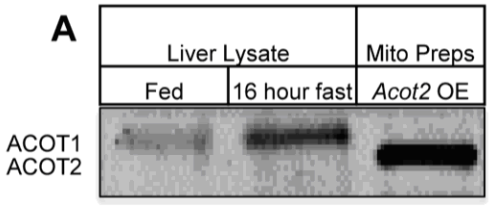
SUPPLEMENTARY DATA

Supplementary Figure 1. ACOT1 antibody distinguishes between ACOT1 and ACOT2. (A) The ACOT1 antibody recognizes mouse ACOT1 and ACOT2. Liver homogenates from fed and 16 hour fasted mice were compared to mitochondrial preps from mice overexpressing *Acot2*.



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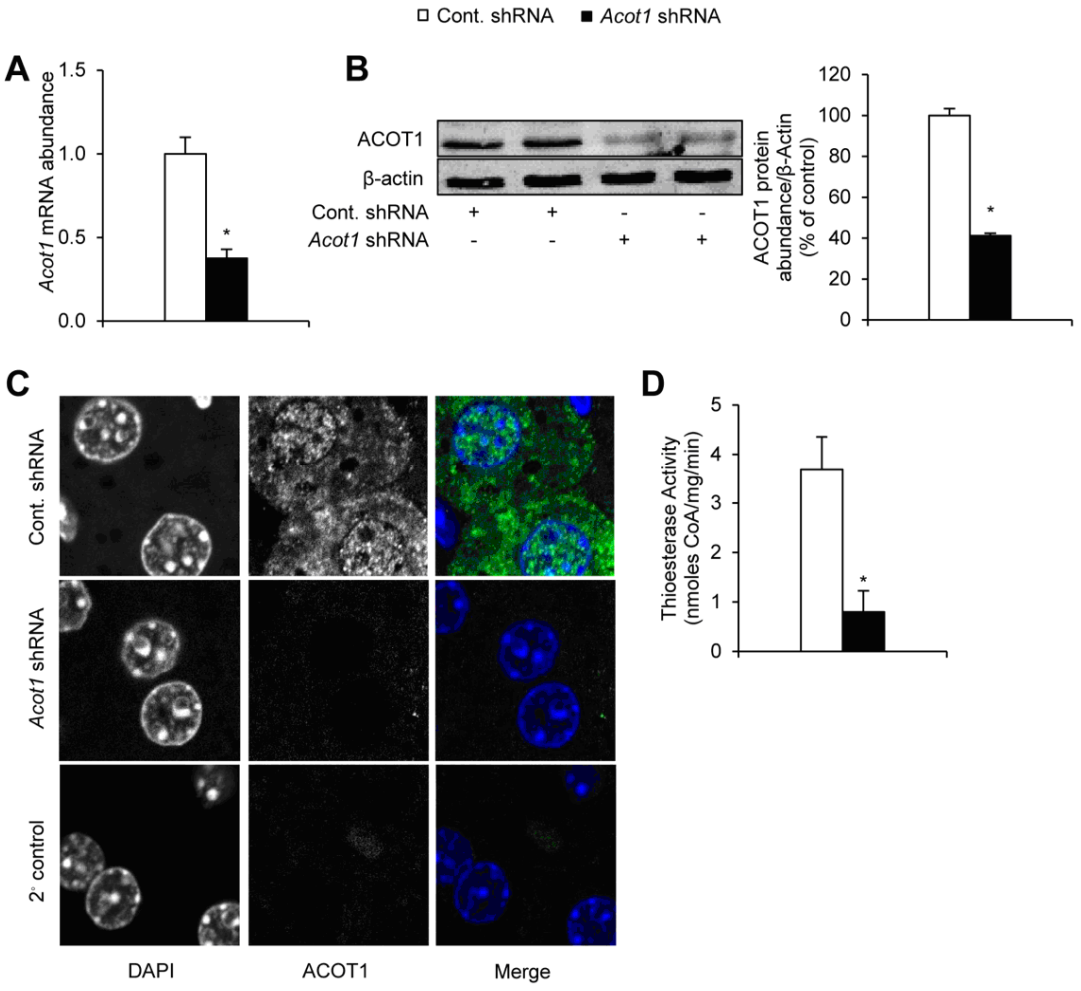
SUPPLEMENTARY DATA

