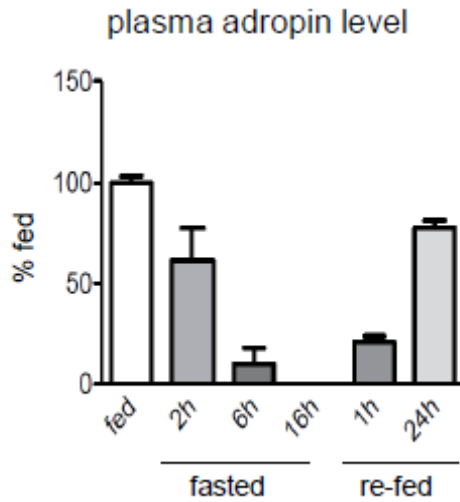
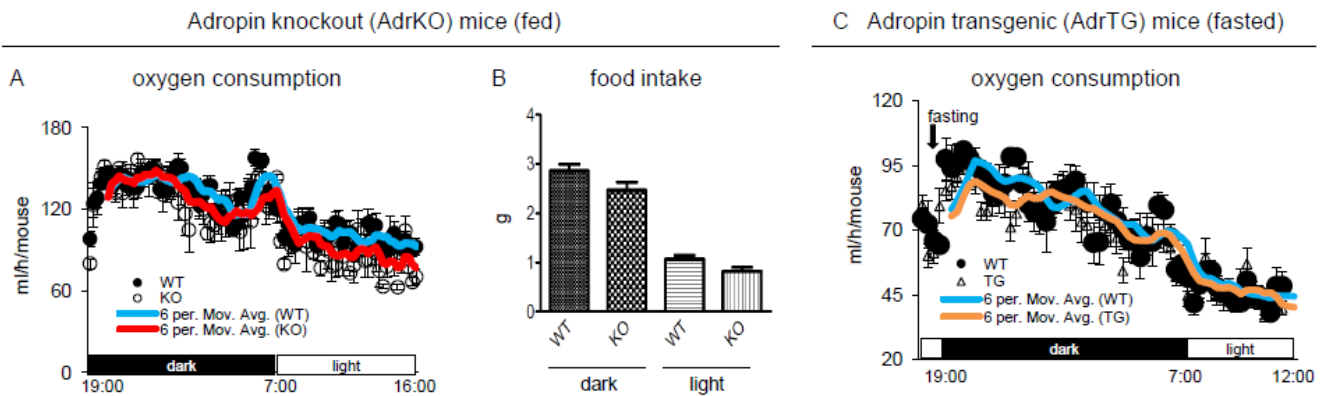


SUPPLEMENTARY DATA

Supplementary Figure 1. Plasma was collected from male wild type B6 mice fasted for 2h, 6h and 16h, or re-fed for 1h or 24h following a 16h-fast (n=3-6). The adropin level in the plasma in the fed state is around 3 ng/ml.



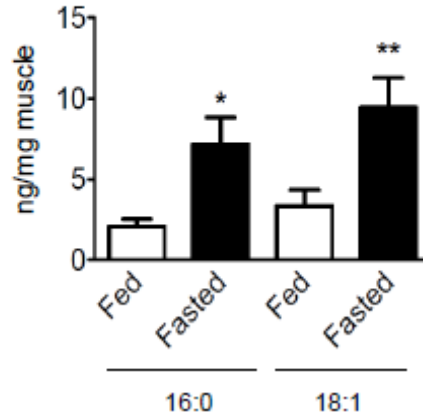
Supplementary Figure 2. Oxygen consumption (A) and accumulated food intake (B) of the *AdrKO* group in the fed state (n=4-9). (C) Oxygen consumption of the *AdrTG* monitored in the fasted state (n=6-10). The 6-period moving average trend line was calculated to represent the data points.



