Foxj3 regulates thermogenesis of brown and beige fat via induction of PGC-1α

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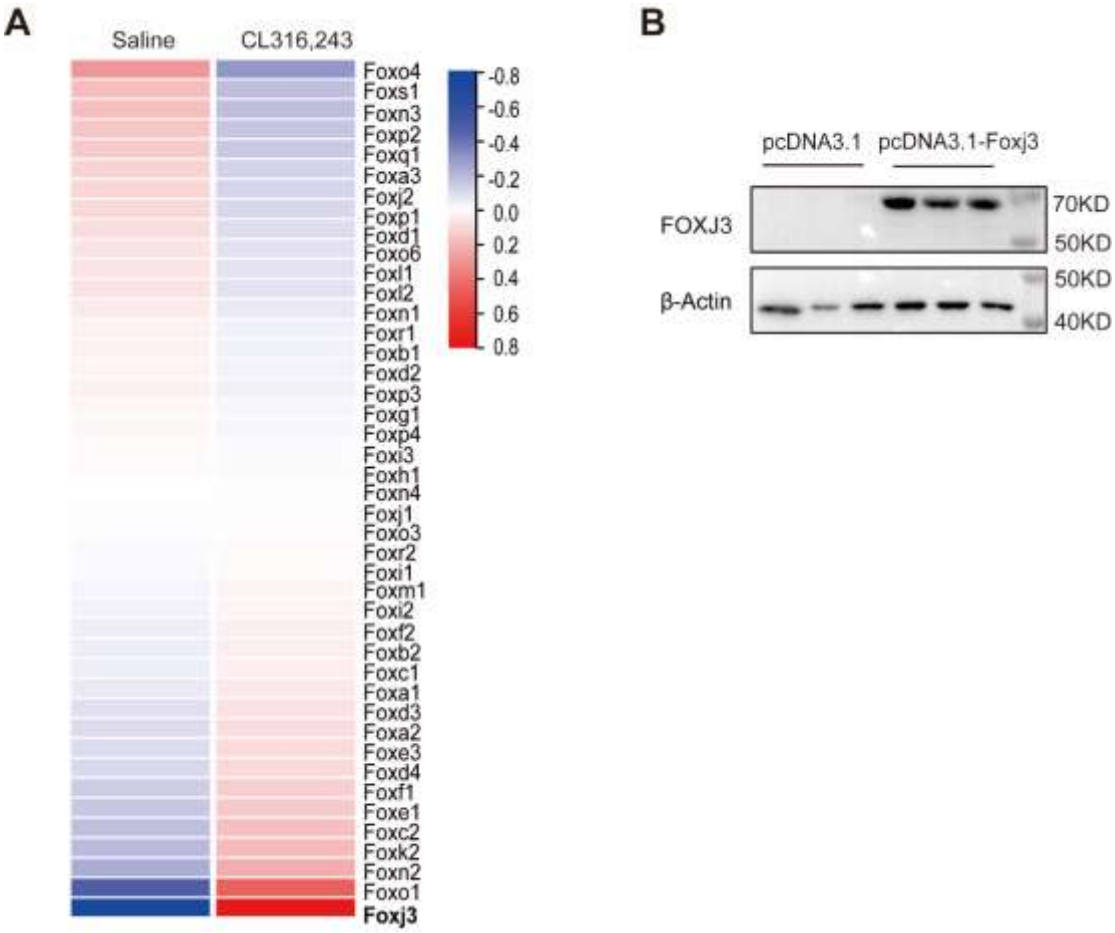
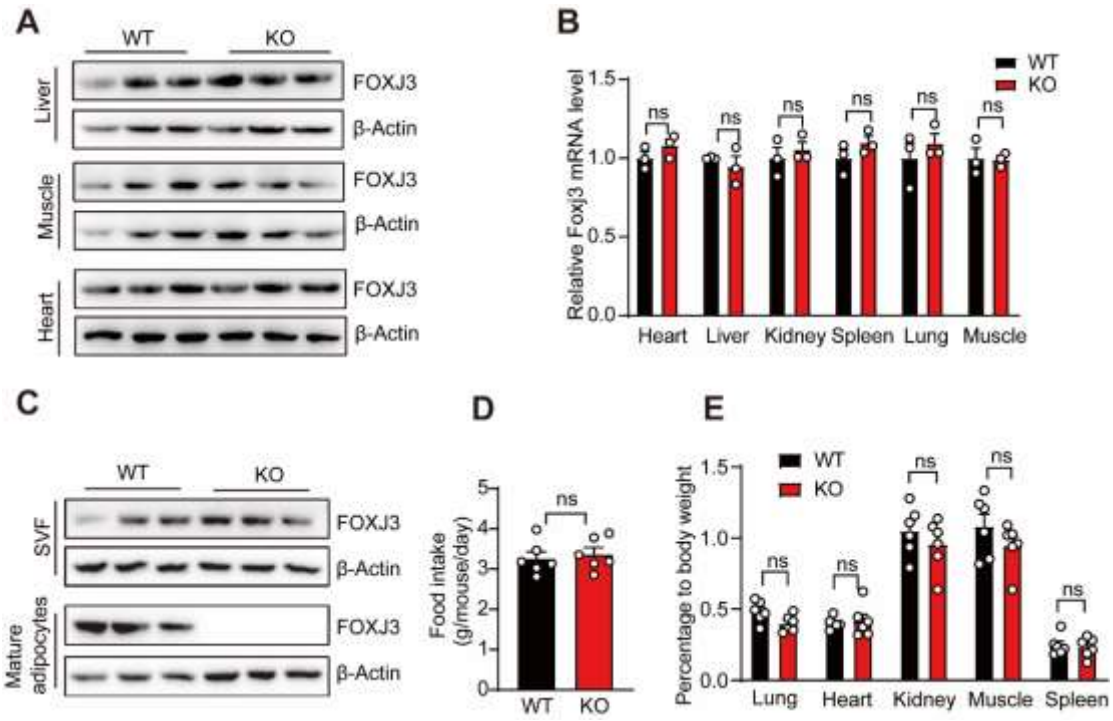
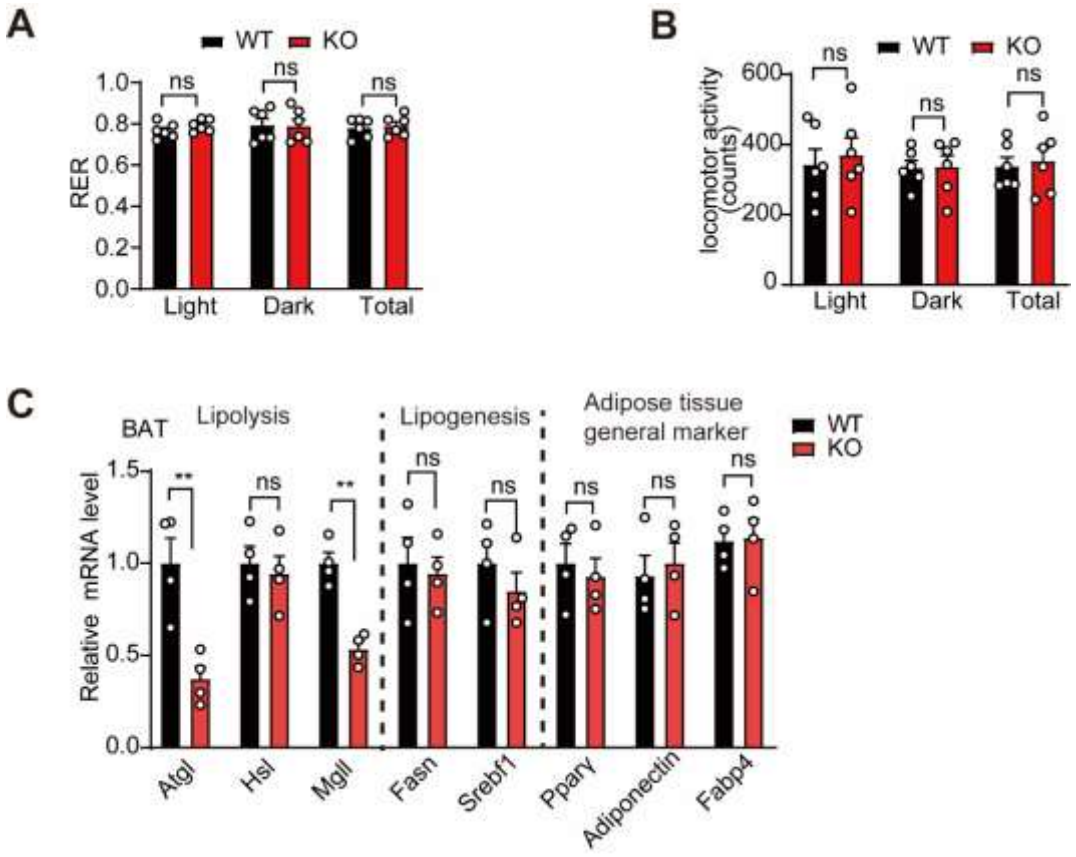
Supplementary Figures



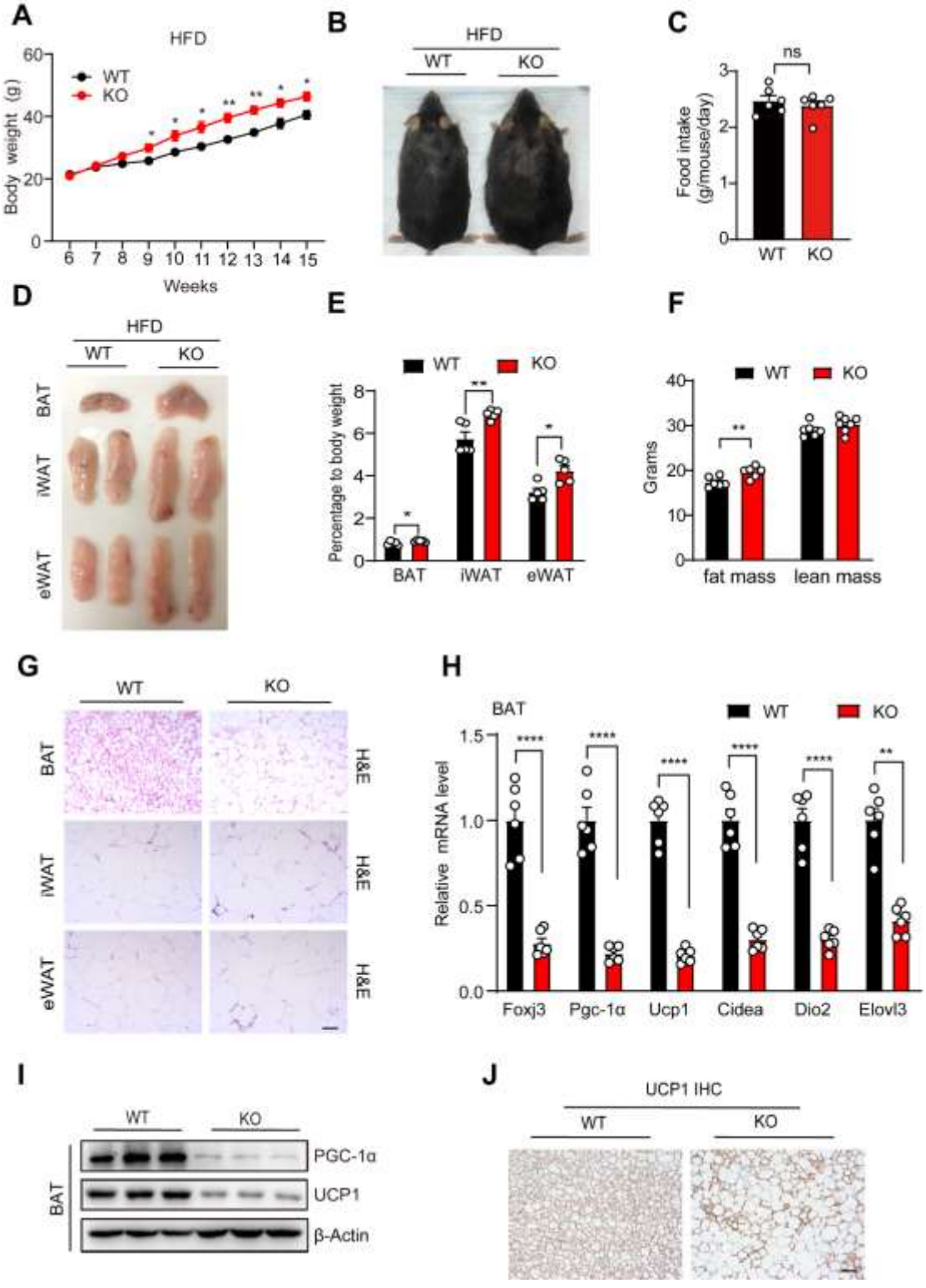
Supplementary Figure 1. Foxj3 expression in brown fat is highly induced by CL316,243. A: Heat map showing the mRNA levels of Fox family members by mRNA microarray analysis of BAT of C57BL/6J mice treated with saline or CL316,243 (1mg/kg) for 4 h. B: Western blot analysis of the protein levels of Foxj3 and β-Actin in HEK293T cell transfected with pcDNA3.1and pcDNA3.1-Foxj3 plasmids for 48h.