Supplementary Figure 2. Hepatic *Acot1* knockdown reduces ACOT1 protein and thioesterase activity. *Acot1* expression is reduced in response to *Acot1* knockdown as analyzed by RT-PCR (A) and Western blotting of liver lysates (B) 7 days post transduction (n=7-9). (C) Immunohistochemistry of ACOT1 from fix liver sections indicates reduced ACOT1 protein with *Acot1* knockdown. (D) *Acot1* knockdown reduced thioesterase activity (n=3-4). *P<0.05.



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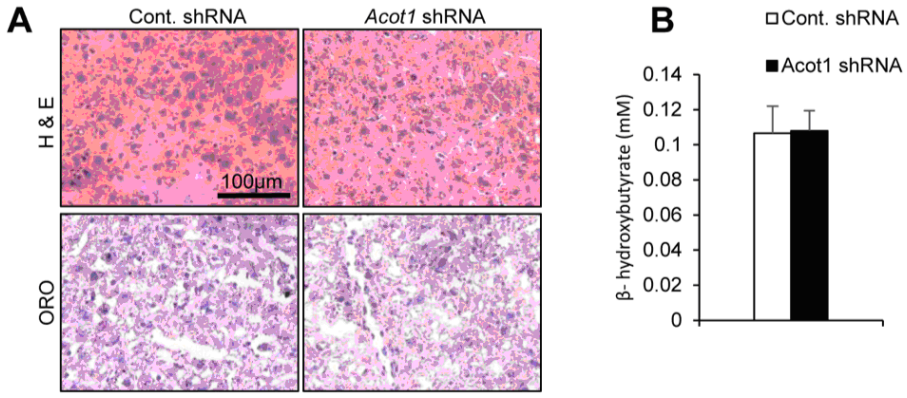
Supplementary Figure 3. *Acot1* knockdown effects on hepatic lipid metabolism in the fed state. Control and *Acot1* knockdown mice were harvested in the fed state. (A) Liver tissue were frozen in Tissue-Tek® O.C.T. for H&E and Oil Red O staining. (B) Serum was assessed for β-hydroxybutyrate.



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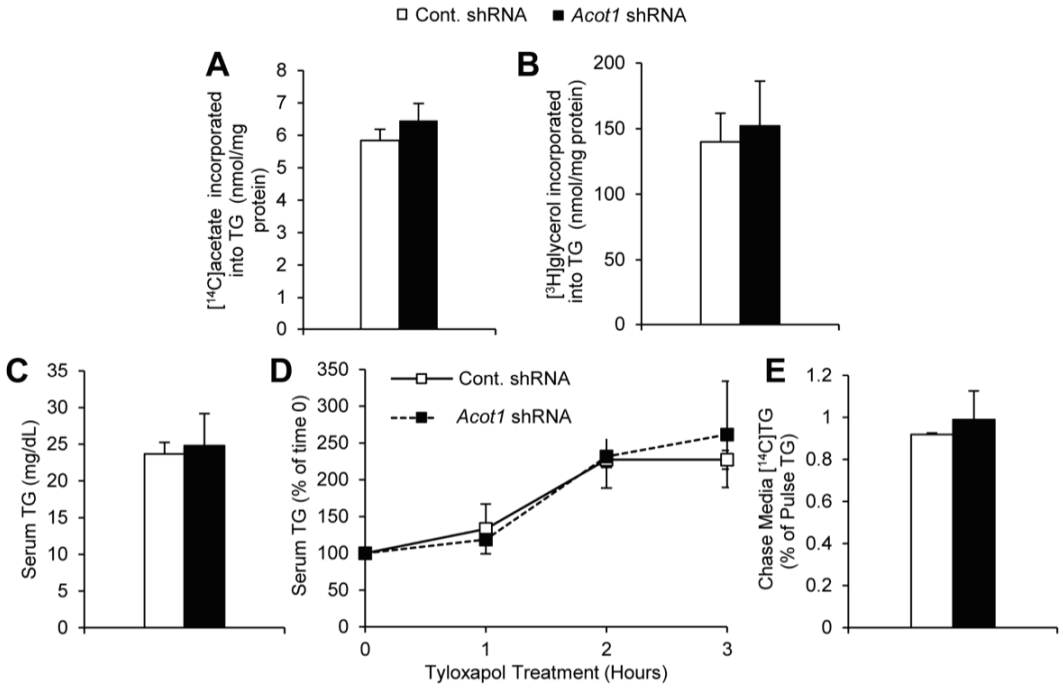
Supplementary Figure 4. Liver specific knockdown of *Acot1* does not affect *de novo* lipogenesis, TG synthesis, or VLDL secretion. Pulse-chase experiments were conducted with [^{14}C]glycerol, [^{14}C]acetate, and [^{14}C]oleate. Incorporation of [^{14}C]acetate (A) and [^{14}C]glycerol (B) into the triglyceride (TG) fraction after the 2 hour pulse was similar between treatments. (C-D) *Acot1* knockdown does not alter fasting serum TG (C) or changes in serum TG in response to tyloxapol to measure rates of VLDL secretion (D). Media [^{14}C]TG after the 6 hour chase was similar between groups (E).



