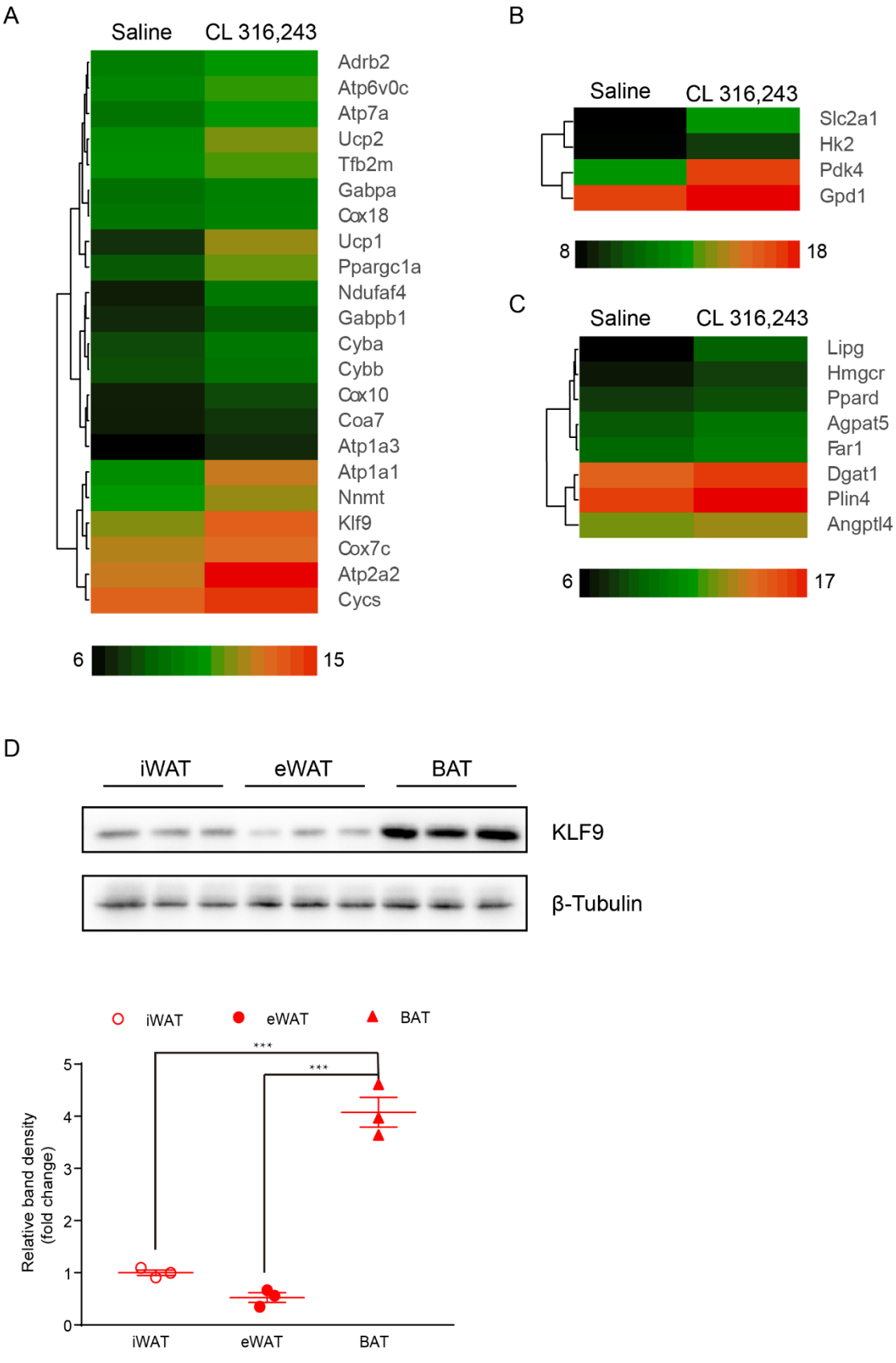
