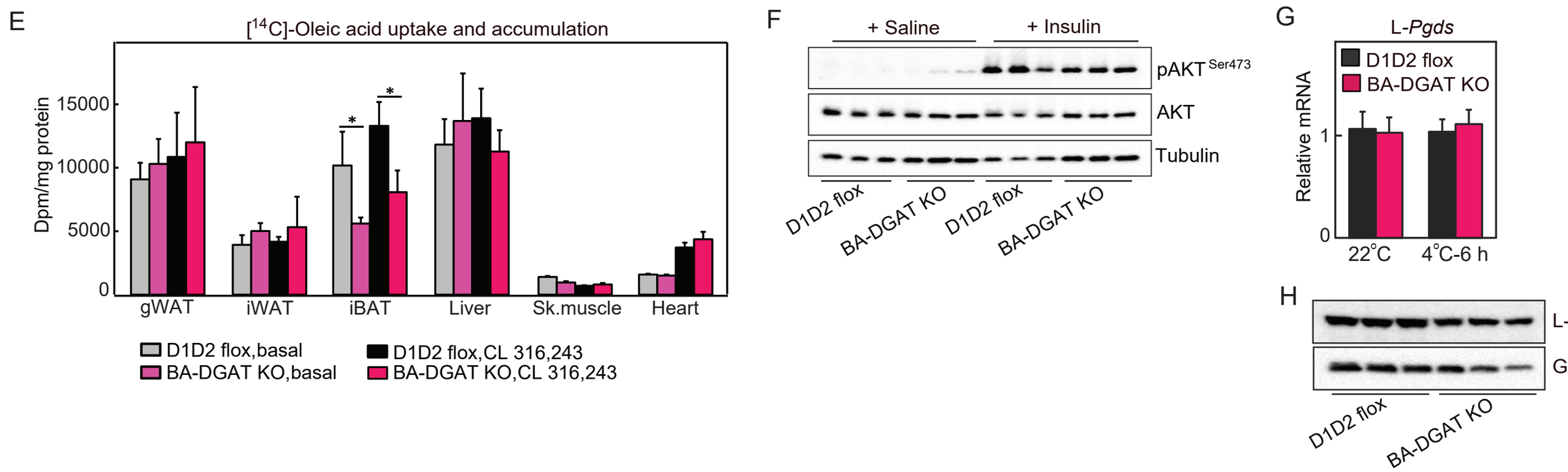
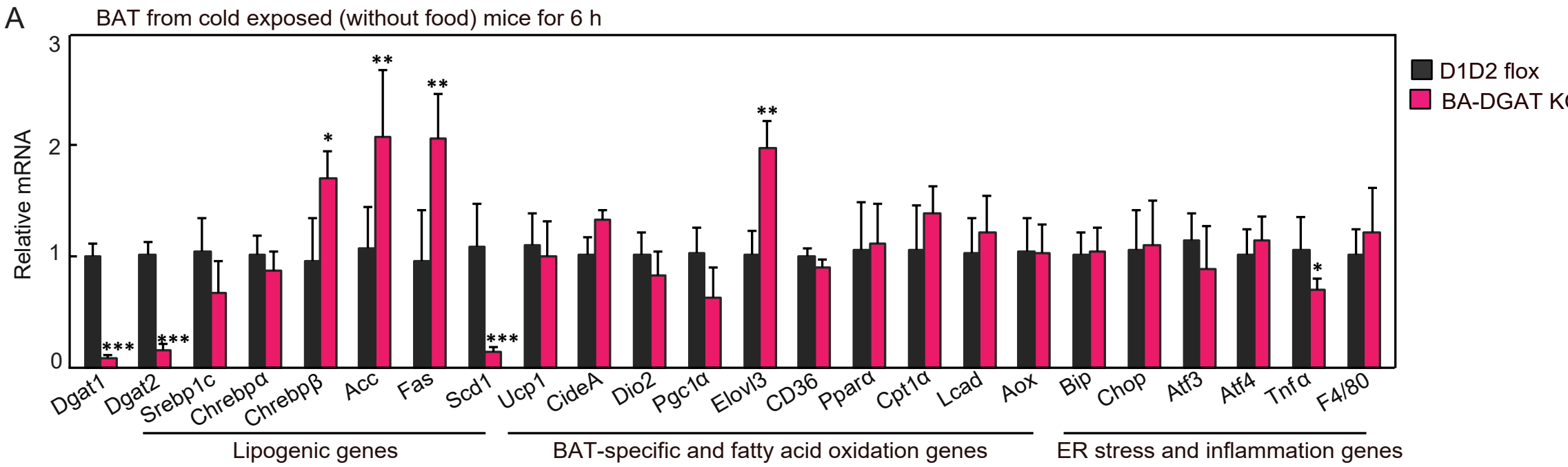