SUPPLEMENTARY DATA

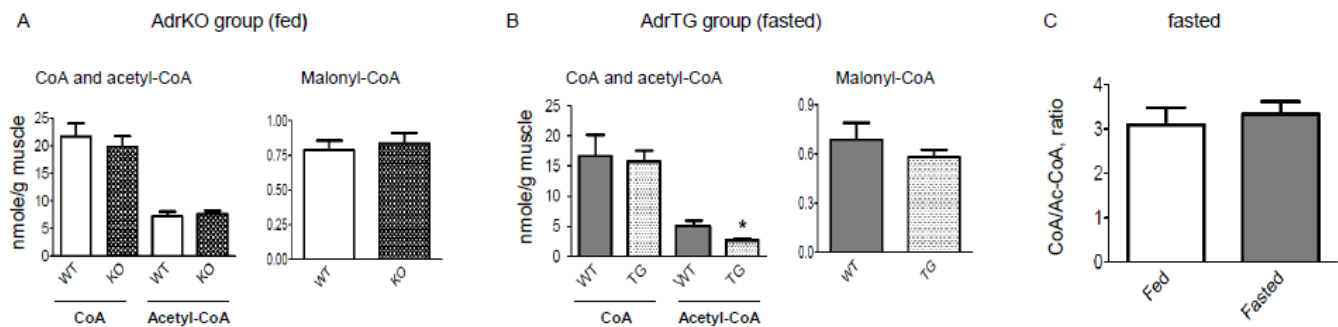
Supplementary Figure 3. Levels of long-chain acylcarnitines [16:0 (palmitoylcarnitine) and 18:1 (oleoylcarnitine)] in skeletal muscle of fasted and fed mice (n=4). Before euthanasia, both the fed and the fasted mice had received 3 injections of vehicle used to inject adropin³⁴⁻⁷⁶. The fasted mice also serve as the vehicle control group in Figure 6E.

long-chain acylcarnitine levels
in fasted wild type mice



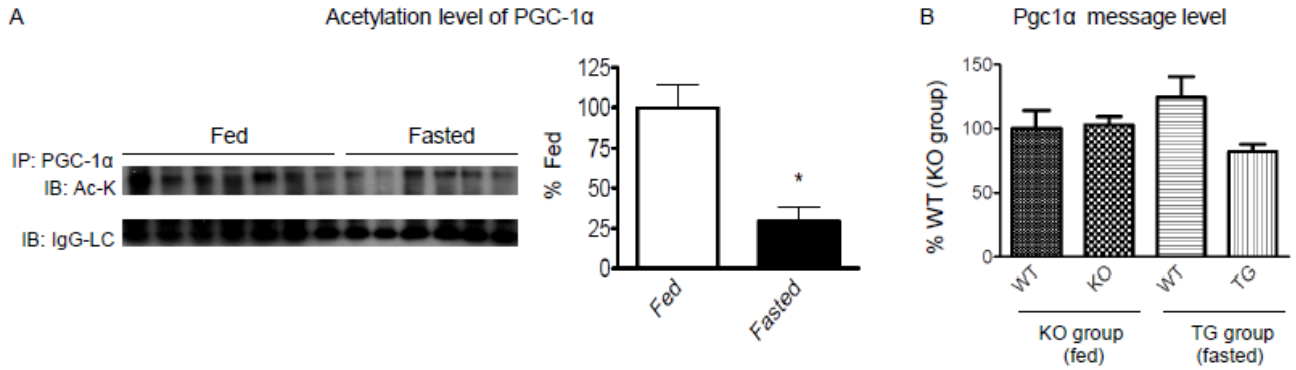
Supplementary Figure 4. The levels of free CoA and short-chain acyl-CoA's including acetyl-CoA (Ac-CoA) and malonyl-CoA in the ADrKO group (A, n=7-8) and the ADrTG group (B, n=6-10). The ADrKO group had been maintained in the fed state when they were euthanized, while the ADrTG group had been fasted overnight (16h) before euthanasia. (C) The CoA/acetyl-CoA (Ac-CoA) ratio for of the fed and fasted WT mice (n=6-7/group).

