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Supplementary Materials for

TFEB drives PGC-1α expression in adipocytes to protect against diet-induced metabolic dysfunction

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This PDF file includes:

SUPPLEMENTAL FIGURES

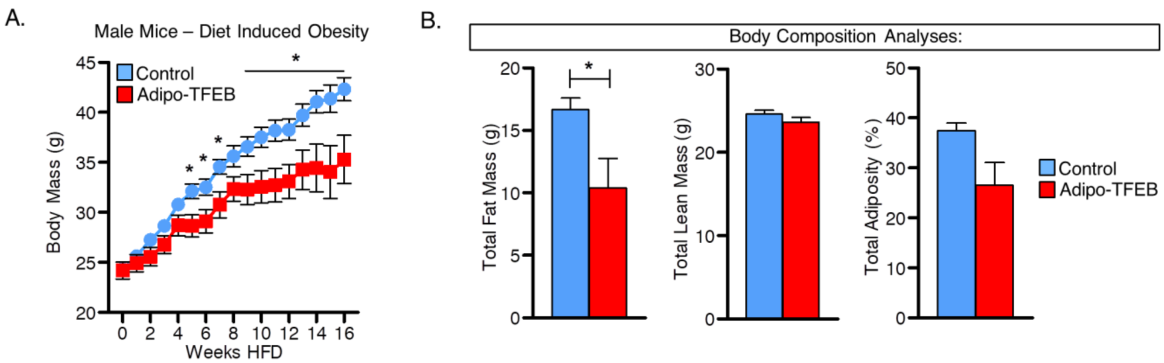


Fig. S1. WD-induced obesity in male mice. A. Body mass of male mice fed a Western diet for 16 weeks (n=16 control mice, 8 Adipo-TFEB mice). **B.** Total fat mass, lean mass and total adiposity in Western diet-fed male mice (n=16 control mice, 8 Adipo-TFEB mice). Data presented as mean ±SEM. *Student's two-tailed t-test $P < 0.05$.