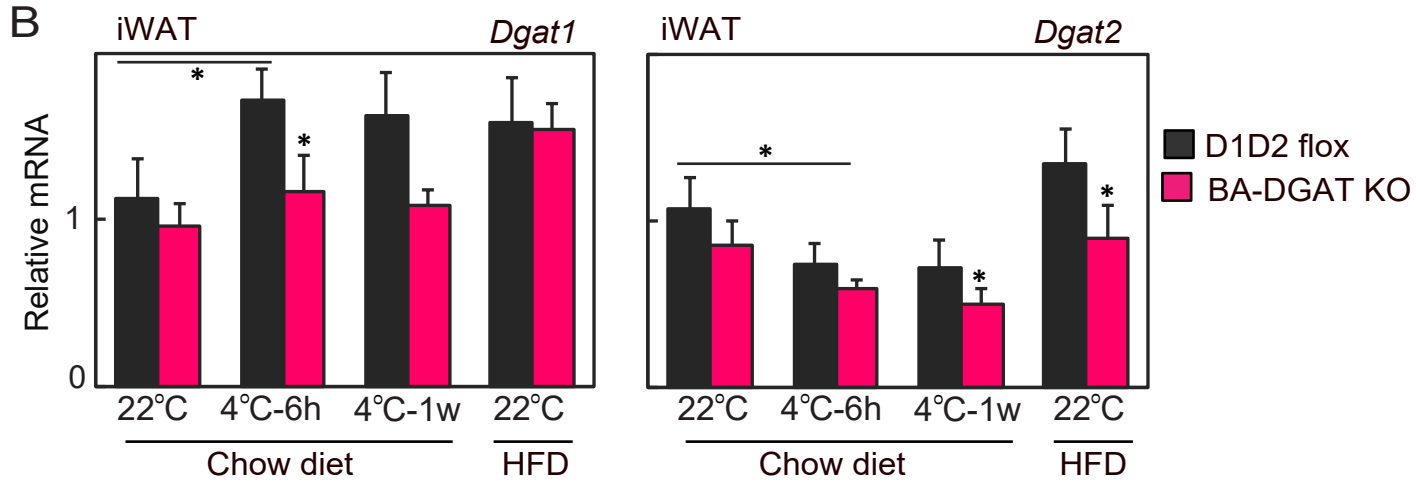
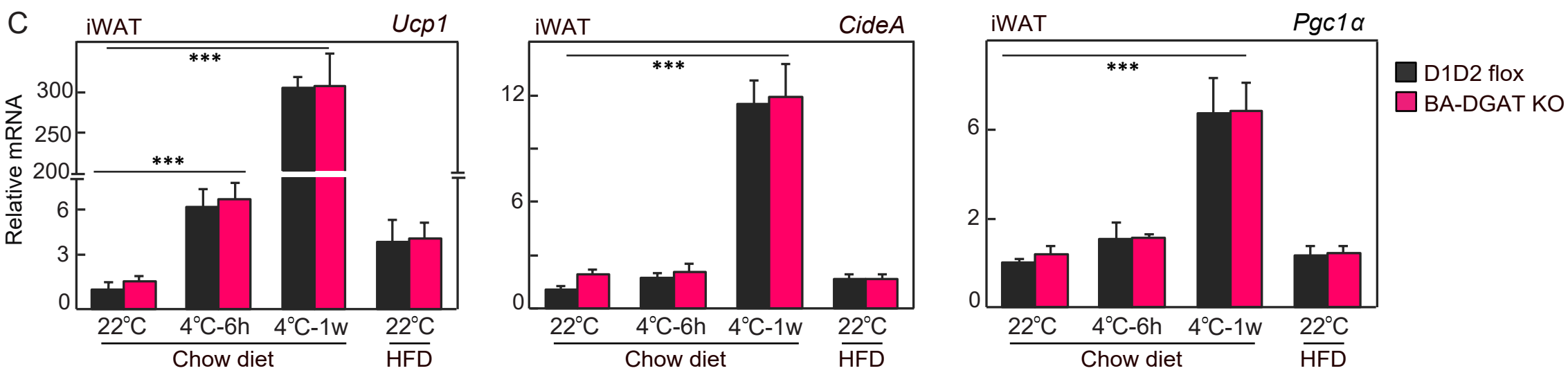