short-chain acyl-CoA levels



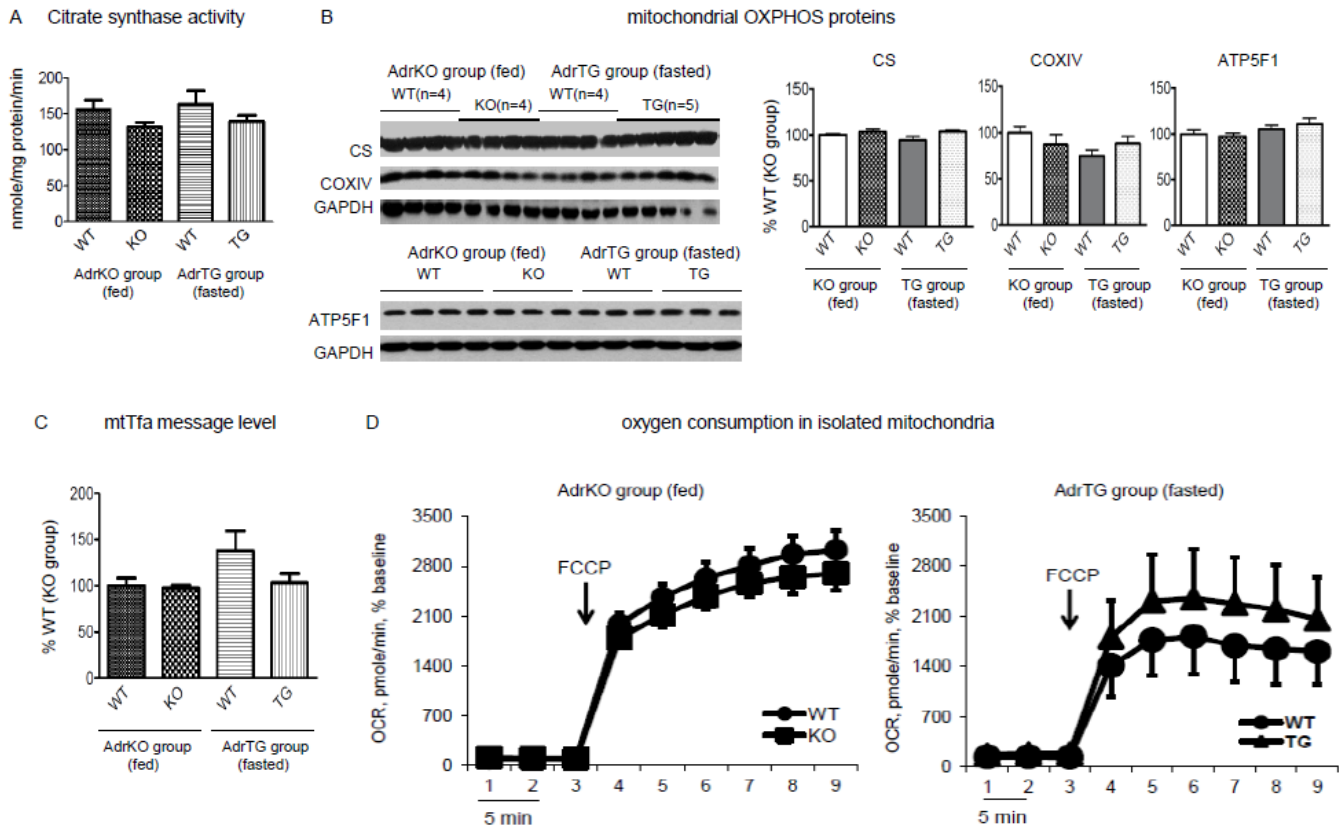
SUPPLEMENTARY DATA

Supplementary Figure 5. (A) Acetylation levels of PGC-1 α in muscle under fed or fasted condition was compared in WT mice (n=6-7). The left panel shows the western blot, while the right panel shows quantified data expressed as a percent of fed mice. The protein samples of the fed mice are the ones from the WT mice in the AdrKO group, and the protein samples of the fasted mice are the ones from the WT mice in the AdrTG group. (B) *Pgc-1 α* message levels in muscle (AdrKO group, n=6; AdrTG group, n=5-7).