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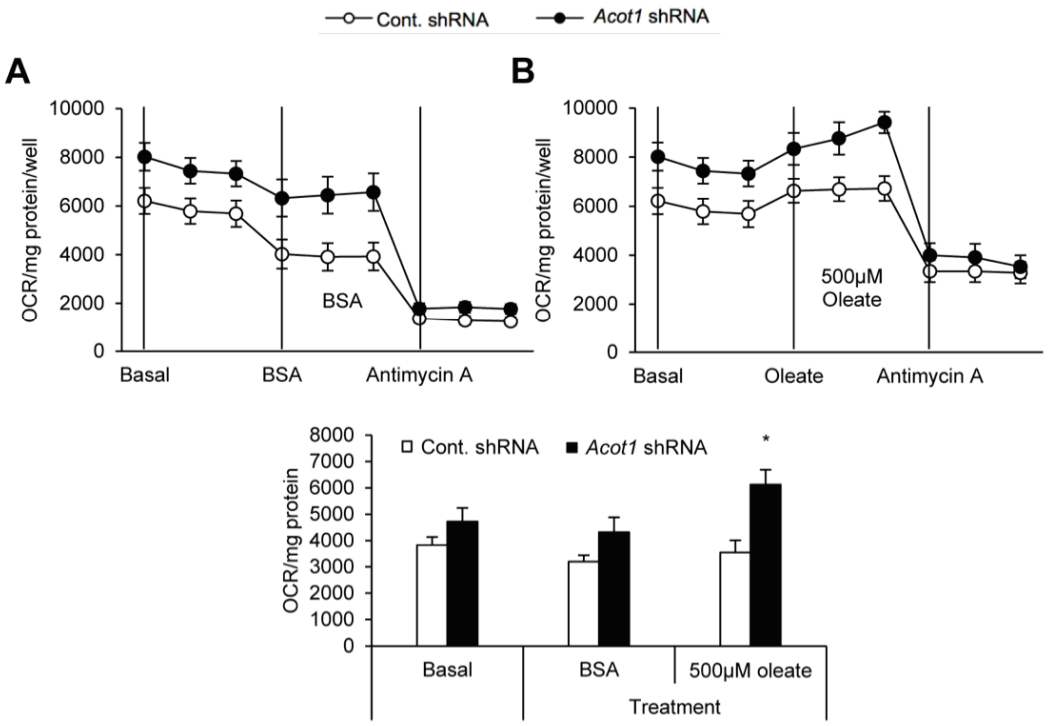
Supplementary Figure 5. Hepatic *Acot1* knockdown increases oxygen consumption. Prim hepatocytes were isolated from control and *Acot1* knockdown mice. OCR was determined iseahorse analyzer XF in the presence of (A) BSA control or (B) 500 uM oleate.