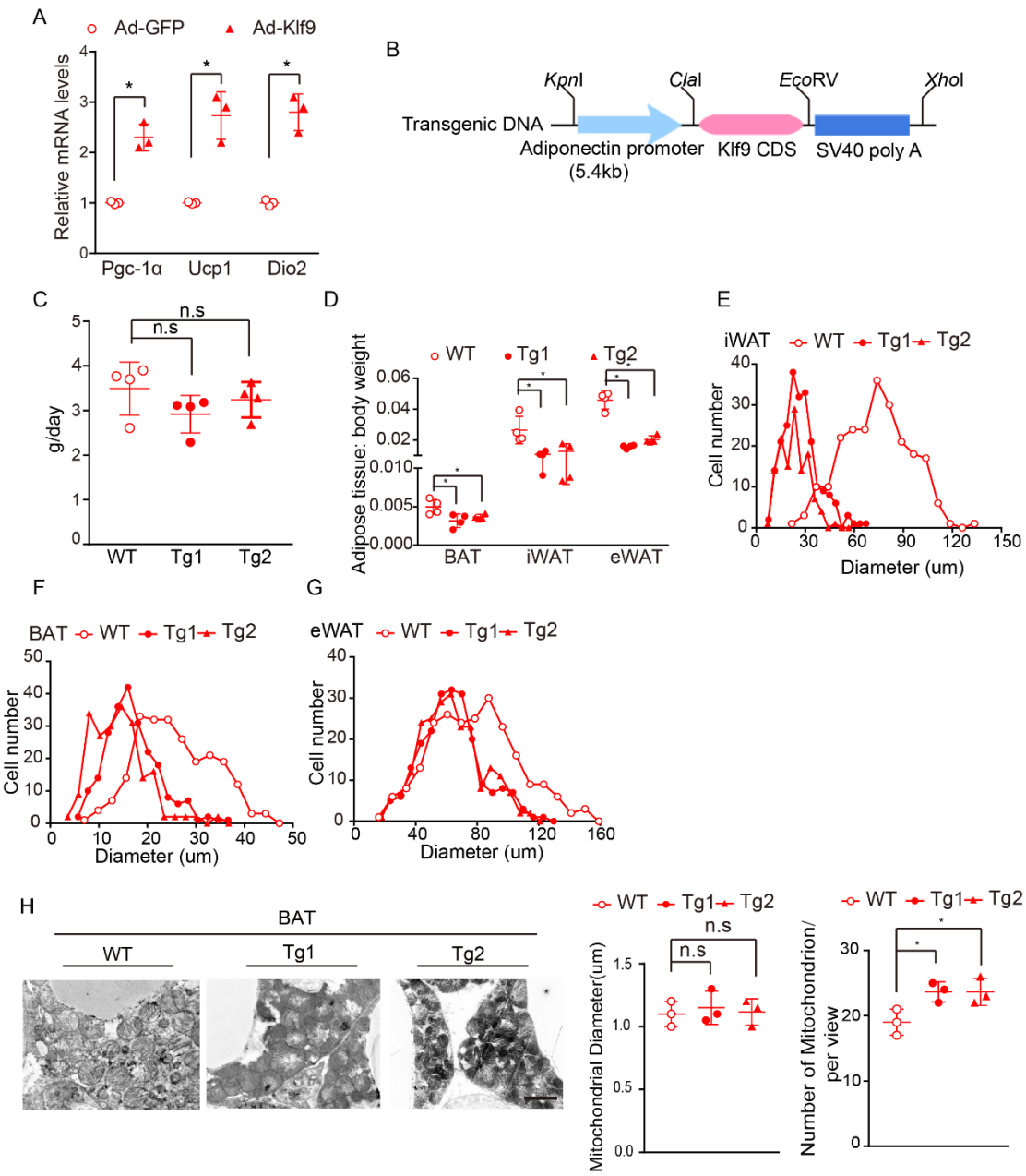
Supplemental Figures

