Supplemental Data
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The Nuclear Orphan Receptor COUP-TFII Plays an Essential Role in Adipogenesis, Glucose Homeostasis, and Energy Metabolism

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Experimental Procedures

shRNA constructs

The short hairpin RNA (shRNA) for mouse COUP-TFII (5'-AGGTAACGTGATTGATTCAGTATCTTA-3') was cloned into pSUPER.retro (OligoEngine) according to the manufacturer's instructions. The shRNA plasmid was co-transfected with pCL into 293T cells to produce retroviral supernatants. 48h after transfection, the culture medium was filtered through a 0.45μm filter and the viral supernatant was used for 3T3-L1 cells infection.

Triglyceride quantification

Lipids in EWAT and liver were extracted by using a 2:1 Chloroform:Methanol mixture (V/V) (Folch et al., 1957). Relative triglyceride content in differentiated cells was quantified by using an adipogenesis assay kit (Chemicon) following the manufacturer's instructions.

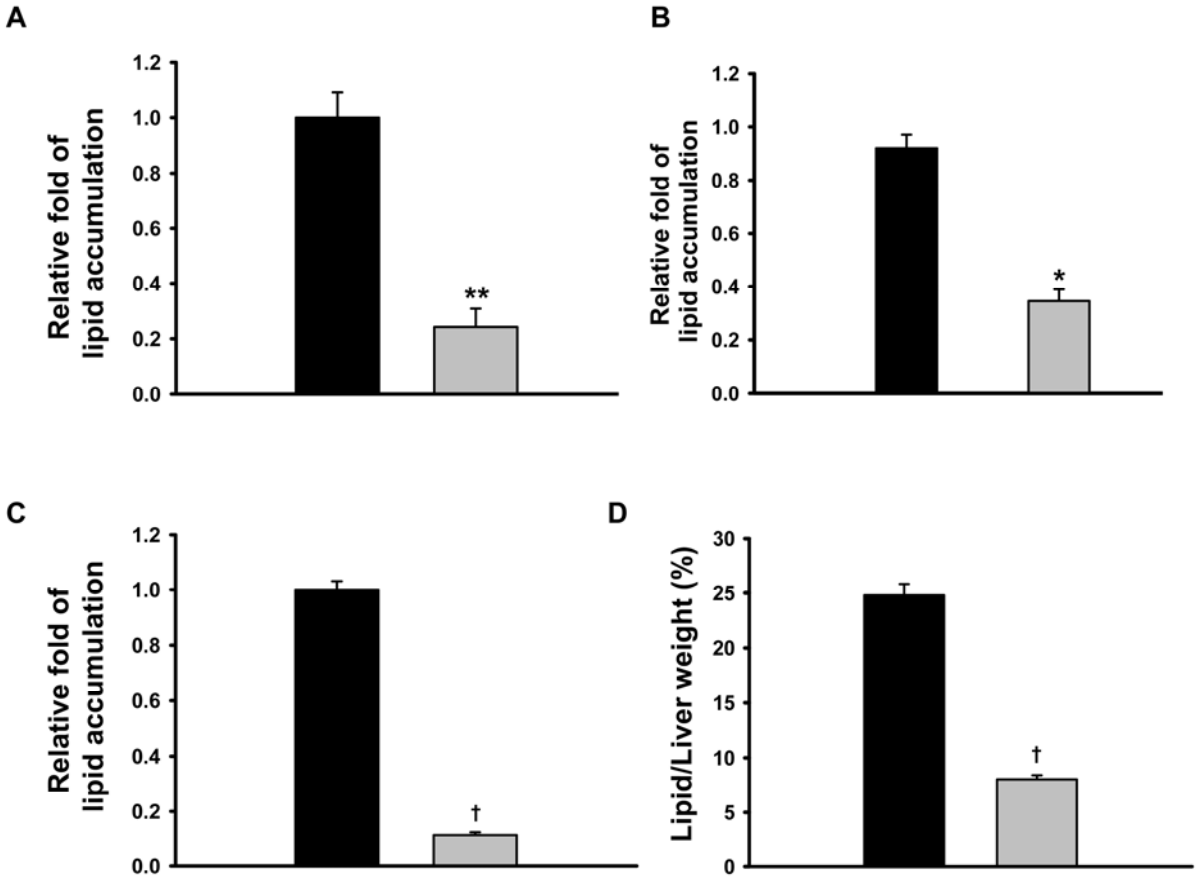

Figure S1. Triglyceride quantification

(A). Corresponds to the Figure 2C lipid accumulation in EWAT of two-week-old *COUP-TFII*$^{+/+}$ (black box, n=3) and *COUP-TFII*$^{+/-}$ mice (gray box, n=3). Lipid is too low to be determined in 3-day-old mice and 5-day-old mice.

(B). Corresponds to Figure 3B (right), black box represent 3T3-L1 cells transfected with control siRNA, and gray box represent cells transfected with COUP-TFII siRNA (triplicates).

(C). Corresponds to Figure 3D (right), black box represent *COUP-TFII* $^{flox/flox}$ MEFs treated with 4-OH-TM, gray box represent *ROSA26* $^{CRE-ERT2/+}$, *COUP-TFII* $^{flox/flox}$ MEFs treated with 4-OH-TM (triplicates).

(D). Corresponds to Fig 5E. Black box represents *COUP-TFII*$^{+/+}$ (n=5) and gray box represents *COUP-TFII*$^{+/-}$ mice (n=5). Data in (A-D) represent mean ± SEM. * $p<0.05$, ** $p<0.01$, † $p<0.0001$.