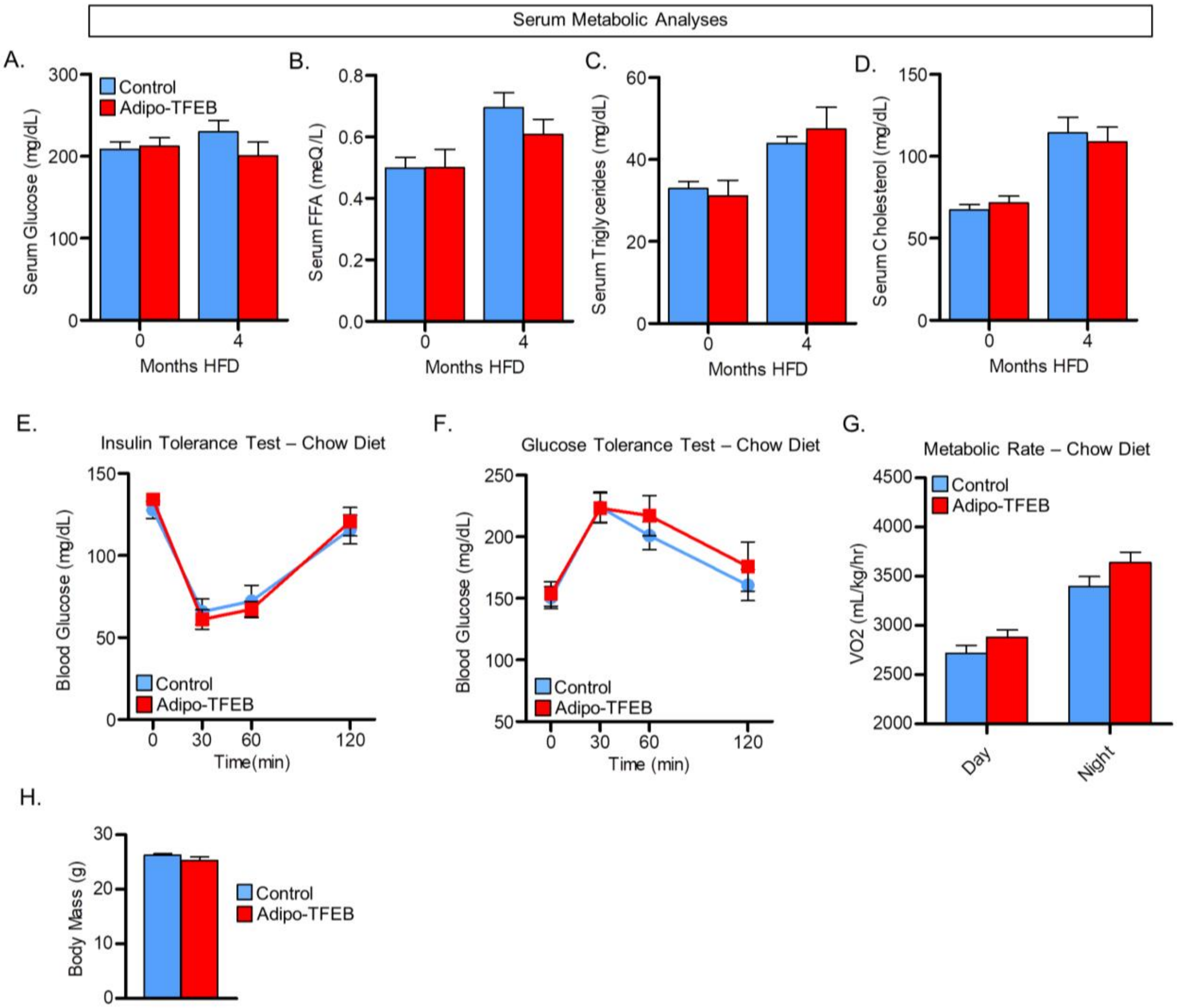
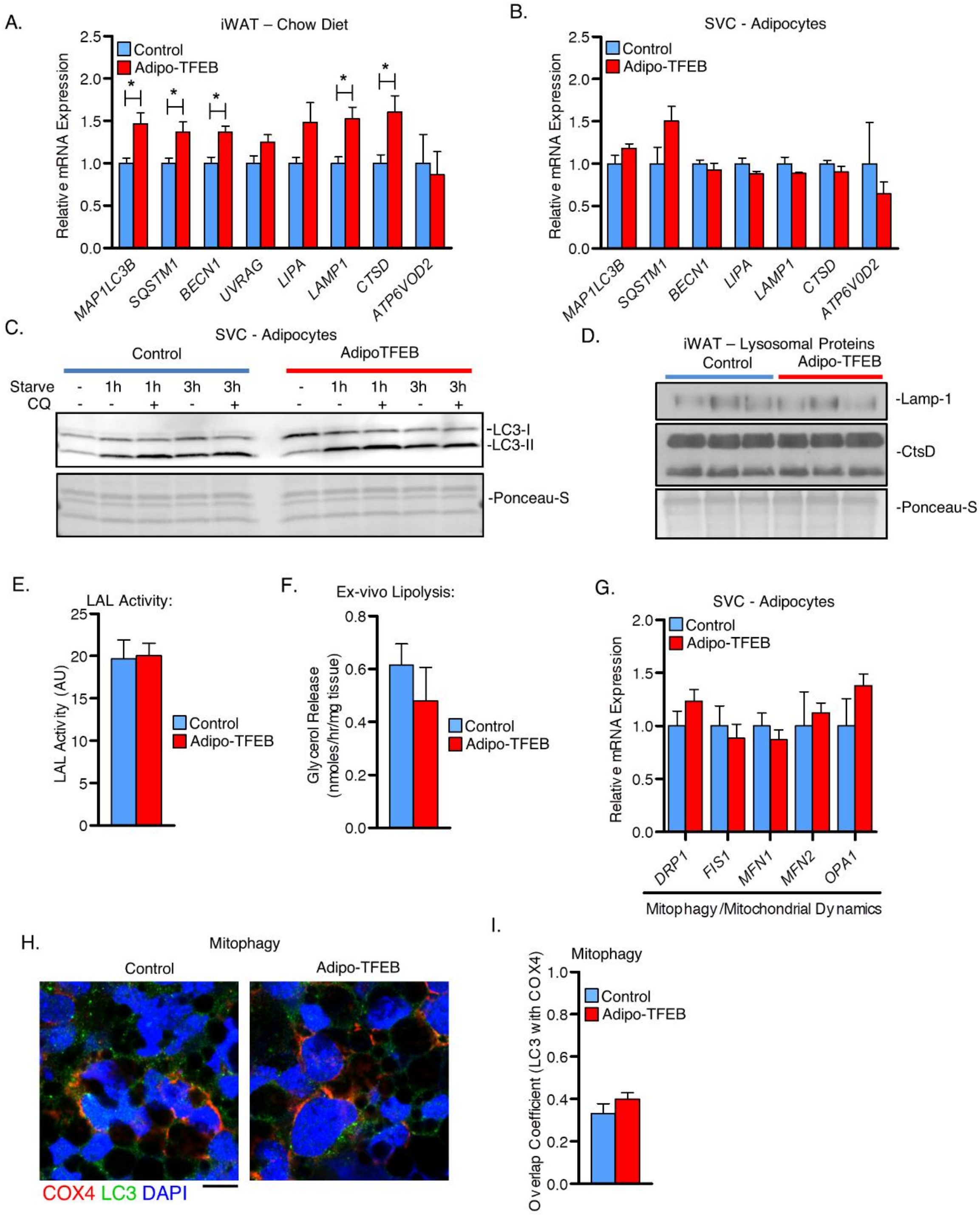