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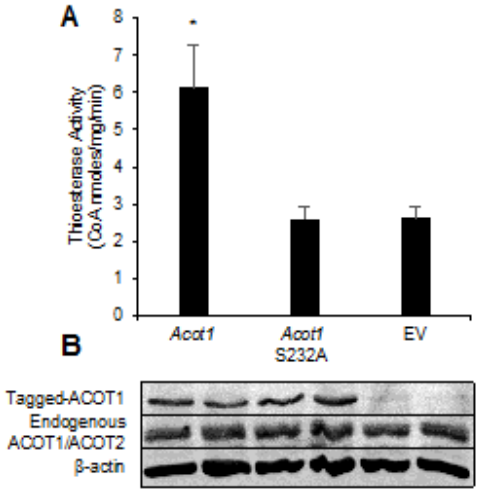
Supplementary Figure 6. *Acot1* point mutation S232A. *Acot1*-DsRed Express N1 (*Acot1*) and*Acot1*-S232A-DsRed Express N1 (*Acot1* S232A) express similarly in COS7 cells (A) compared to the DsRed Express N1 (EV) yet *Acot1*-S232A has no thioesterase activity.



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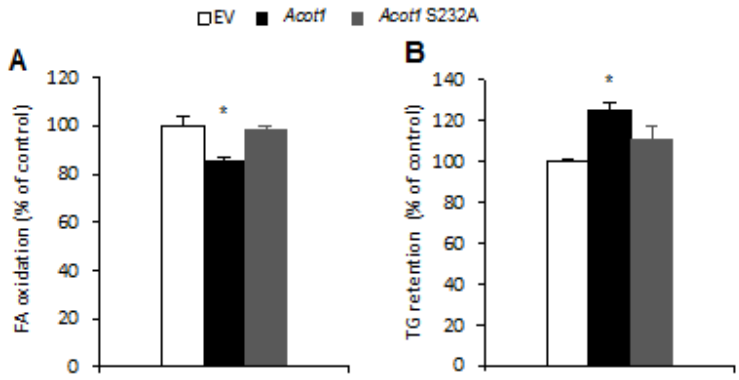
SUPPLEMENTARY DATA

Supplementary Figure 7. ACOT1 overexpression on lipid trafficking. L cells were transfected with *Acot1*-DsRed Express N1 (*Acot1*) and *Acot1*-S232A-DsRed Express N1 (*Acot1* S232A) and treated with trace [^{14}C]oleate along with 500 μM unlabeled oleate bound to BSA (3:1 molar ratio) overnight, then chased for 6 hours with isotope free media. Compared to the DsRed Express N1 (EV), and catalytically dead mutant, *Acot1*-S232A-DsRed Express N1 (*Acot1* S232A), *Acot1*-DsRed Express N1 (*Acot1*) had less (A) FA oxidation and greater (B) TG retention.