Supplementary Figure 2. Ablation of Foxj3 in adipose tissue predisposes mice to obesity, insulin resistance, and hepatosteatosis on a chow diet. A: Western blot analysis of the protein levels of Foxj3 in liver, muscle and heart of the mice described in Figure 2A. B: Quantitative PCR analysis of mRNA levels of Foxj3 in different tissues from the mice described in Figure 2A (n = 3/group). C: Western blot analysis of the protein levels of Foxj3 in mature adipocytes or SVF cells isolated from newborn 24-hour Foxj3$^{ad-/-}$ and Foxj3$^{flox/flox}$ mice. D: The amount of food intake of the mice described in Figure 2C (n = 6/group). E: The ratio of weight of lung, heart, kidney, muscle, and spleen to body weight from the mice described in Figure 2C (n = 6/group). Data are represented as mean ± SEM. Abbreviations: n.s., not significant.



Supplementary Figure 3. Foxj3 deficiency in fat reduces whole-body energy expenditure and impairs the thermogenic function of BAT. A: Respiratory exchange ratio of the mice described in Figure 3A (n = 6/group). B: Locomotor activity of the mice described in Figure 3A (n = 6/group). C: Quantitative PCR analysis of mRNA levels of genes involved in lipolysis, lipogenesis and adipogenesis in BAT of the mice described in Figure 3G (n = 4/group). Data are mean ± SEM. *P < 0.05, **P < 0.01 by two-tailed Student's t-test (C). Abbreviations: n.s., not significant.