Fig. S2. Metabolic phenotyping of Adipo-TFEB mice. Fasting serum levels of **A.** Glucose **B.** Free Fatty Acids **C.** Triglycerides **D.** Cholesterol in female mice immediately prior to the initiation of or following 4 months Western Diet (n=11-21 mice per group). **E.** Insulin tolerance test in chow-fed 8 week old male mice (n= 8 Control, 6 Adipo-TFEB mice). **F.** Glucose tolerance test in chow-fed 9 week old male mice (n=8 Control, 6 Adipo-TFEB mice). **G.** Metabolic Rate (VO_2) measured over given 12h intervals in 10 week old chow-fed male mice (n=7 Control, 5 Adipo-TFEB mice). **H.** Body mass in mice from (G) (n=7 Control, 5 Adipo-TFEB mice). Data presented as mean ±SEM. *Student's two-tailed t-test $P < 0.05$.



A.
iWAT – Chow Diet
Control
Adipo-TFEB
Relative mRNA Expression
MAP1LC3B
SQSTM1
BECN1
UVRAG
LIPA
LAMP1
CTSD
ATP6V0D2
B.
SVC - Adipocytes
Control
Adipo-TFEB
Relative mRNA Expression
MAP1LC3B
SQSTM1
BECN1
LIPA
LAMP1
CTSD
ATP6V0D2
C.
SVC - Adipocytes
Control
AdipoTFEB
Starve
CQ
-LC3-I
-LC3-II
-Ponceau-S
D.
iWAT – Lysosomal Proteins
Control
Adipo-TFEB
-Lamp-1
-CtsD
-Ponceau-S
E.
LAL Activity:
LAL Activity (AU)
Control
Adipo-TFEB
F.
Ex-vivo Lipolysis:
Glycerol Release (nmoles/hr/mg tissue)
Control
Adipo-TFEB
G.
SVC - Adipocytes
Control
Adipo-TFEB
Relative mRNA Expression
DRP1
FIS1
MFN1
MFN2
OPA1
Mitophagy/Mitochondrial Dynamics
H.
Mitophagy
Control
Adipo-TFEB
COX4 LC3 DAPI
I.
Mitophagy
Overlap Coefficient (LC3 with COX4)
Control
Adipo-TFEB