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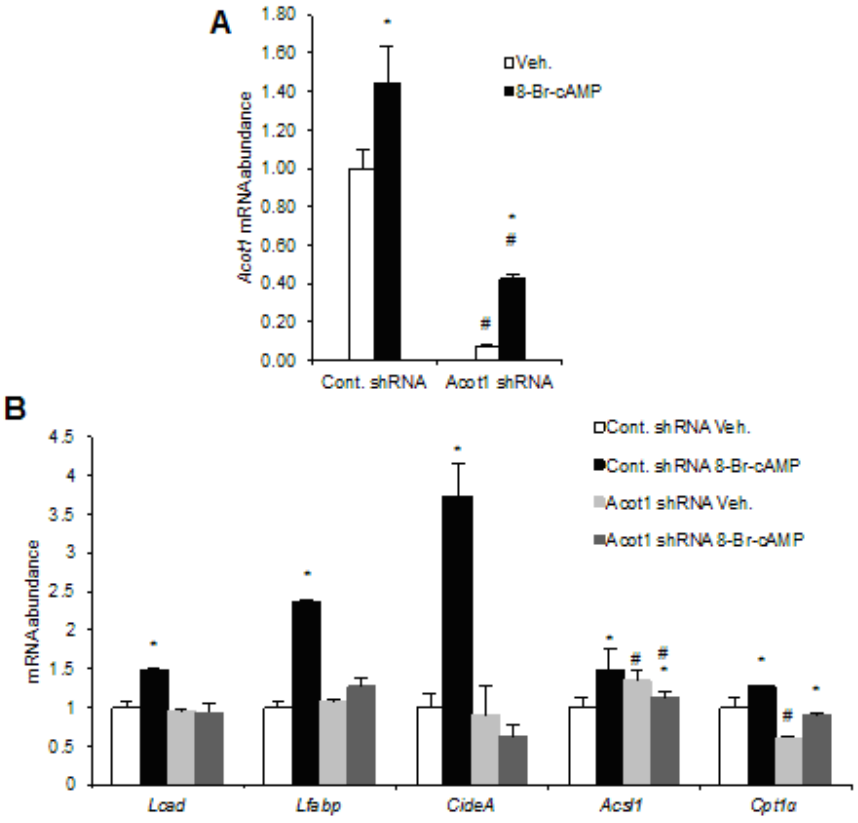
Supplementary Figure 8. *Acot1* knockdown prevent cAMP induced PPARα target gene expression. AML12 cells were transduced with control shRNA or *Acot1* shRNA. Twenty-four hours later cells were treated with 500 μM oleate overnight followed by vehicle (veh) or 8-Br-cAMP for 6 hours prior to harvest. (A) *Acot1* expression in control and *Acot1* knockdown AML12 cells. (B) PPARα target gene expression. *P<0.05 vs. veh. #P<0.05 vs. control shRNA.



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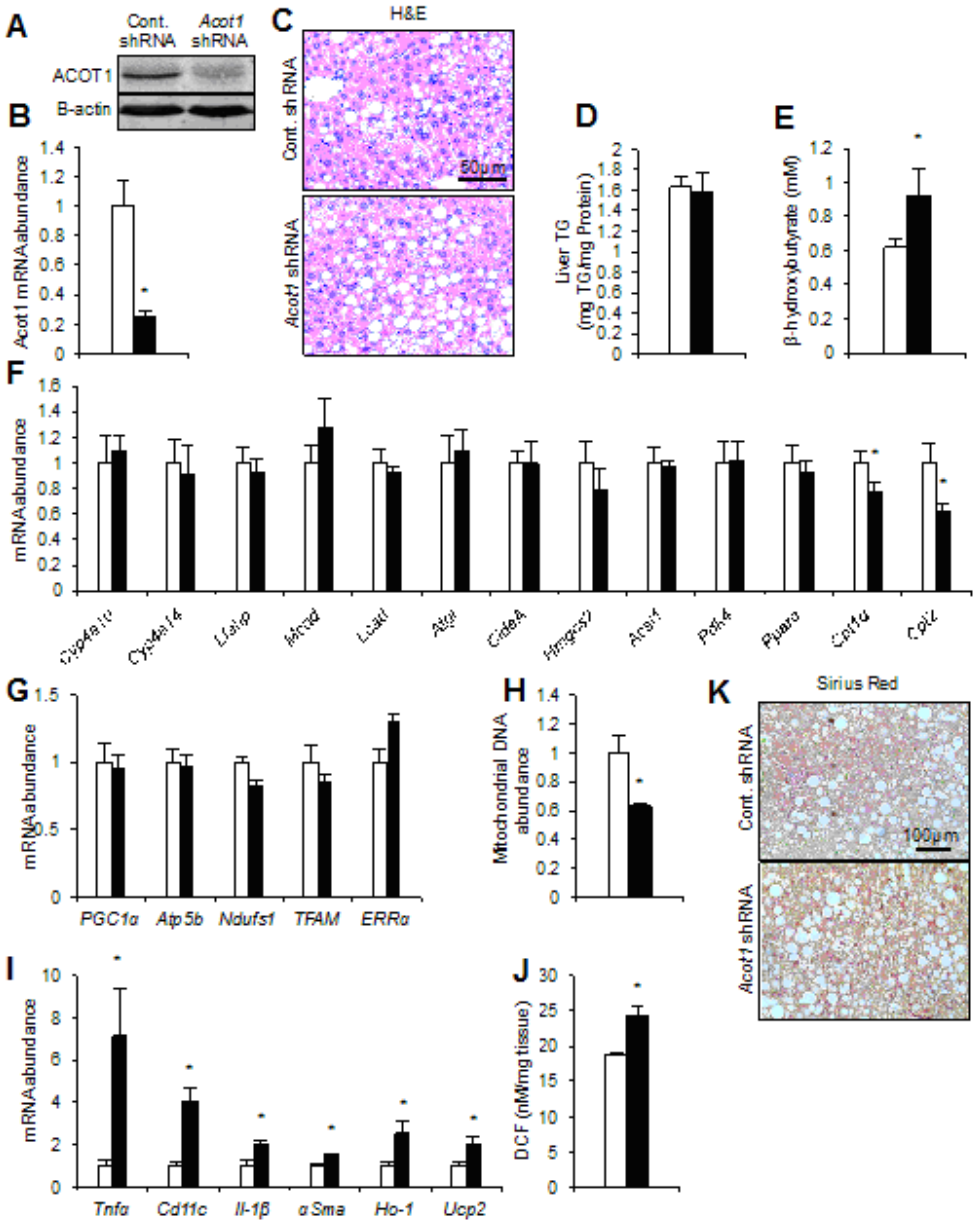
SUPPLEMENTARY DATA