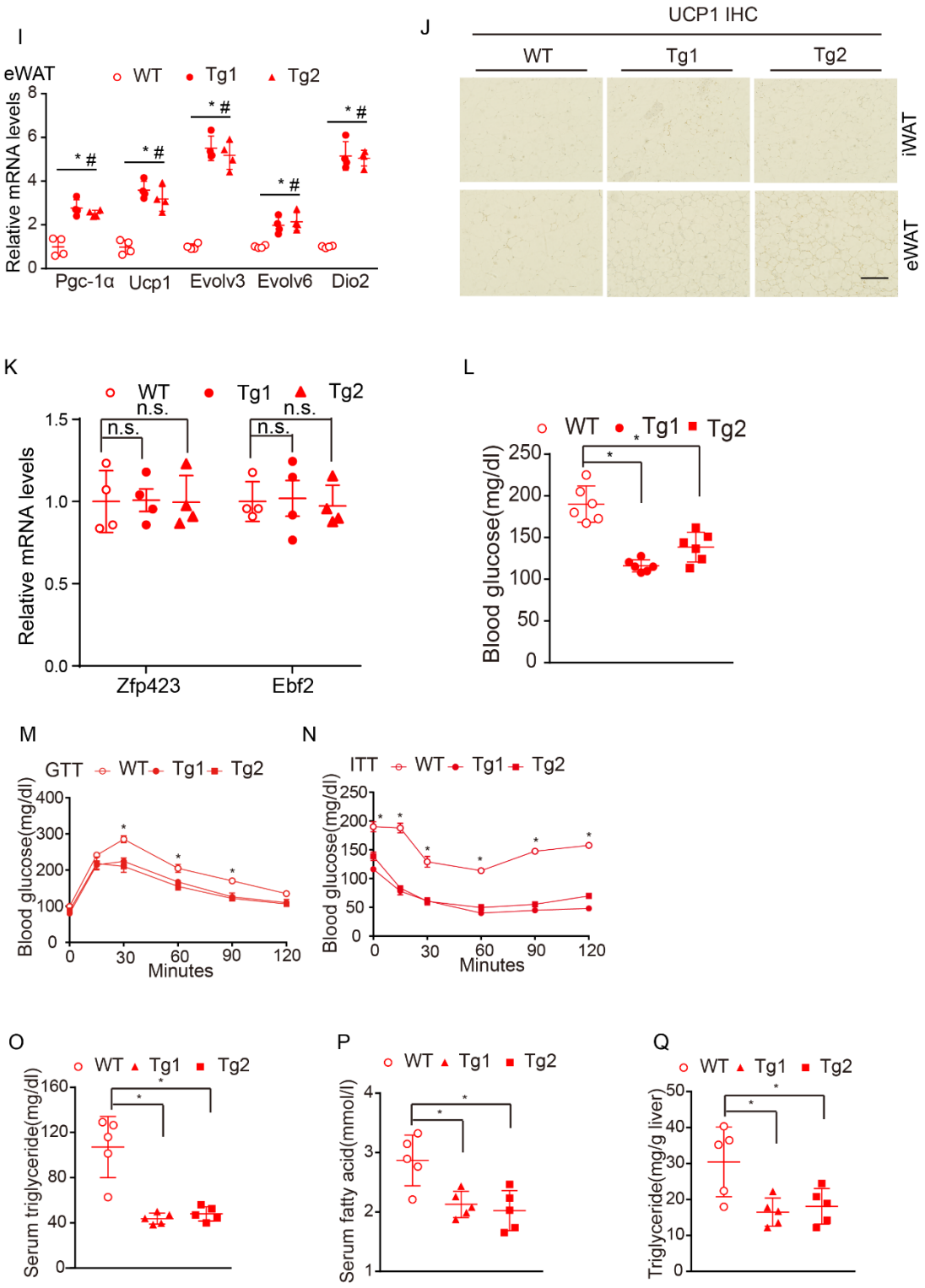


Supplementary Figure 1. β3-adrenergic agonist regulates the expression of Klf9

and other genes involved in glucose, lipid and energy metabolism in adipose of C57 BL/6J mice

(A-C) Heat map showing differentially expressed genes involved in energy metabolism (A), glucose metabolism (B) and lipid metabolism (C) by Affymetrix microarray analysis of iWAT of C57BL/6J mice treated with saline or CL316,243 (1 mg/kg) for 4 hours. mRNA Microarray analysis was performed at CapitalBio Technology (Beijing). (D) Western blot analysis of Klf9 protein levels in iWAT, eWAT and BAT of C57BL/6J mice (upper panel) and quantification of the target protein bands relative to tubulin control using ImageJ software (bottom panel). Throughout, data are presented as mean ± s.e.m. ***$P < 0.001$ by two-tailed Student's t-test (D).