Fig. S3. Analyses of the autophagy-lysosome system and mitophagy in Adipo-TFEB mice. All analyses were performed in tissues from 8 week old chow fed female mice. **A.** Transcriptional characterization of TFEB-target autophagy-lysosome genes in iWAT (n=8 Control, 8 Adipo-TFEB mice). **B.** Autophagy-lysosome genes in stromal-vascular adipocytes from iWAT (n=3 biological replicates/group). **C.** Autophagic flux analysis in stromal vascular adipocytes that were starved and treated with chloroquine (50 μM) as indicated. Results are representative of two independent experiments. **D.** Protein expression of lysosome markers in iWAT (n=3 8-week old female mice for each genotype). **E.** Lysosomal acid lipase activity in iWAT (n=3 8-week old female mice for each genotype). **F.** Lipolysis as assessed by glycerol release from WAT explants (n=3 control, 4 Adipo-TFEB mice). **G.** Gene expression of mitophagy and mitochondrial dynamics genes in SVC-Adipocytes (n=3 biological replicates/group and representative of at least 2 independent experiments**)**. **H.** Immunofluorescent colocalization analyses assessing recruitment of autophagosomes (LC3) to mitochondria (COX4). Scale bar length - 10μM. **I.** Overlap coefficient of LC3 with COX4 (n>35 cells/group from >10 different fields. Data presented as mean ±SEM. *Student's two-tailed t-test $P < 0.05$.

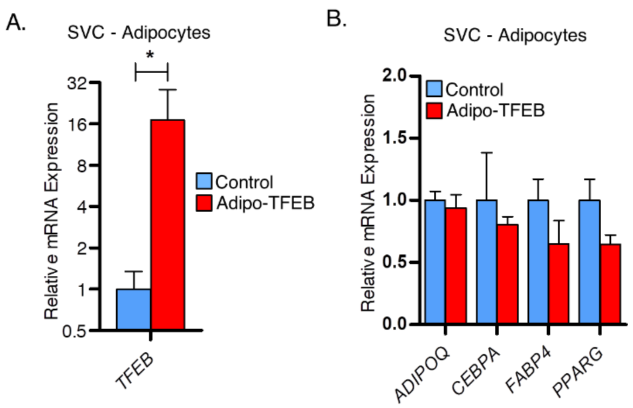


Fig. S4. iWAT stromal vascular adipocyte culture model. A. Demonstration of TFEB overexpression and **B.** similar adipogenic efficiency in cultured, differentiated SVC adipocytes. n=3 biological replicates/group and representative of at least 2 independent experiments. Data presented as mean ±SEM. *Student's two-tailed t-test $P < 0.05$.