A
HFD
WT
KO
Body weight (g)
Weeks
B
HFD
WT
KO
C
ns
Food intake (g/mouse/day)
WT
KO
D
HFD
WT
KO
BAT
iWAT
eWAT
E
WT
KO
Percentage to body weight
BAT
iWAT
eWAT
F
WT
KO
Grams
fat mass
lean mass
G
WT
KO
BAT
iWAT
eWAT
H&E
H
BAT
WT
KO
Relative mRNA level
Foxj3
Pgc-1α
Ucp1
Cidea
Dio2
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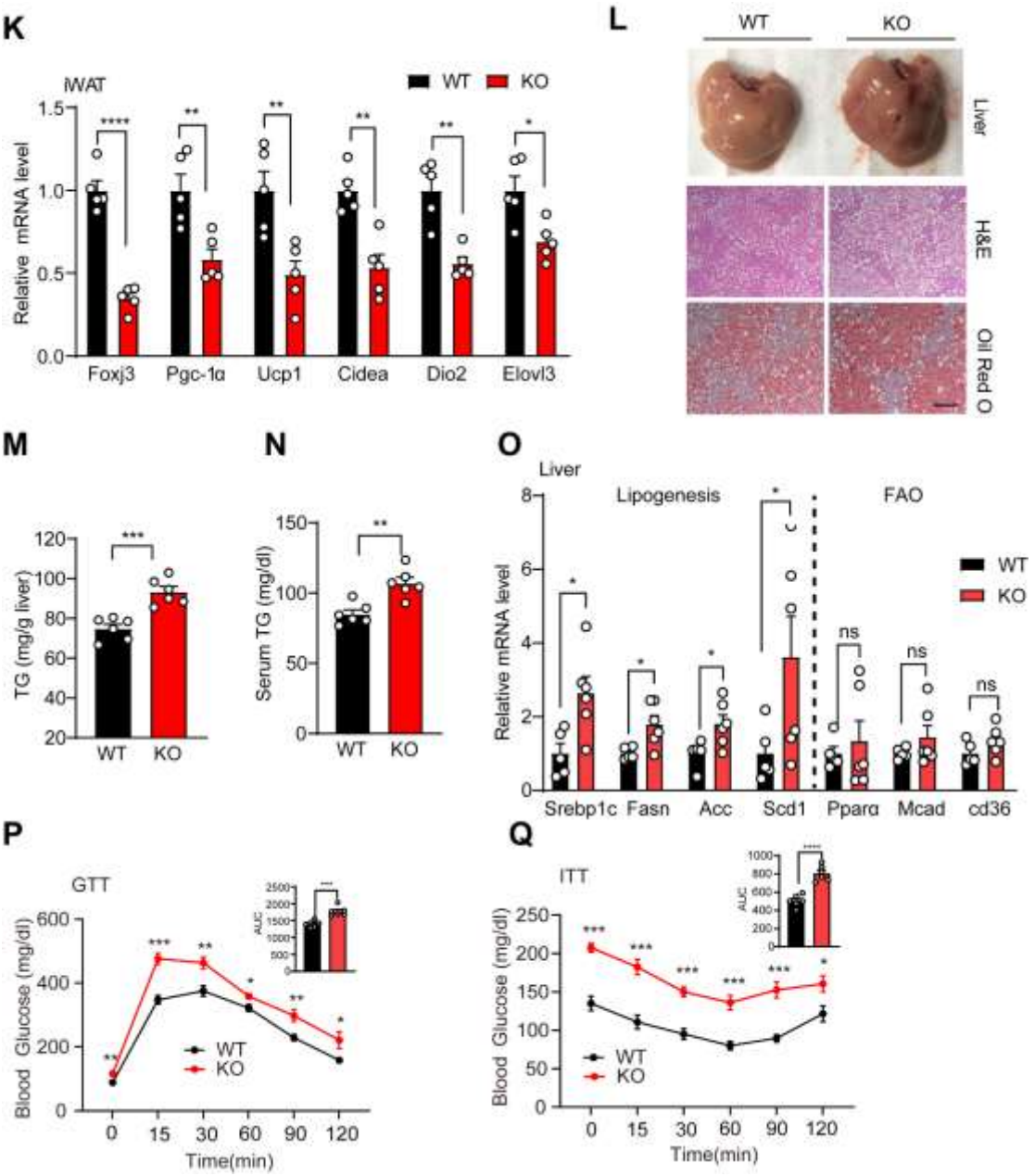
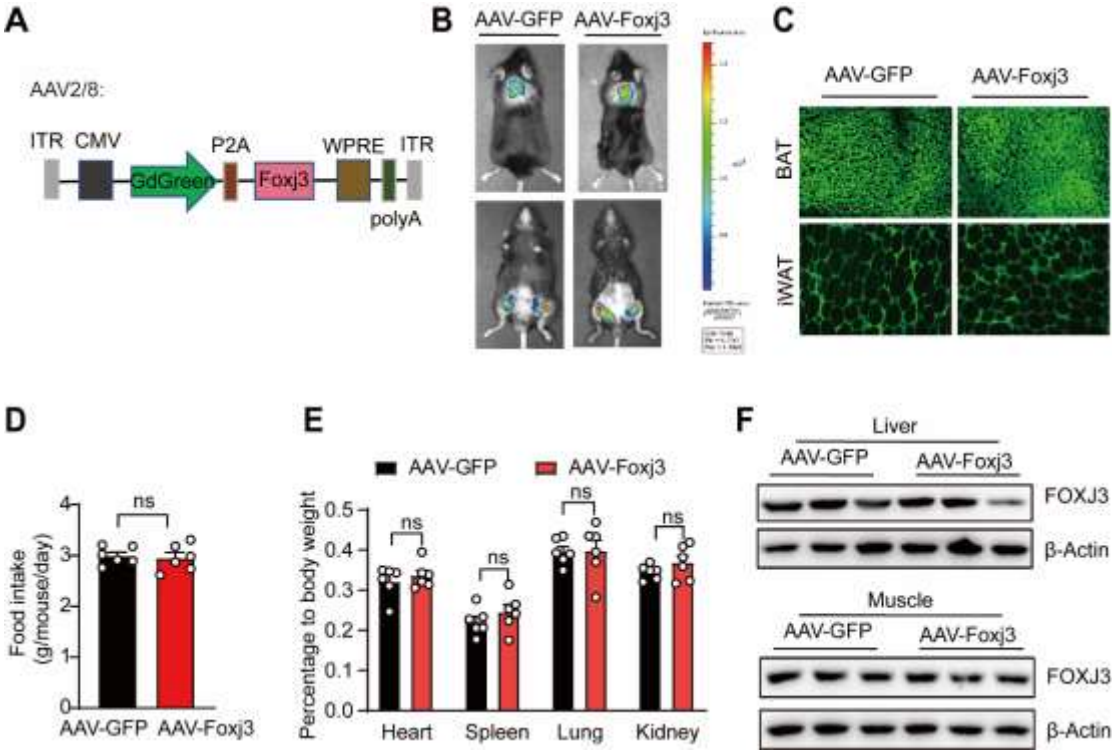
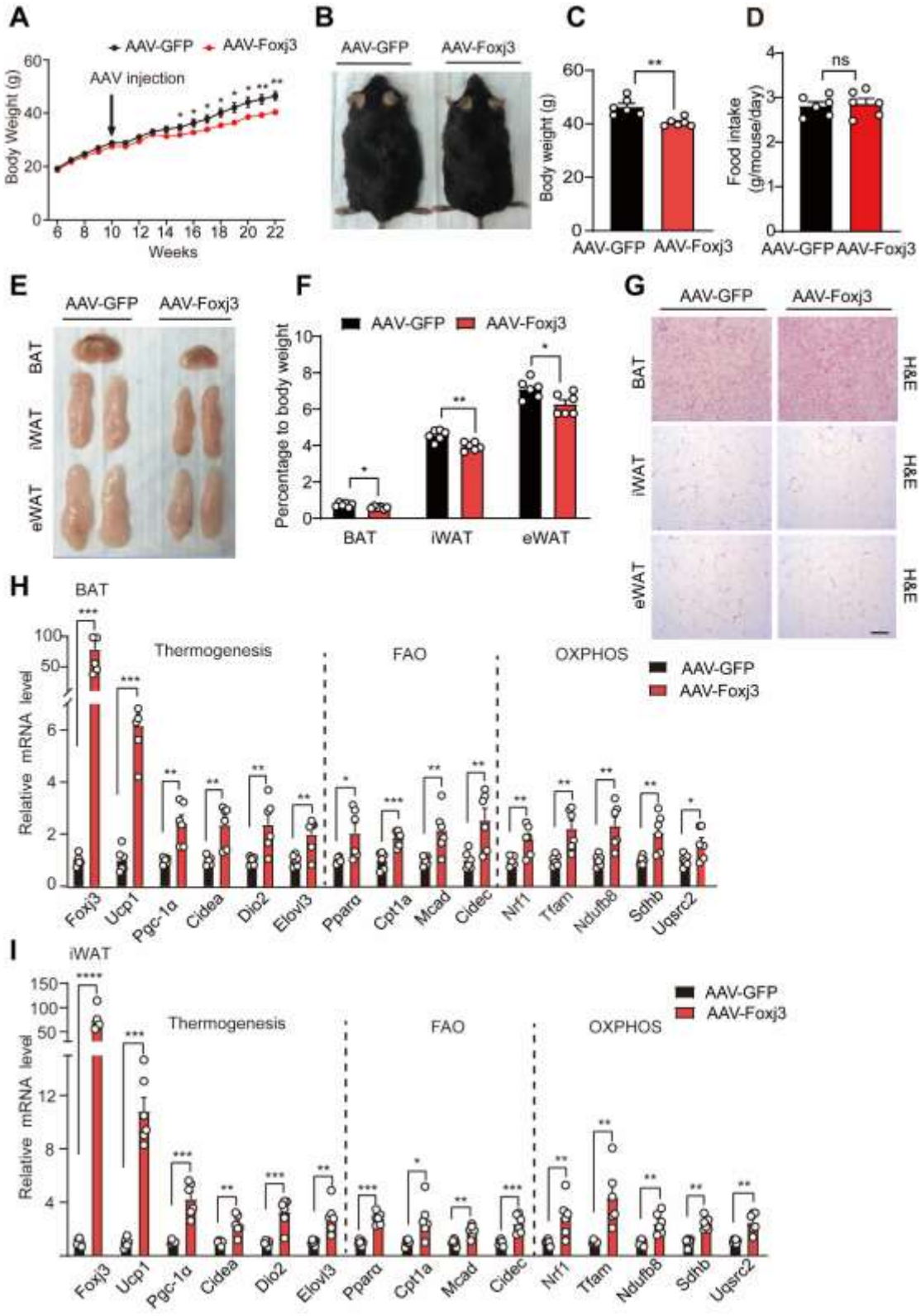


Supplementary Figure 4. Adipose-specific Foxj3 ablation aggravates HFD-induced obesity and insulin resistance. A: The growth curve of Foxj3$^{ad-/-}$ and Foxj3$^{flox/flox}$ mice fed a high-fat diet starting at 6 weeks of age (n = 6/group). B: A representative photograph of Foxj3$^{ad-/-}$ and Foxj3$^{flox/flox}$ mice fed an HFD for 2 months. C: Food intake of the mice described in B (n = 6/group). D: Gross appearance of interscapular BAT and inguinal and epididymal fat pads from mice in B. E: The ratio of weight of BAT, iWAT, and eWAT to body weight from the mice in B (n = 5/group). F: MRI assay

of body composition of mice in B (n = 6/group). G: H&E staining of paraffin-embedded BAT and inguinal and epididymal fat pad sections from the mice in B. Scalebar: 50μm. H: Quantitative PCR analysis of thermogenic genes of BAT of the mice in B (n = 6/group). I: Representative Western blot analysis of thermogenic genes in BAT of the mice described in B. J: Representative images of UCP1 immunohistochemistry (IHC) of BAT from the mice in B. Scalebar: 50μm. K: Quantitative PCR analysis of thermogenic genes of iWAT of the mice in B (n = 5/group). L: Representative gross morphology, H&E and Oil Red O staining of livers from the mice in B. Scalebar: 100μm. M and N: Biochemical analysis showing hepatic TG (M) and serum TG (N) content from the mice in B (n = 6/group). O: Quantitative PCR analysis of Srebp1c, Fasn, Acc, Scd1, Pparα, and Mcad in the livers of the mice in B (n = 6/group). P and Q: Blood glucose levels during GTT (P) and ITT (Q) in the mice in B (n = 6/group). The insert graphs represent the area under the curve (AUC). Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by two-tailed Student's t-test (A, E-F, H, K and M-Q). Abbreviations: n.s., not significant.



Supplementary Figure 5. Overexpression of Foxj3 in fat increases energy expenditure. A: Schematic representation of the basic components of Foxj3 insert packaged inside recombinant AAV gene transfer vector. The vector was single-stranded, contained ITR elements from AAV serotypes 2, and was packaged in serotype 8 capsids. B: Representive photographic image of the mice injected with AAV-GFP or AAV-Foxj3 under a fluorescent signal via the IVIS Spectrum system (PerkinElmer). C: The fluorescent intensity in frozen adipose tissue sections from the mice in B. D: Daily food intake of the mice described in Figure 4B (n = 6/group). E: The ratio of weight of heart, spleen, lung and kidney