Figure S2. Related to Figures 2 and 3. Analysis of Insulin signalling in BAT and Indirect calorimetry analysis of BA-DGAT KO mice.
(A-D) Oxygen consumption, carbon dioxide release, energy expenditure and RER in basal or CL 316,243-administered mice. Each data point represents the mean of six readings per hour (n=4).
(E) [^{14}C]-oleic acid uptake by tissues in basal or in CL 316,243-administered mice (n=3).
(F) Western blot analysis of insulin signaling in BAT of basal or insulin-administered mice (n=3).
(G) mRNA levels of L-*Pgds* in BAT of mice housed at room temperature (22°C) or cold exposed for 6 hours (4°C-6h) (n=6).
(H) Immunoblot analysis of L-PGDS in BAT of mice housed at room temperature (n=3).
Data are presented as mean ± SD. *p<0.05, **p<0.01.

Supplementary Figure 3







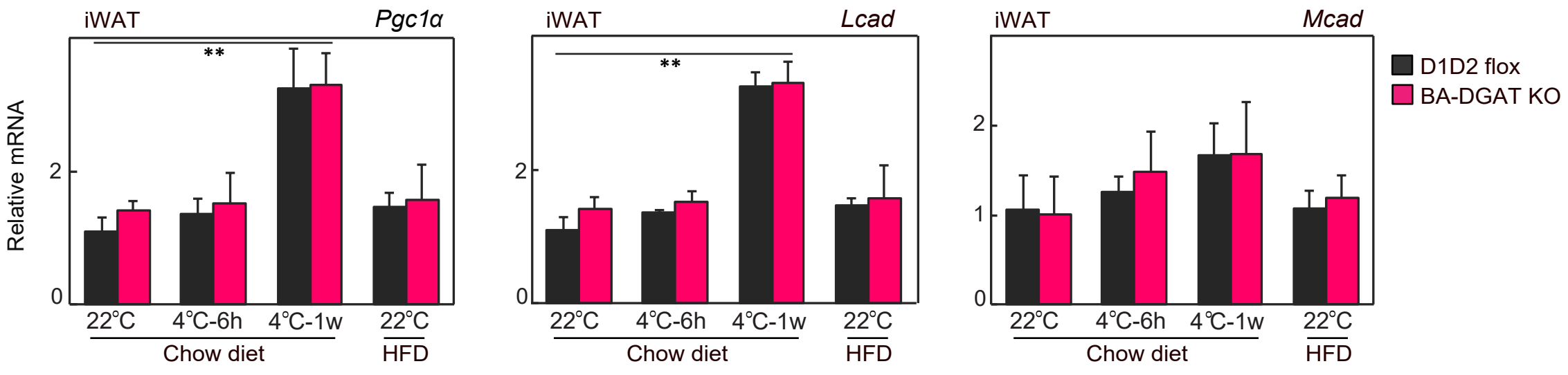


Figure S3. Related to Figure 3. Quantitative Real-Time PCR Analysis of genes in BAT and iWAT.
(A) mRNA levels in BAT of cold exposed (without food) mice for 6 hours (n=6).
(B) *Dgat1* and *Dgat2* mRNA levels in iWAT of acutely or chronically cold exposed or high-fat-diet fed mice (n=6).
(C) mRNA levels of thermogenic genes in iWAT of acutely or chronically cold exposed or high-fat-diet fed mice (n=6).
Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure 4