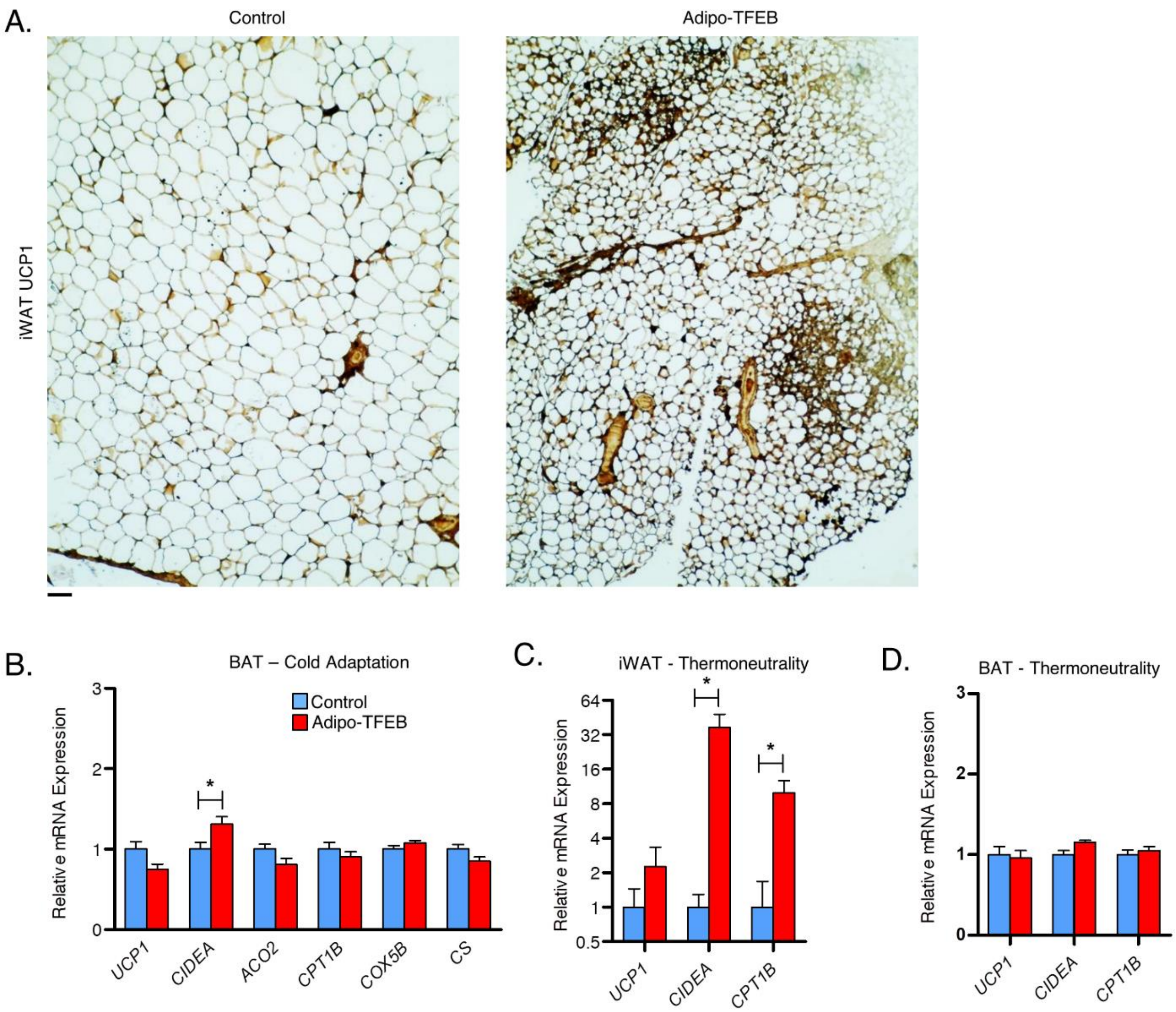


Fig. S5. UCP-1 expression and transcriptional responses in cold and thermoneutrality. A. Uncropped images of UCP1 immunohistochemistry in iWAT presented in Fig. 4. **B.** Mitochondrial and lipid oxidation gene expression in BAT of 3-month old male mice (n=8 Control, 6 Adipo-TFEB mice) following 48h group housing at 4ºC. **C.** iWAT and **D.** BAT gene expression in 10 week old male mice housed at thermoneutrality (30ºC) for 2 weeks (n=4 Control, 3 Adipo-TFEB mice). Data presented as mean ±SEM. *Student's two-tailed t-test P < 0.05.