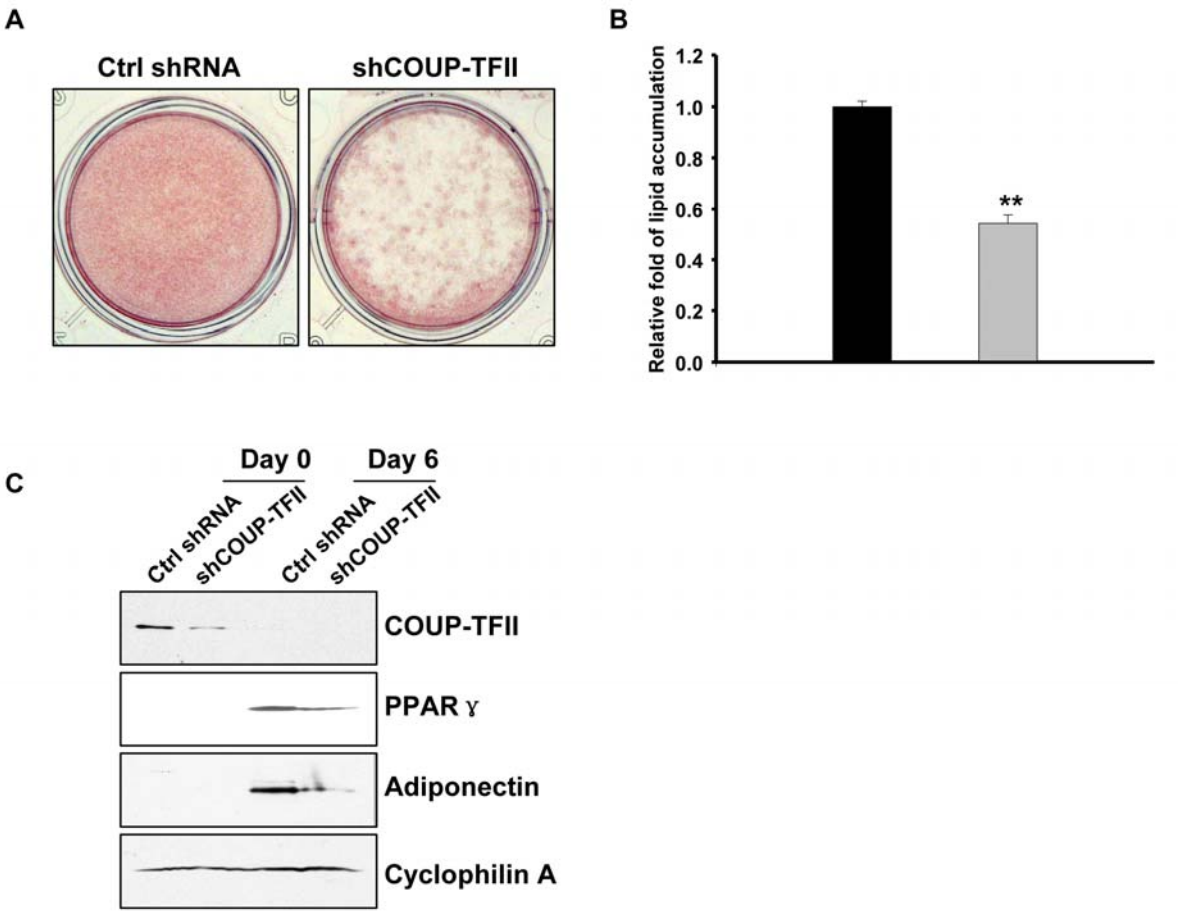

Figure S2. Knock down COUP-TFII in 3T3-L1 cells using retrovirus system resulted in a decreased adipogenesis

(A). 3T3-L1 cells were transduced with retrovirus expressing shRNA to COUP-TFII or a control shRNA before induction of adipogenesis with differentiation cock tail, Oil-Red-O staining of the cells 8 days after induction. (B). Triglyceride quantification to evaluate adipogenesis with triplicates induction plates by using adipogenesis assay kit (Chemicon), black box represent control shRNA transduced cells, gray box represent shCOUP-TFII transduced cells. Data represent mean ± SEM. **$p<0.01$. (C). Western blot analysis for COUP-TFII, and adipocyte markers, PPARγ and adiponection in 3T3-L1 cells before induction (day 0) and 6 days after induction. Cyclophilin A was used as the loading control.

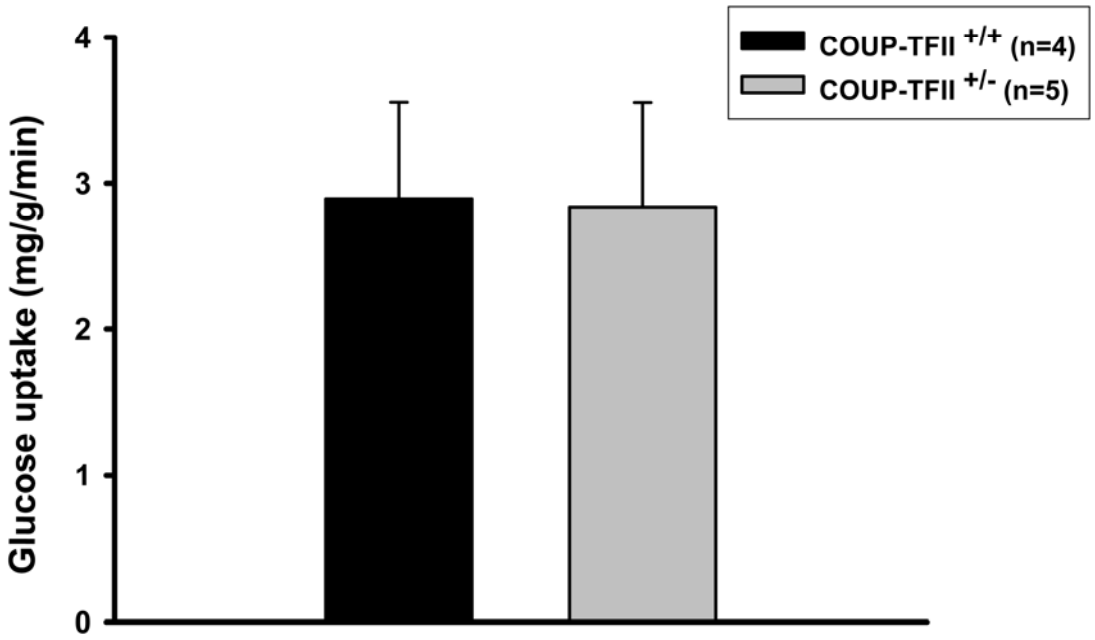

Figure S3. Liver glucose uptake using euglycemic-hyperinsulinemic-clamp analysis

Data represent mean ± SEM.