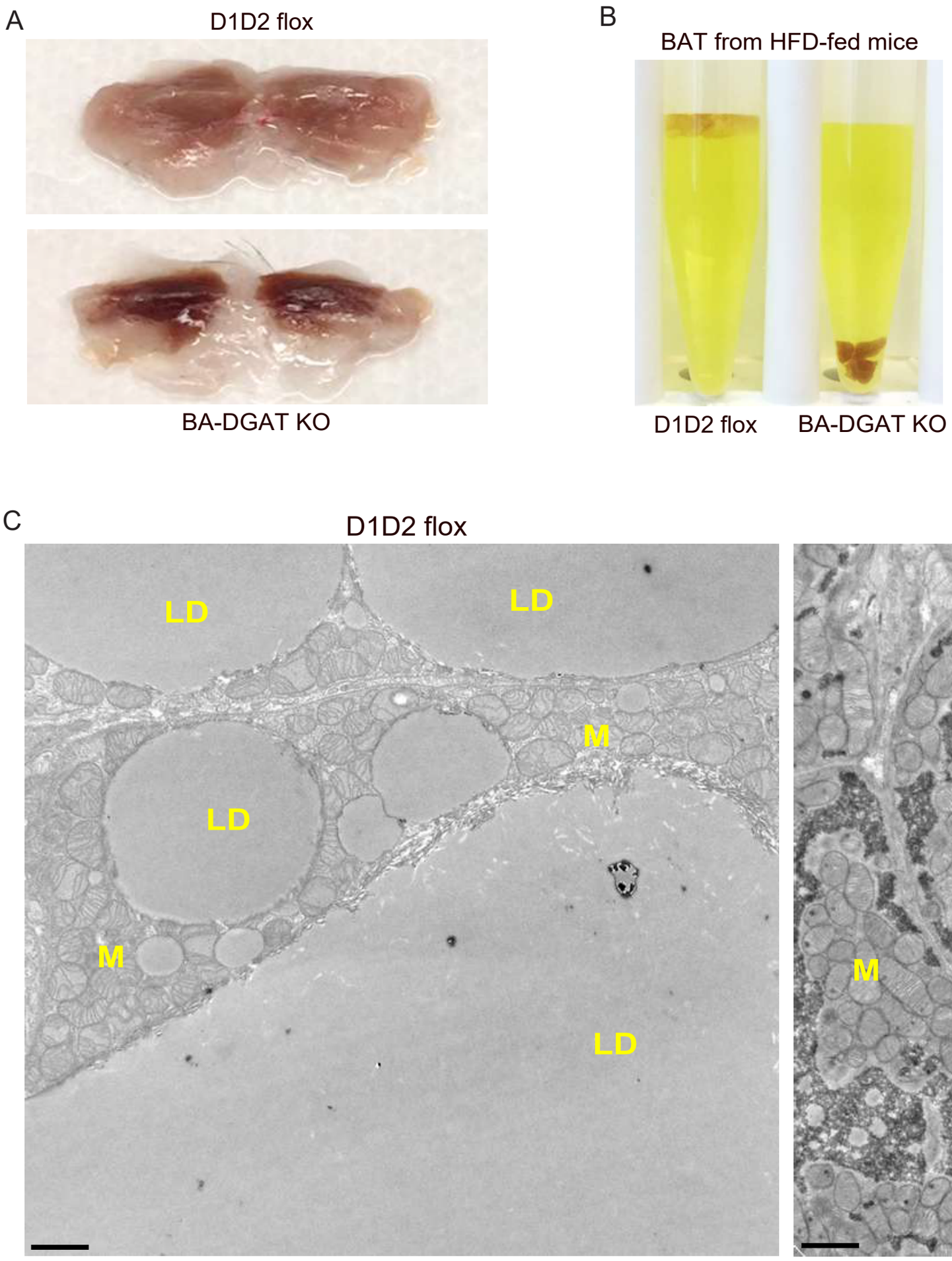


Figure S4. Related to Figure 4. Gross Appearance of BAT and TEM images of BAT from HFD-fed mice.
(A) Gross appearance of BAT from 12 weeks HFD-fed mice.
(B) BAT of HFD-fed BA-DGAT KO mice sinks in an aqueous buffer with fixative (1.25% formaldehyde, 2.5 % glutaraldehyde and 0.03% picric acid in 0.1 M sodium cacodylate buffer, pH 7.4, density of the fixative is 1.01 g/ml) used to fix BAT tissue for electron microscopy.
(C) TEM images of BAT from 12 weeks HFD-fed mice; scale bars, 2 μm. LD, lipid droplet; M, mitochondria; N, nucleus; Gly, glycogen.

Supplementary Table S1. Related to Figure 1

Plasma metabolites from *ad libitum* chow diet fed mice

Plasma metabolites	D1D2 flox	BA-DGAT KO
Glucose (mg/dl)	158 ± 9.5	146 ± 8.5*
Free fatty acids (m mol/l)	0.42 ± 0.09	0.41 ± 0.07
Free glycerol (m mol/l)	0.17 ± 0.06	0.19 ± 0.03
Triglycerides (mg/dl)	41 ± 8.8	31 ± 4.5*
Total cholesterol (mg/dl)	88 ± 18	77 ± 10
Insulin (ng/ml)	0.61 ± 0.07	0.59 ± 0.05

(n=8-11 mice) values are mean ± SD. *p<0.05

Supplementary Table S2. Related to STAR Method RNA Extraction and Quantitative Real-Time PCR