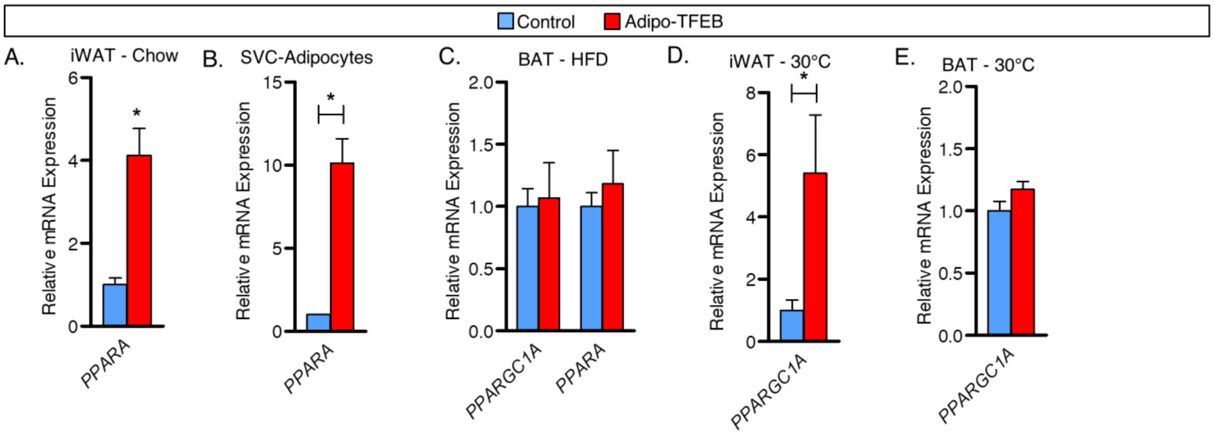


Fig. S6. Transcriptional response of PGC-1α and PPARα to TFEB overexpression.
A. *PPARA* mRNA in inguinal adipose from 8 week old female chow-fed mice (n=9 Control, 7 Adipo-TFEB mice). **B.** Stromal vascular adipocytes (n=3 biological replicates/group and representative of two independent experiments. **C.** Interscapular brown adipose tissue from female mice fed a Western diet for 4 months (n=9 Control, 7 Adipo-TFEB mice). **D.** iWAT and **E.** BAT PPPARGC1α expression in 10-week old mice housed at thermoneutrality (30ºC) for 2 weeks (n=4 Control, 3 Adipo-TFEB mice). Data presented as mean ±SEM. *Student's two-tailed t-test P < 0.05.