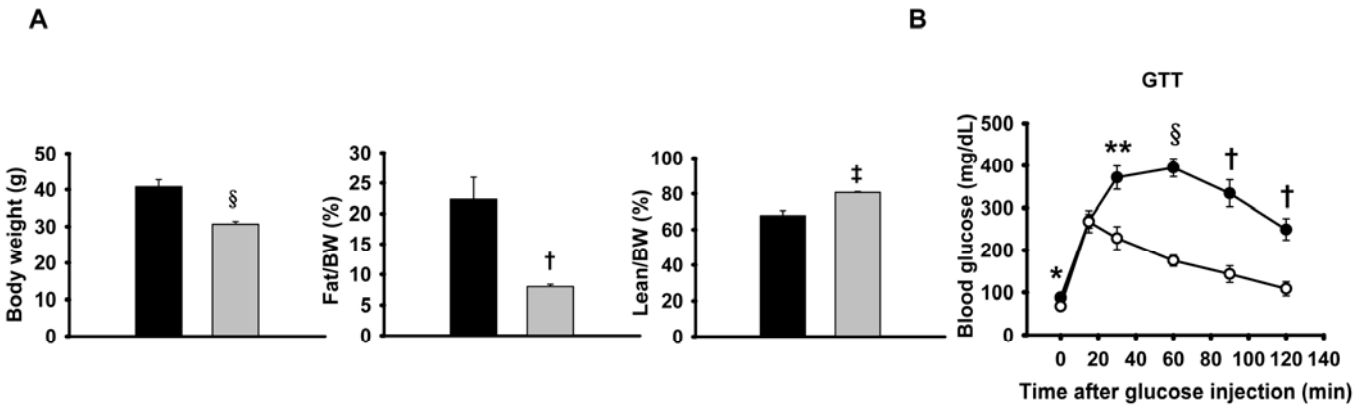

Figure S4. *COUP-TFII* $^{+/-}$ mice are resistant to age-related obesity

(A). Body weight (left panel) and MRI analysis of body fat and lean content (center and right panels) of 18-month-old *COUP-TFII*$^{+/+}$ (black box, n=6) and *COUP-TFII*$^{+/-}$ (gray box, n=7) littermates mice fed a normal chow. † $p<0.005$; ‡ $p<0.001$; § $p<0.0005$.(B). GTT on 9-month-old littermates of *COUP-TFII*$^{+/+}$ (filled circle, n=4) and *COUP-TFII*$^{+/-}$ (opened circle, n=4) mice. * $p<0.05$; ** $p<0.01$; † $p<0.005$; § $p<0.0005$. Data in (A) and (B) represent mean ± SEM.