Primers used for quantitative Real-Time PCR analysis of mouse genes

Gene		Primer sequence (5'–3')
Cyclophilin	Fwd:	GGAGATGGCACAGGAGGAAA
	Rev:	CCGTAGTGCTTCAGTTTGAAGTTCT
Dgat1	Fwd:	GGAATATCCCCGTGCACAA
	Rev:	CATTTGCTGCTGCCATGTC
Dgat2	Fwd:	CCGCAAAGGCTTTGTGAA
	Rev:	GGAATAAGTGGGAACCAGATCAG
Glut1	Fwd:	GGCCAGATTCATCAACTGGAT
	Rev:	GCTCCAAGGCTGTACCCTAAG
Glut4	Fwd:	GTGACTGGAACACTGGTCCTA
	Rev:	CCAGCCAGTTGCATTGTAG
Glycogen synthase	Fwd:	GAACGCAGTGCTTTTCGAGG
	Rev:	CCAGATAGTAGTTGTCACCCCAT
Glycogenin	Fwd:	TGGAGTCTTTGTCTATCAACCCT
	Rev:	TTGCCCAGCCACTAAAATATGT
Srebp1c	Fwd:	GGAGCCATGGATTGCACATT
	Rev:	GGCCCGGGAAGTCACTGT
Chrebpα	Fwd:	CGACACTCACCCACCTCTTC
	Rev:	TTGTTCAGCCGGATCTTGTC
Chrebpβ	Fwd:	TCTGCAGATCGCGTGGAG
	Rev:	CTTGTCCCGGCATAGCAAC
Fas	Fwd:	GGAGGTGGTGATAGCCGGTAT
	Rev:	TGGGTAATCCATAGAGCCCAG
Acc	Fwd:	GATGAACCATCTCCGTTGGC
	Rev:	GACCCAATTATGAATCGGGAGTG
Elovl3	Fwd:	TTCTCACGCGGGTTAAAAATGG
	Rev:	GAGCAACAGATAGACGACCAC
Scd1	Fwd:	TTCTTGCGATACACTCTGGTGC
	Rev:	CGGGATTGAATGTTCTTGTCGT
Ucp1	Fwd:	AGGCTTCCAGTACCATTAGGT
	Rev:	CTGAGTGAGGCAAAGCTGATTT
CideA	Fwd:	TGCTCTTCTGTATCGCCCAGT
	Rev:	GCCGTGTTAAGGAATCTGCTG
Dio2	Fwd:	CAGTGTGGTGCACGTCTCCAATC
	Rev:	TGAACCAAAGTTGACCACCAG
Pgc1α	Fwd:	TTCATCTGAGTATGGAGTCGCT
	Rev:	GGGGGTGAAACCACTTTTGTAA
Pparα	Fwd:	AATGCAATTCGCTTTGGAAG
	Rev:	GGCCTTGACCTTGTTCATGT
Cpt1α	Fwd:	GAACCCCAACATCCCCAAAC
	Rev:	TCCTGGCATTGTCCTGGAAT
Mcad	Fwd:	AGGTTTCAAGATCGCAATGG
	Rev:	CTCCTTGGTGCTCCACTAGC
Lcad	Fwd:	TCCATGGCAAAATACTGGGC
	Rev:	TTGCAATCGGGTACTCCCAC
Aox	Fwd:	CTCATCTTCGAGGCTTGGAAACCAC
	Rev:	ATTTCACGGATAGGGACAAGAAAGGC
Cd36	Fwd:	ATGGGCTGTGATCGGAACTG
	Rev:	GTCTTCCCAATAAGCATGTCTCC
Bip	Fwd:	ACTTGGGGACCACCTATTCCT
	Rev:	ATCGCCAATCAGACGCTCC
Chop	Fwd:	CCACCACACCTGAAAGCAGAA
	Rev:	AGGTGAAAGGCAGGGACTCA
Atf3	Fwd:	GAGGATTTTGCTAACCTGACACC
	Rev:	TTGACGGTAACTGACTCCAGC
Atf4	Fwd:	CCTTCGACCAGTCGGGTTTG
	Rev:	CTGTCCCGGAAAAGGCATCC
Cd68	Fwd:	TGTCTGATCTTGCTAGGACCG
	Rev:	GAGAGTAACGGCCTTTTTGTGA
Tnfα	Fwd:	CCCTCACACTCAGATCATCTTCT
	Rev:	GCTACGACGTGGGCTACAG
F4/80	Fwd:	TGACTCACCTTGTGGTCCTAA
	Rev:	CTTCCCAGAATCCAGTCTTTCC
L-*Pgds*	Fwd:	GACAAGTTTCTGGGGCGCTGGT
	Rev:	GCTGTAGAGGGTGGCCATGCGG

Fwd, forward; Rev, reverse.