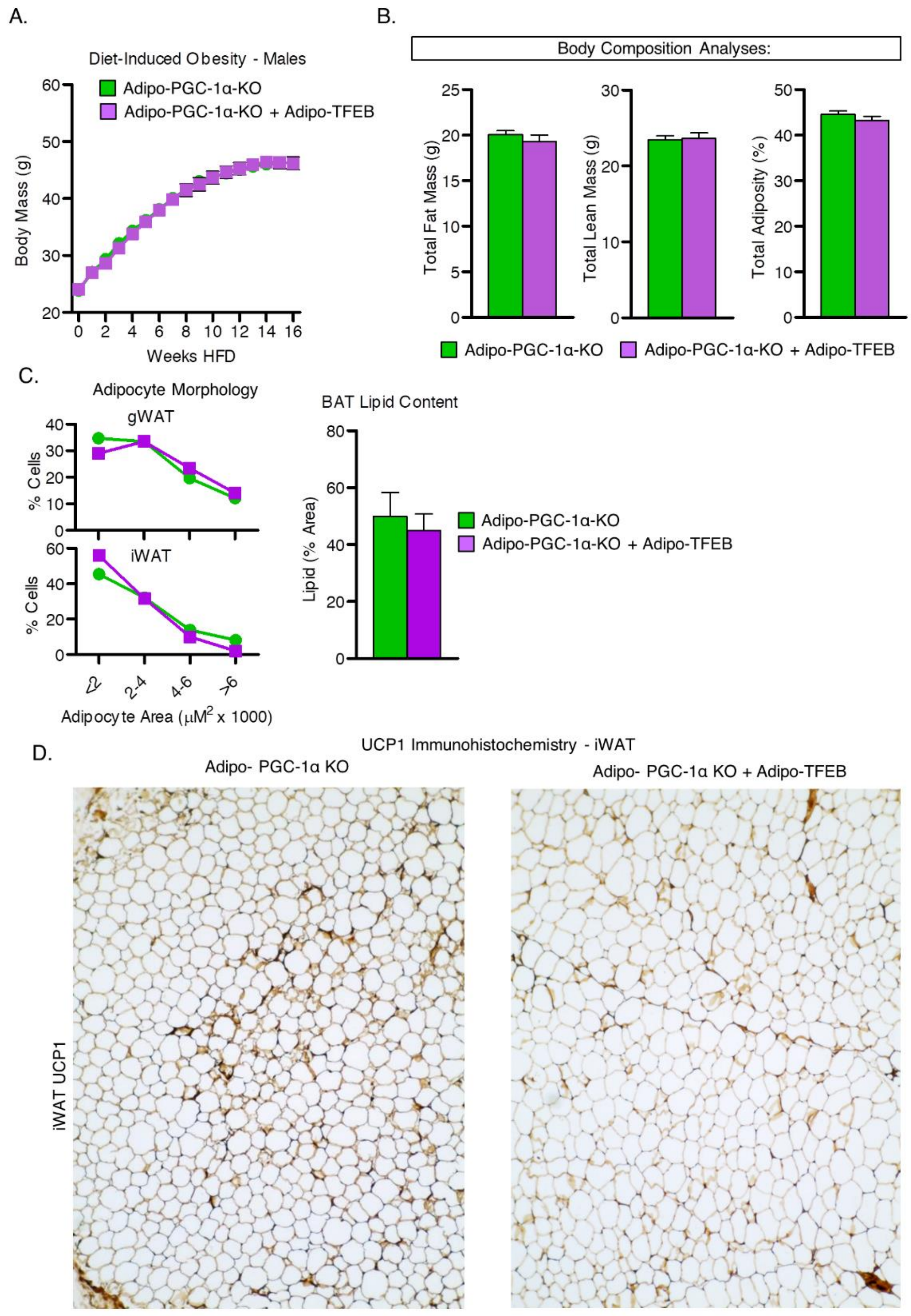


Fig. S7. Additional analyses in mice with concurrent PGC-1α deficiency.
A. Body mass of male mice fed a Western diet for 16 weeks (n=15 Adipo-PGC-1α-KO,12 Adipo-PGC-1α-KO + Adipo-TFEB mice) **B.** Total fat mass, lean mass and total adiposity in Western diet-fed male mice (n=10 Adipo-PGC-1α-KO, 8 Adipo-PGC-1α-KO + Adipo-TFEB mice). **C.** gWAT and iWAT adipocyte sizing and BAT lipid content in Western diet-fed female (pooled from n=6 mice/group). **D.** Uncropped UCP1 immunohistochemistry images from Fig. 8.

Table S1. PCR primers used in the present study.