A
Ad-GFP
Ad-Klf9
Relative mRNA levels
Pgc-1α
Ucp1
Dio2
B
KpnI
ClaI
EcoRV
XhoI
Transgenic DNA
Adiponectin promoter
(5.4kb)
Klf9 CDS
SV40 poly A
C
n.s
n.s
g/day
WT
Tg1
Tg2
D
WT
Tg1
Tg2
Adipose tissue: body weight
BAT
iWAT
eWAT
E
iWAT
WT
Tg1
Tg2
Cell number
Diameter (um)
F
BAT
WT
Tg1
Tg2
Cell number
Diameter (um)
G
eWAT
WT
Tg1
Tg2
Cell number
Diameter (um)
H
BAT
WT
Tg1
Tg2
WT
Tg1
Tg2
n.s
n.s
Mitochondrial Diameter(um)
WT
Tg1
Tg2
Number of Mitochondrion/
per view

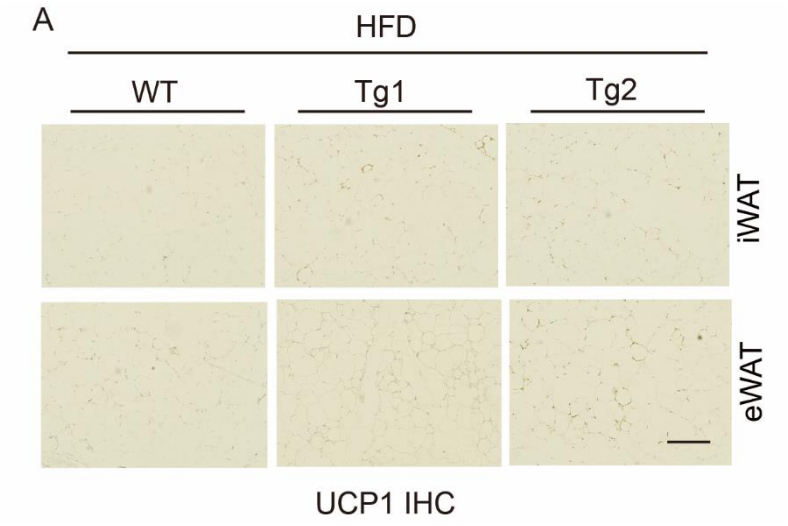


Supplementary Figure 2. Characteristics of adipose-specific Klf9 transgenic mice fed a chow diet