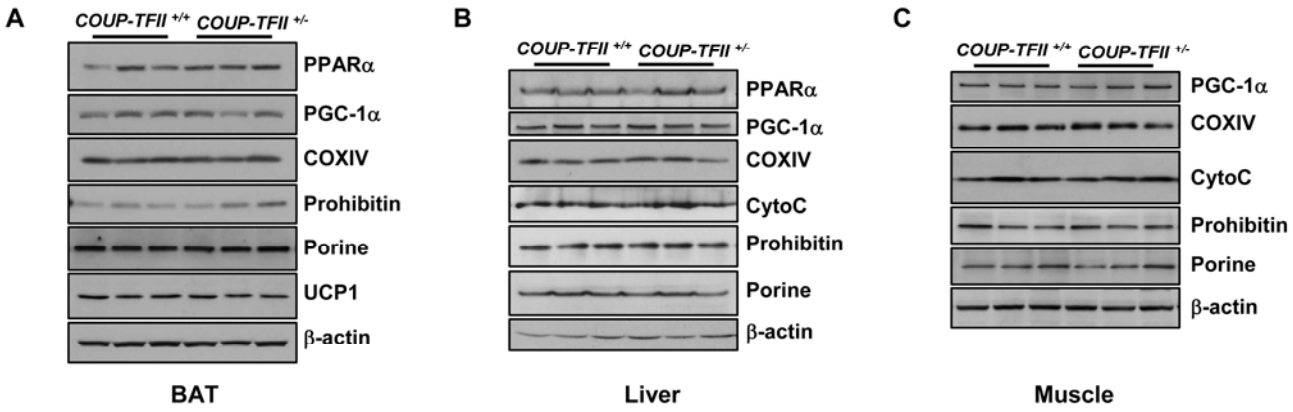

Figure S5. Expression of mitochondrial proteins in *COUP-TFII* $^{+/-}$ mice

Immunoblots of the BAT (A), liver (B), and skeletal muscle (C) (all in triplicates) extracts for PGC-1α, COXVI, cytochrome c, porine, prohibitin. β-actin was used as the loading control. Tissues were collected from littermates of *COUP-TFII* $^{+/+}$ and *COUP-TFII* $^{+/-}$ mice.

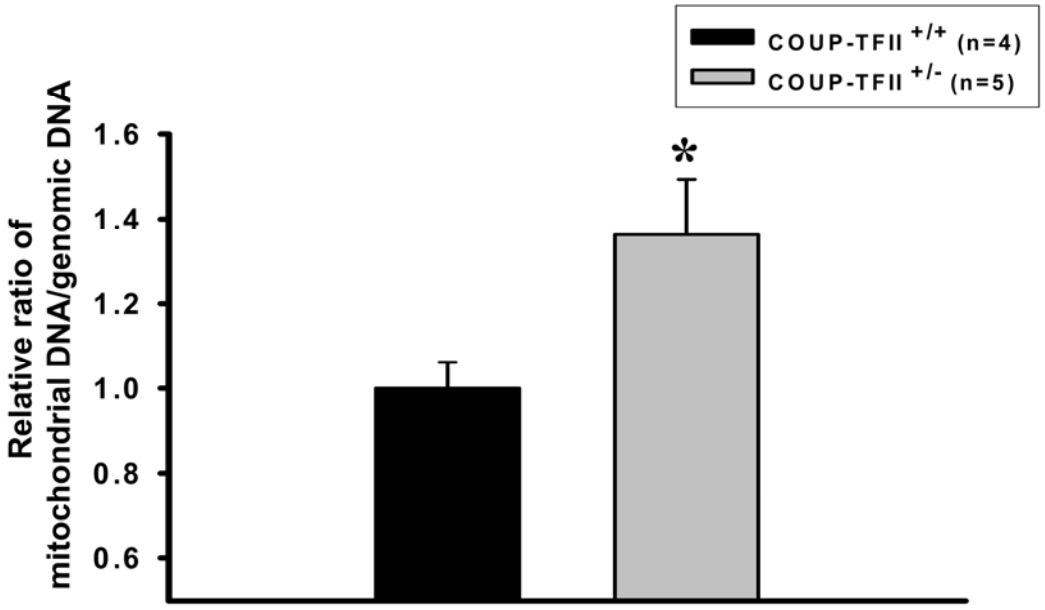

Figure S6. Analysis of the relative ratio of mitochondrial DNA/genomic DNA by real-time PCR

Real-time PCR analysis of COXII (encoded by mtDNA) versus cyclophilin A gene number in EWAT genomic DNA from both *COUP-TFII* $^{+/+}$ and *COUP-TFII* $^{+/-}$ littermates. Data represent mean ± SEM. * $p<0.05$.

Reference:

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. *226*:497-509.