to body weight from the mice described in Figure 4B. (n = 6/group). F: Representative Western blot analysis of protein levels of Foxj3 in liver and muscle of the mice described in Figure 4B. Data are mean ± SEM. Abbreviations: n.s., not significant.


A
AAV-GFP
AAV-Foxj3
AAV injection
Body Weight (g)
Weeks
B
AAV-GFP
AAV-Foxj3
C
Body weight (g)
AAV-GFP
AAV-Foxj3
D
Food intake (g/mouse/day)
ns
AAV-GFP
AAV-Foxj3
E
AAV-GFP
AAV-Foxj3
BAT
iWAT
eWAT
F
AAV-GFP
AAV-Foxj3
Percentage to body weight
BAT
iWAT
eWAT
G
AAV-GFP
AAV-Foxj3
BAT
iWAT
eWAT
H&E
H
BAT
Thermogenesis
FAO
OXPHOS
AAV-GFP
AAV-Foxj3
Relative mRNA level
Foxj3
Ucp1
Pgc-1α
Cidea
Dio2
Elovl3
Pparα
Cpt1a
Mcad
Cidec
Nrf1
Tfam
Ndufb8
Sdhb
Uqsrc2
I
iWAT
Thermogenesis
FAO
OXPHOS
AAV-GFP
AAV-Foxj3
Relative mRNA level
Foxj3
Ucp1
Pgc-1α
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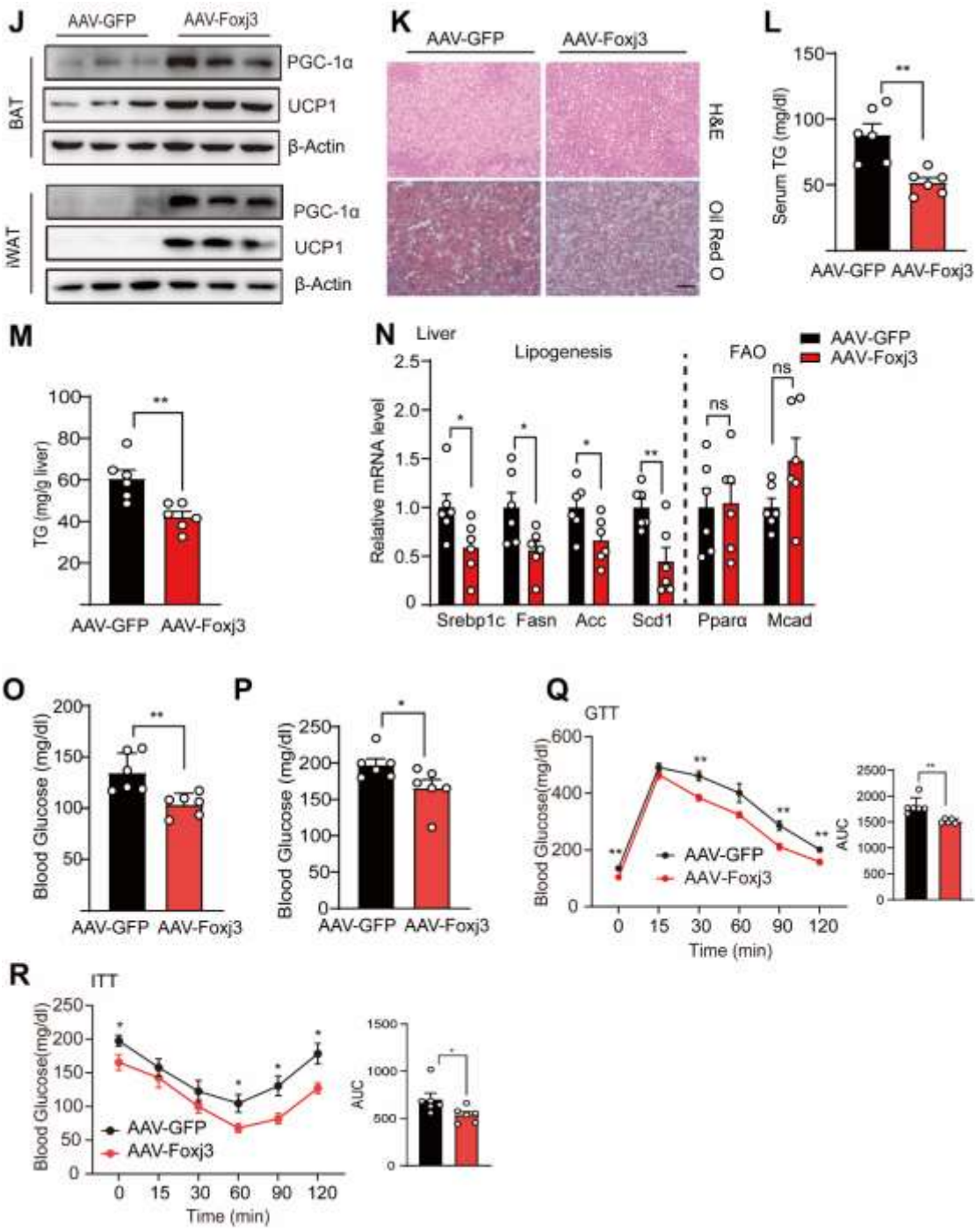

Supplementary Figure 6. Overexpression of Foxj3 in fat protects mice against DIO and metabolic disorders. 10-week-old C57BL/6J mice fed a HFD for 4 weeks were subjected to in situ injection of AAV-Foxj3 or AAV-GFP into the BAT and iWAT and fed a HFD for 12-week. A: The growth curve of AAV-Foxj3- and AAV-GFP-injected mice during 12-week HFD feeding (n = 6/group). B: A representative photograph of 22-week-