(A) Quantitative PCR analysis of mRNA levels of PGC-1α, Ucp1 and Dio2 in

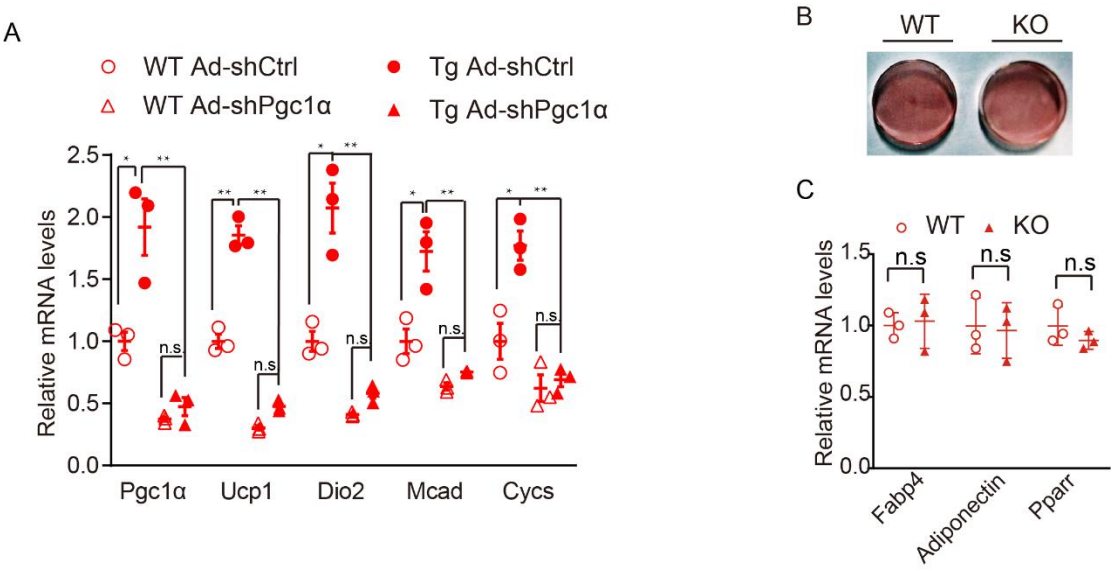
differentiated primary brown cell isolated from WT mice infected with Ad-GFP and Ad-Klf9 (n=3/group). (B) Schematic illustration of Klf9 transgenic construct containing full-length Klf9 cDNA driven by adiponectin promoter. (C) The amount of food intake of WT and Klf9 transgenic mice described in Figure 2C(n=4/group). (D) The ratio of weight of brown, inguinal and epididymal adipose tissue to body weight of mice in WT and Klf9 transgenic mice described in Figure 2C (n=4/group). (E-G) Quantification of the adipocyte size of iWAT (E), BAT (F) and eWAT (G) of the mice in Figure 2C (Data were collected from H&E-stained sections from five individual mice, three fields per mouse, 10–15 cells per field in each group, using Image J software). (H) Transmission electron microscopy showing mitochondrial morphology of BAT from WT and Klf9 transgenic mice (left panel) described in Figure 2C, followed with quantifications of mitochondrial diameter (middle panel) and mitochondrial number (right panel).(Scale bar: 5000x for 2μm.) (n = 3/group). (I) Quantitative PCR analysis of mRNA levels of genes involved in thermogenesis in the eWAT of mice described in Figure 2C (n=4/group). (J) Representative images of UCP1 immunohistochemistry of BAT from mice treated as in Figure 2C. (K) Quantitative PCR analysis of mRNA levels of Zfp423, Ebf2 in BAT from mice in 2C (n=3/group). (L) Blood glucose levels of WT and Klf9 transgenic mice fasted for 6 hours (n=6/group). (M, N) Blood glucose levels during GTT (M) and ITT(N) performed in WT and Klf9 transgenic mice described in Figure 2C (n=6/group). (O-Q) Serum concentrations of triglyceride (O), free fatty acids (P), (FFAs) and hepatic triglyceride (Q) in WT and Klf9 transgenic mice described in Figure 2C (n=5/group).

Throughout, data are presented as mean ± s.e.m. *P < 0.05 by two-tailed Student's t-test (A, C, D, H, I, K-Q).