Supplementary Figure 9. Hepatic *Acot1* knockdown exacerbates complications on a high fat diet. *Acot1* expression is reduced as measured by (A) Western blotting and (B) RT-PCR (n=8-10). *Acot1* knockdown had no effect on hepatic TG (C) H&E and (D) liver TG quantification. (E) *Acot1* knockdown increased serum β-hydroxybutyrate. *Acot1* knockdown had no effect on (F) PPARα and (G) PGC1α target gene expression, yet significantly reduced total (H) mitochondrial abundance. (I) *Acot1* knockdown increased expression of inflammatory markers and oxidative stress genes. (J) *Acot1* knockdown increased intracellular ROS measured by OxiSelect *in vitro* ROS/RNS Kit (n=4). (K) Sirius Red staining indicated more fibrosis with *Acot1* knockdown.



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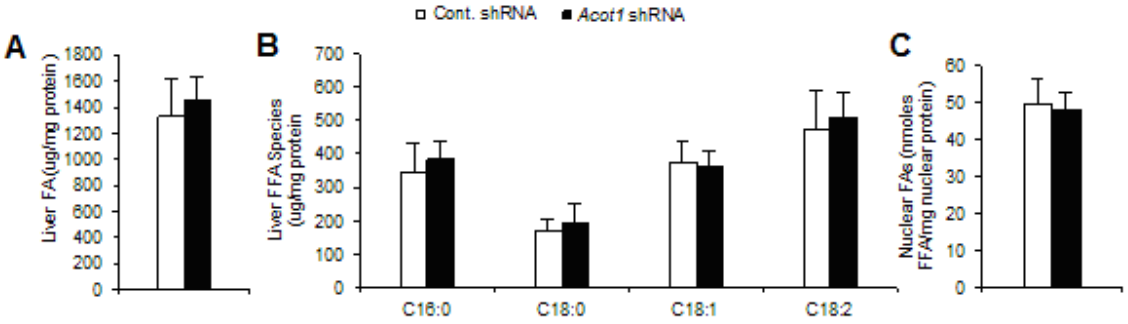
Supplementary Figure 10. Hepatic *Acot1* knockdown had no effect on hepatic FA content. (A) Total liver FAs were not changed with *Acot1* knockdown as well as no significant change in abundance of (B) specific FA species. (C) Nuclear preps were isolated from liver tissue, FAs were extracted via DOLE extraction method, and quantified.



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