old AAV-Foxj3- and AAV-GFP-injected mice fed a HFD. C: Body weight of the mice described in B (n = 6/group). D: Food intake of the mice described in Figure B (n = 6/group). E: Gross appearance of interscapular BAT and inguinal and epididymal fat pads from the mice in B. F: The ratio of weight of BAT, iWAT, and eWAT to body weight from the mice in B (n = 6/group). G: H&E staining of paraffin-embedded BAT and inguinal and epididymal fat pad sections from the mice in B. Scalebar: 100μm. H and I: Quantitative PCR analysis of mRNA levels of genes involved in thermogenesis, fatty acid oxidation, and oxidative phosphorylation in BAT and iWAT of mice described in B (n = 6/group). J: Representative Western blot analysis of thermogenic genes in BAT and iWAT of the mice described in B. K: Representative images of H&E- and Oil Red O-stained hepatic sections of the mice described in B. Scale bar: 100μm. L and M: Serum TG and hepatic TG content of the mice described in B (n = 6/group). N: Quantitative PCR analysis of Srebp1c, Fasn, Acc, Scd1, Pparα and Mcad in the livers of the mice in B (n = 6/group). O and P: Basal blood glucose levels of 6 hour-fasted (O) and 16 hour-fasted (P) mice injected with AAV-GFP or AAV-Foxj3 for 6 weeks (n = 6/group). Q and R: Blood glucose levels during GTT (Q) and ITT (R) in the mice in O (n= 6/group). The insert graphs represent the area under the curve (AUC). Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by two-tailed Student's t-test (A, C, F, H-I, and L-R). Abbreviations: n.s., not significant.

Supplemental Table 1. Primers used in quantitative-PCR, ChIP assay and plasmids construction.