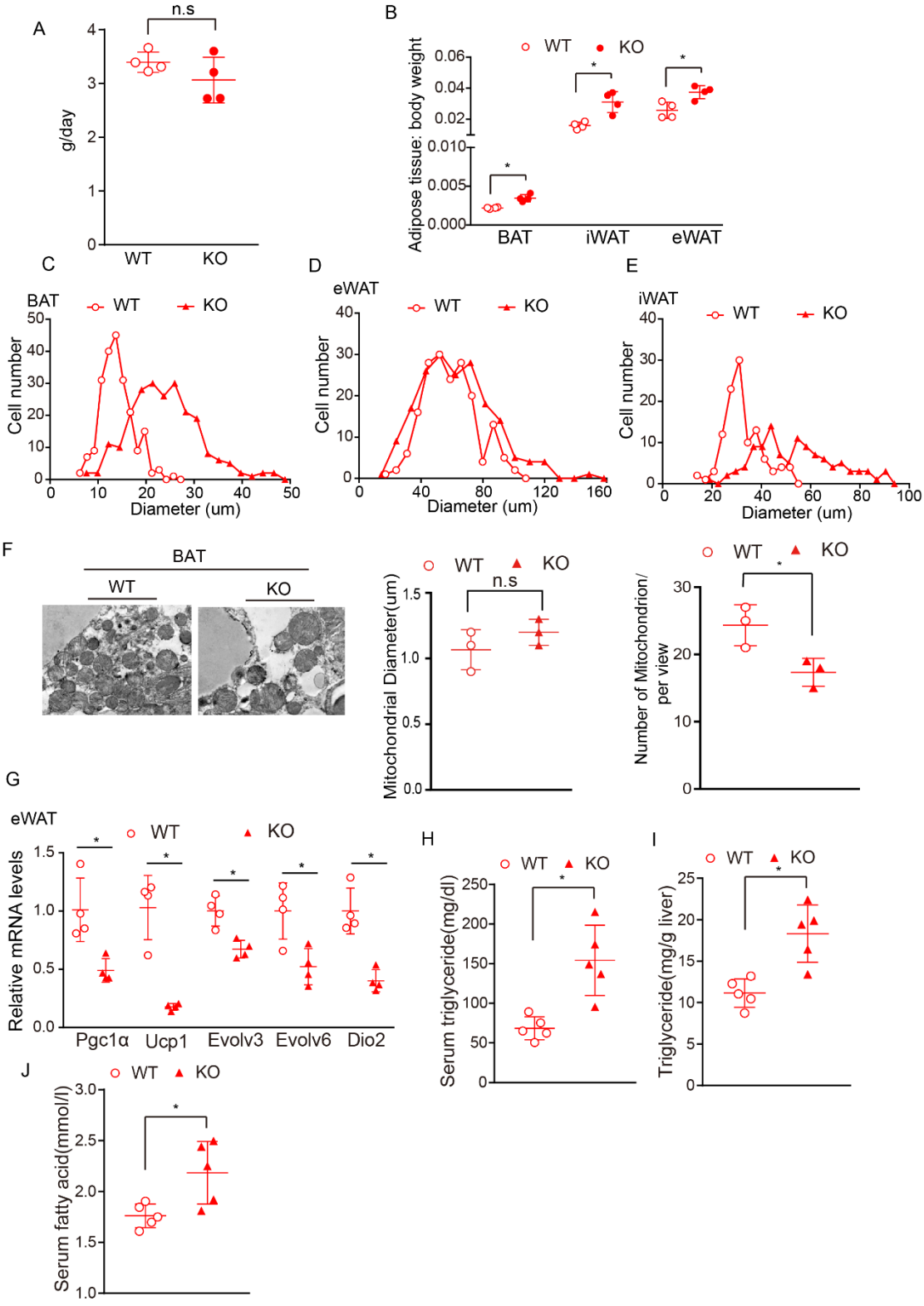
Supplementary Figure 3. Immunohistochemical analysis of UCP1 in iWAT and eWAT of Klf9 transgenic mice fed a high-fat diet

(A) Representative images of UCP1 immunohistochemistry of iWAT and eWAT from mice treated as in Figure 3B.



Supplementary Figure 4. Klf9 is not required for differentiation of primary brown adipocytes

(A) Quantitative PCR analysis of mRNA levels of genes involved in thermogenesis in differentiated brown cell from WT and Klf9 transgenic mice infected with the indicated adenoviruses (B) Oil red staining of differentiated primary brown cells from WT and Klf9-mutant mice. (C) Quantitative PCR analysis of mRNA levels of genes involved in adipogenesis in differentiated cells as described in A (n=3/group). Throughout, data are presented as mean ± s.e.m., *P < 0.05, **P<0.01 by two-way ANOVA (A), n.s. by two-tailed Student's t-test (C).



Supplementary Figure 5. Characteristics of global Klf9-mutant mice

(A) The amount of food intake of 5-month-old WT and global Klf9-mutant mice fed a

chow diet (n=4/group). (B)The ratio of weight of brown, inguinal and epididymal adipose tissue to body weight of 5-month-old WT and global Klf9-mutant mice fed a chow diet (n=4/group). (C-E) Quantification of adipocyte size of BAT (C), eWAT (D) and iWAT (E) of WT and global Klf9 mutant mice (Data were collected from H&E-stained sections from five individual mice, three fields per mouse, 10–15 cells per field in each group, using Image J software). (F) Transmission electron microscopy (left panel) showing mitochondrial morphology of BAT from WT and global Klf9 mutant mice described in B, quantifications of mitochondrial diameter (middle panel) and mitochondrial number (right panel).(Scale bar: 5000x for 2μm.) (n = 3/group) (G) Quantitative PCR analysis of mRNA levels of genes involved in thermogenesis in eWAT of mice as described in B (n=4/group). (H-J) Serum concentrations of triglyceride (H), hepatic triglyceride (I) and free fatty acids (FFAs) (J) of mice as described in B (n=5/group). Throughout, data are presented as mean ± s.e.m. *$P < 0.05$ by two-tailed Student's t-test (A, B and F-J).