qPCR Primers		
	Forward	Reverse
PPARGC1A	5'- AGC CGT GAC CAC TGA CAA CGA G -3'	5'- GCT GCA TGG TTC TGA GTG CTA AG -3'
CS	5'- CAA GCA GCA ACA TGG GAA GA -3'	5'- GTC AGG ATC AAG AAC CGA AGT CT -3'
PPARD	5'- GGT CAT AGC TCT GCC ACC AT -3'	5'- ACT CAG AGG CTC CTG CTC AC -3'
PPARG	5'- TCA GAG GGA CAA GGA TTC ATG A -3'	5'- CAC CAA AGG GCT TCC GCA GGC T -3'
UCP1	5'- GTG AAG GTC AGA ATG CAA GC -3'	5'- AGG GCC CCC TTC ATG AGG TC -3'
ACO2	5'- GTT GGA CCC ACC CAA AGA T -3'	5'- GGT CCG TGG TAT TCC ACA ATA G -3'
CPT1B	5'- TGT CTA CCT CCG AAG CAG GA -3'	5'- GCT GCT TGC ACA TTT GTG TT -3'
PPARA	5'- AGT TCG GGA ACA AGA CGT TG -3'	5'- CAG TGG GGA GAG AGG ACA GA -3'
DIO2	5'- CAG TGT GGT GCA CGT CTC CAA TC -3'	5'- TGA ACC AAA GTT GAC CAC CAG -3'
CIDEA	5'- TGC TCT TCT GTA TCG CCC AGT -3'	5'- GCC GTG TTA AGG AAT CTG CTG -3'
PRDM16	5'- CAG CAC GGT GAA GCC ATT C -3'	5'- GCG TGC ATC CGC TTG TG -3'
TFEB	5'- GGT GCA GTC CTA CCT GGA GA -3'	5'- GTG GGC AGC AAA CTT GTT CC -3'
36B4	5'- ATC CCT GAC GCA CCG CCG TGA -3'	5'- TGC ATC TGC TTG GAG CCC ACG TT -3'
P62	5'- GCT GCC CTA TAC CCA CAT CT -3'	5'- GGC CTT CAT CCG AGA AAC -3'
ATP6VOD2	5'- CAG AGC TGT ACT TCA ATG TGG AC -3'	5'- AGG TCT CAC ACT GCA CTA GGT -3'
COX5B	5'- ATC GCT GAC TCT CGC CTT T -3'	5'- CGT CCA TCA GCA ACA AGA GA -3'
MAP1LC3B	5'- CGT CCT GGA CAA GAC CAA GT -3'	5'- ATT GCT GTC CCG AAT GTC TC -3'
BECN	5'- AAT CTA AGG AGT TGC CGT TAT AC -3'	5'- CCA GTG TCT TCA ATC TTG CC -3'
LAMP1	5'- ACA TCA GCC CAA ATG ACA CA -3'	5'- GGC TAG AGC TGG CAT TCA TC -3'
CTSD	5'- GCT TCC GGT CTT TGA CAA CCT -3'	5'- CAC CAA GCA TTA GTT CTC CTC C -3'
UVRAG	5'- CAT CCA AAC GCA CAG AAG AA -3'	5'- TTG CAC ACT GGG CTC TAT GA -3'
LIPA	5'- CTA GAA TCT GCC AGC AAG CC -3'	5'- AGT ATT CAC CGA ATC CCT CG -3'
OPA1	5'- ATA CTG GGA TCT GCT GTT GG-3'	5'- AAG TCA GGC ACA ATC CAC TT-3'
DRP1	5'- TCA GAT CGT CGT AGT GGG AA-3'	5'- TCT TCT GGT GAA ACG TGG AC-3'
FIS1	5'- AAG TAT GTG CGA GGG CTG T-3'	5'- TGC CTA CCA GTC CAT CTT TC-3'
MFN1	5'- CCT ACT GCT CCT TCT AAC CCA-3'	5'- AGG GAC GCC AAT CCT GTG A-3'
MFN2	5'- ATG TTA CCA CGG AGC TGG AC-3'	5'- AAC TGC TTC TCC GTC TGC AT-3'
ADIPOQ	5'- CGA TTG TCA GTG GAT CTG ACG-3'	5'- CAA CAG TAG CAT CCT GAG CCC T-3'
FABP4	5'- ACA AGC TGG TGG TGG AAT GTG-3'	5'- CCT TTG GCT CAT GCC CTT T-3'
CEBPA	5'- TGC GCA AGA GCC GAG ATA AA-3'	5'- CCT TCT GTT GCG TCT CCA CG-3'
Genotyping Primers		
Cre	5'-GCA TTA CCG GTC GAT GCA ACG AGT GAT GAG-3'	5'-GAG TGA ACG AAC CTG GTC GAA ATC AGT GCG-3'
PGC-1α flox	5'-GTG GTA GAA GTA TCC AGT AGG CAG AGA TTT ATG AC - 3'	5'-GTG TCT GGT TTG ACA ATC TGC TAG GTC - 3'
TFEB Tg	5'-CAT CAA CCC TGA GAT GCA GAT GCC TAA CAC-3'	5'-TGT GAT TGT CTT TCT TCT GCC GCT CCT TGG-3'