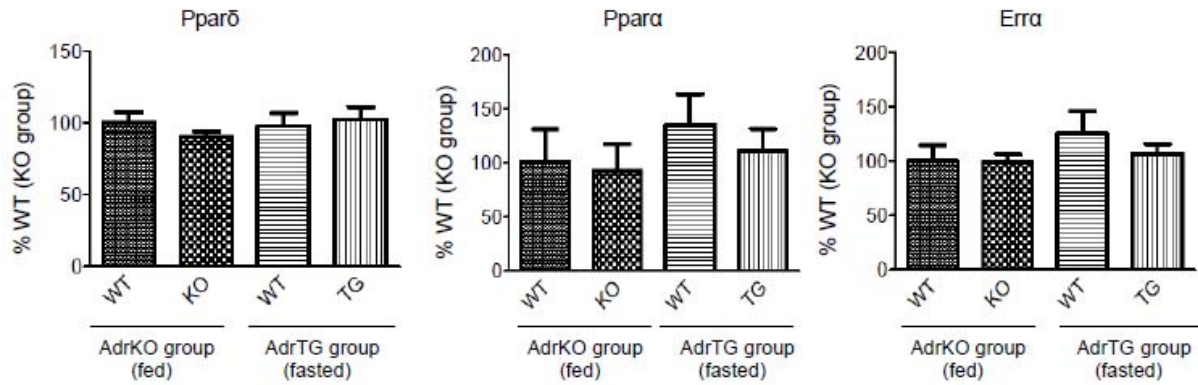
SUPPLEMENTARY DATA

Supplementary Figure 6. (A) Muscle citrate synthase activities (AdrKO group, n=8; AdrTG group, n=6-10). The AdrKO group were maintained in fed state when they were euthanized, while the AdrTG group were fasted overnight (16h) before euthanasia. (B) Analysis of OXPHOS proteins including citrate synthase (CS) (n=4-5), cytochrome c oxidase subunit IV (COXIV) (n=4-5), and b subunit of ATP synthase proton channel (ATP5F1) (n=3-4) measured by western blot. (C) Mitochondrial transcription factor A (mtTfa) message levels in muscle (AdrKO group, n=6; AdrTG group, n=4-7). (D) Pyruvate (10mM)/malate (5mM)-based oxygen consumption rates (OCR) in basal state and upon FCCP (4 μ M) stimulation measured using mitochondria isolated from muscle (AdrKO group, n=4; AdrTG group, n=6-10).



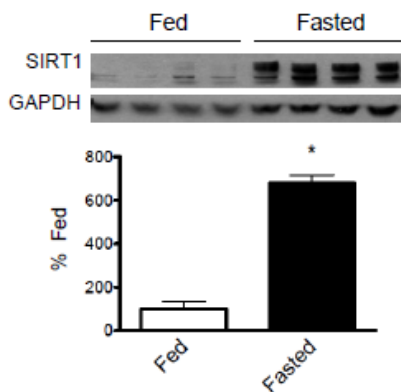
SUPPLEMENTARY DATA

Supplementary Figure 7. The message levels of *Ppar δ* , *Ppara* and *Err α* in muscle (AdrKO group, n=6; AdrTG group, n=5-7). The AdrKO group were maintained in fed state when they were euthanized, and the AdrTG group were fasted overnight (16h) before euthanasia. *Ppar δ* , peroxisome proliferator-activated receptor delta; *Ppara*, peroxisome proliferator-activated receptor alpha; *Err α* , estrogen-related receptor alpha.

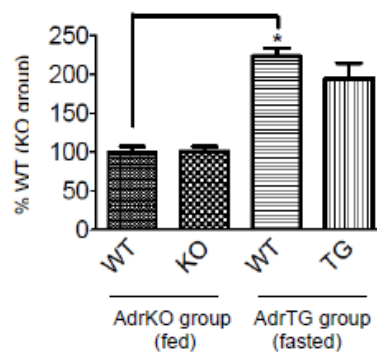


Supplementary Figure 8. (A) Muscle SIRT1 protein levels under fed or fasted conditions (n=5-7). The AdrKO group were maintained in fed state when they were euthanized, and the AdrTG group were fasted overnight (16h) before euthanasia. The protein samples of the fed mice are those used for the WT mice in the AdrKO group, and the protein samples of the fasted mice are the WT mice in the AdrTG group [note: some gel blots from the WT mice in the AdrTG group (shown in Fig.5A) are presented under the fasted group]. (B) *Sirt1* message levels in muscle were measured in the AdrKO (n=6) and the AdrTG (n=5-7) group. (C) Message levels of *Sirt1* in muscle following acute adropin injections in fasted mice (see the details in Figure 6), (n=5).

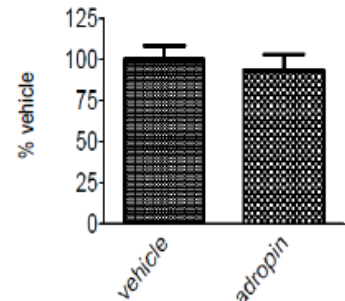
A SIRT1 protein level in fasted state



B *Sirt1* message level



C *Sirt1* message level



SUPPLEMENTARY DATA

Supplementary Figure 9. (A) Levels of phosphorylated AMPK (pAMPK) and total AMPK (AMPK) in muscle measured in wild type mice fasted for 2h, 6h and 16h (n=3-4/group). The fed mice, i.e. t=0, was used as the control. All the levels are presented as “% t=0”. (B) The AdrKO group were maintained in fed state when they were euthanized, and the adropin TG group were fasted overnight (16h) before euthanasia. The levels of pAMPK (n=4) and AMPK (n=5-6) in muscle were then measured by western blot (n=3).