Supplementary Table 1. List of specific primers used for real-time quantitative PCR gene expression analysis and ChIP analysis.

Gene symbol	Forward primer	Reverse primer
Klf9	5’-GCACAAGTGCCCCTACAGT-3’	5’-TGTATGCACTCTGTAATGGGCTTT-3’
Ucp1	5’-AGGCTTCCAGTACCATTAGGT-3’	5’-CTGAGTGAGGCAAAGCTGATTT-3’
Ppargc1a	5’-TGATGTGAATGACTTGGATACAGACA-3’	5’-GCTCATTGTTGTACTGGTTGGATATG-3’
Tfam	5’-GAAGGGAATGGGAAAGGTAGA -3’	5’-AACAGGACATGGAAAGCAGAT -3’
Nrf1	5’-GCACCTTTGGAGAATGTGGT-3’	5’-CTGAGCCTGGGTCATTTTGT-3’
Tfb2m	5’-CCAGAGTGGTTGCCTTTGA-3’	5’-TTCCTCTGTAAGGGCTCCA-3’
Cidea	5’-GCCGTGTTAAGGAATCTGCTG-3’	5’-TGCTCTTCTGTATCGCCCAGT-3’
Dio2	5’-CAGTGTGGTGCACGTCTCCAATC-3’	5’-TGAACCAAAGTTGACCACCAG-3’
Elovl3	5’-TTCTCACGCGGGTTAAAAATGG-3’	5’-GAGCAACAGATAGACGACCAC-3’
Cpt1a	5’-GAACCCCAACATCCCCAAAC-3’	5’-TCCTGGCATTCTCCTGGAAT-3’
Mcad	5’-AACACTTACTATGCCTCGATTGCA-3’	5’-CCATAGCCTCCGAAAATCTGAA-3’
Pparα	5’-ACAAGGCCTCAGGGTACCA-3’	5’-GCCGAAAGAAGCCCTTACAG-3’
Uqcrc2	5’-AAAGTTGCCCCGAAGGTTAAA-3’	5’-GAGCATAGTTTTCCAGAGAAGCA-3’
Ndufb8	5’-TGTTGCCGGGGTCATATCCTA-3’	5’-AGCATCGGGTAGTCGCCATA-3’
Sdhb	5’-AATTTGCCATTTACCGATGGGA-3’	5’-AGCATCCAACACCATAGGTCC-3’
Cycs	5’-GCAAGCATAAGACTGGACCA-3’	5’-TTGTTGGCATCTGTGTAAGAG-3’
Prdm16	5’-GAAGGTGTCCAAACTGACAATGC-3’	5’-CGTCACTTTTGGCTAGCTTCCT-3’
Atp5ase	5’-TCTCCATGCCTCTAACACTCG-3’	5’-CCAGGTCAACAGACGTGTCAG-3’

Acox1	5’-TCCAGACTTCCAACATGAGGA-3’	5’-CTGGGCGTAGGTGCCAATTA-3’
Elovl6	5’-GAAAAGCAGTTCAACGAGAACG-3’	5’-AGATGCCGACCACCAAAGATA-3’
Cidec	5’-ATGGACTACGCCATGAAGTCT-3’	5’-CGGTGCTAACACGACAGGG-3’
Cox8b	5’-TGTGGGGATCTCAGCCATAGT-3’	5’-AGTGGGCTAAGACCCATCCTG-3’
Pparg	5’-TCAGCTCTGTGGACCTCTCC-3’	5’-AACCCTTGCATCCTTCACAAG-3’
Zfp423	5’- CAGGCCCACAAGAAGAACAAG-3’	5’- GTATCCTCGCAGTAGTCGCACA-3’
Ebf2	5’- GCTGCGGGAACCGGAACGAGA-3’	5’- ACACGACCTGGAACCGCCTCA-3’
36B4	5’-GAGGAATCAGATGAGGATATGGGA-3’	5’- AAGCAGGCTGACTTGGTTGC-3’
Pgc-1α ChIP primers	5’-AAGACAGGTGCCTTCAGTTC-3’	5’-CCAGGAATCATTGCATCTGA-3’