Gene symbol	Forward primer	Reverse primer
Foxj3	5’-AGAGAAGCTGGCAGTGGTTG-3’	5’-CCCTTTCCGGGATCATCCTT-3’
Ucp1	5’-AGGCTTCCAGTACCATTAGGT-3’	5’-CTGAGTGAGGCAAAGCTGATTT-3’
Pgc-1α	5’-TGATGTGAATGACTTGGATACAGACA-3’	5’-GCTCATTGTTGTACTGGTTGGATATG-3’
Cidea	5’-GCCGTGTTAAGGAATCTGCTG-3’	5’-TGCTCTTCTGTATCGCCCAGT-3’
Dio2	5’-CAGTGTGGTGCACGTCTCCAATC-3’	5’-TGAACCAAAGTTGACCACCAG-3’
Elovl3	5’-TTCTCACGCGGGTTAAAAATGG-3’	5’-GAGCAACAGATAGACGACCAC-3’
Prdm16	5’-GAAGGTGTCCAAACTGACAATGC-3’	5’-CGTCACTTTTGGCTAGCTTCCT-3’
Pparα	5’-ACAAGGCCTCAGGGTACCA-3’	5’-GCCGAAAGAAGCCCTTACAG-3’
Cpt1a	5’-GAACCCCAACATCCCCAAAC-3’	5’-TCCTGGCATTCTCCTGGAAT-3’
Mcad	5’-AACACTTACTATGCCTCGATTGCA-3’	5’-CCATAGCCTCCGAAAATCTGAA-3’
Cidec	5’-ATGGACTACGCCATGAAGTCT-3’	5’-CGGTGCTAACACGACAGGG-3’
Nrf1	5’-GCACCTTTGGAGAATGTGGT-3’	5’-CTGAGCCTGGGTCATTTTGT-3’
Tfam	5’-GAAGGGAATGGGAAAGGTAGA -3’	5’-AACAGGACATGGAAAGCAGAT -3’
Tfb2m	5’-CCAGAGTGGTTGCCTTTGA-3’	5’-TTCCTCTGTAAGGGCTCCA-3’
Ndufb8	5’-TGTTGCCGGGGTCATATCCTA-3’	5’-AGCATCGGGTAGTCGCCATA-3’
Sdhb	5’-AATTTGCCATTTACCGATGGGA-3’	5’-AGCATCCAACACCATAGGTCC-3’
Atgl	5’-CATGATGGTGCCCTATACTC-3’	5’-GTGAGAGGTTGTTTCGTACC-3’
Hsl	5’-AAGGACTTGAGCAACTCAGA-3’	5’-TTGACTATGGGTGACGTGTA-3’
Mgll	5’-GACGGACAGTACCTCTTTTG-3’	5’-AGAAAAGTAGGTTGGCCTCT-3’
Fasn	5’-GCTGCGGAAACTTCAGGAAAT-3’	5’-AGAGACGTGTCACTCCTGGACTT-3’
Scd1	5’-CCTGCCTCTTCGCGTTTGT-3’	5’-GGCGTGCCTTGTACGTTCT-3
Acc	5’-AGGAAGATGGCGTCCGCTCTG-3’	5’-GGTGAGATGTGCTGGGTCAT-3’
Cd36	5'-GGCCAAGCTATTGCGACAT-3’	5'-CAGATCCGAACACAGCGTAGA-3’
Srebp1c	5’-GGAGCCATGGATTGCACATT-3’	5’-GGCCCGGGAAGTCACTGT-3’
Pparγ	5’-TCAGCTCTGTGGACCTCTCC-3’	5’-AACCCTTGCATCCTTCACAAG-3’
Adiponectin	5’-GTTCCCAATGTACCCATTCGC-3’	5’-TGTTGCAGTAGAACTTGCCAG-3’
Fabp4	5’-AAGGTGAAGAGCATCATAACCCT-3’	5’-TCACGCCTTTCATAACACATTCC-3’

36B4	5’-GAGGAATCAGATGAGGATATGGGA-3’	5’- AAGCAGGCTGACTTGGTTGC-3’
Cox2 (mtDNA)	5’-ATAACCGAGTCGTTCTGCCAAT-3’	5’-TTTCAGAGCATTGGCCATAGAA-3’
Rps18 (mtDNA)	5’-TGTGTTAGGGGACTGGTGGACA-3’	5’-CATCACCCACTTACCCCCAAAA-3’
Pgc-1α ChIP primers	5’-GCTGGCTTCAGTCACAGTGT -3’	5’-TTGCTGCACAAACTCCTGAC-3’
Pgc-1α Promoter -2100Luc	5’-TACTACGCGTGTTGTCTCTCTCTCTCTCTGA-3’	5’-TACTCTCGAGCCAGCTCCCGAATGACGC-3’
Ucp1 ChIP primers	5’-GTCACCCAAATCTGAAGGT-3’	5’-ATAGCTGTAGGATGAACGTC-3’
Ucp1 Promoter -1727Luc	5’-GGCTAGCCCCAACTGCTTTGTGACAATC -3’	5’-CCCTCGAGGGGAAGGTGATGATATCTGCCAG-3’
Ucp1 Promoter -500Luc	5’ GGCTAGCCGTCACCCAAATCTGAAGGT-3’	5’-CCCTCGAGGGGAAGGTGATGATATCTGCCAG-3’
Ucp1 Promoter -400Luc	5’-GGCTAGCCAGGGGGCCCGGGACTGG-3’	5’-CCCTCGAGGGGAAGGTGATGATATCTGCCAG-3’