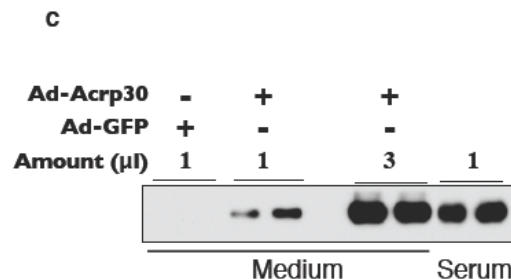
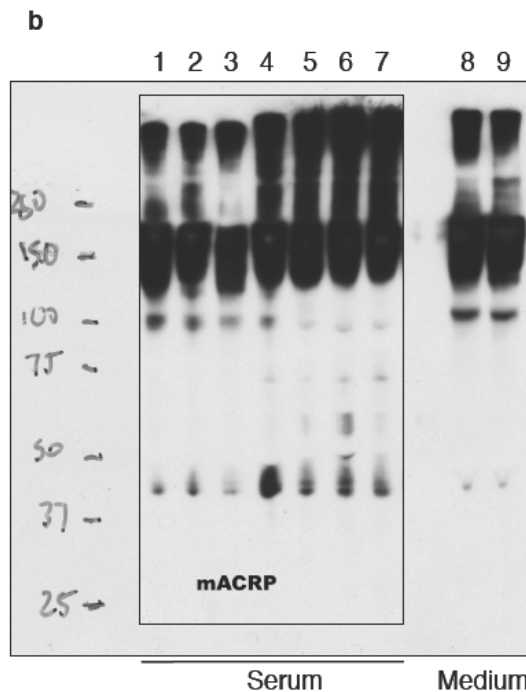
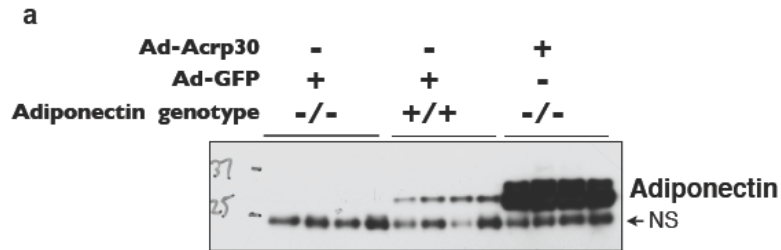


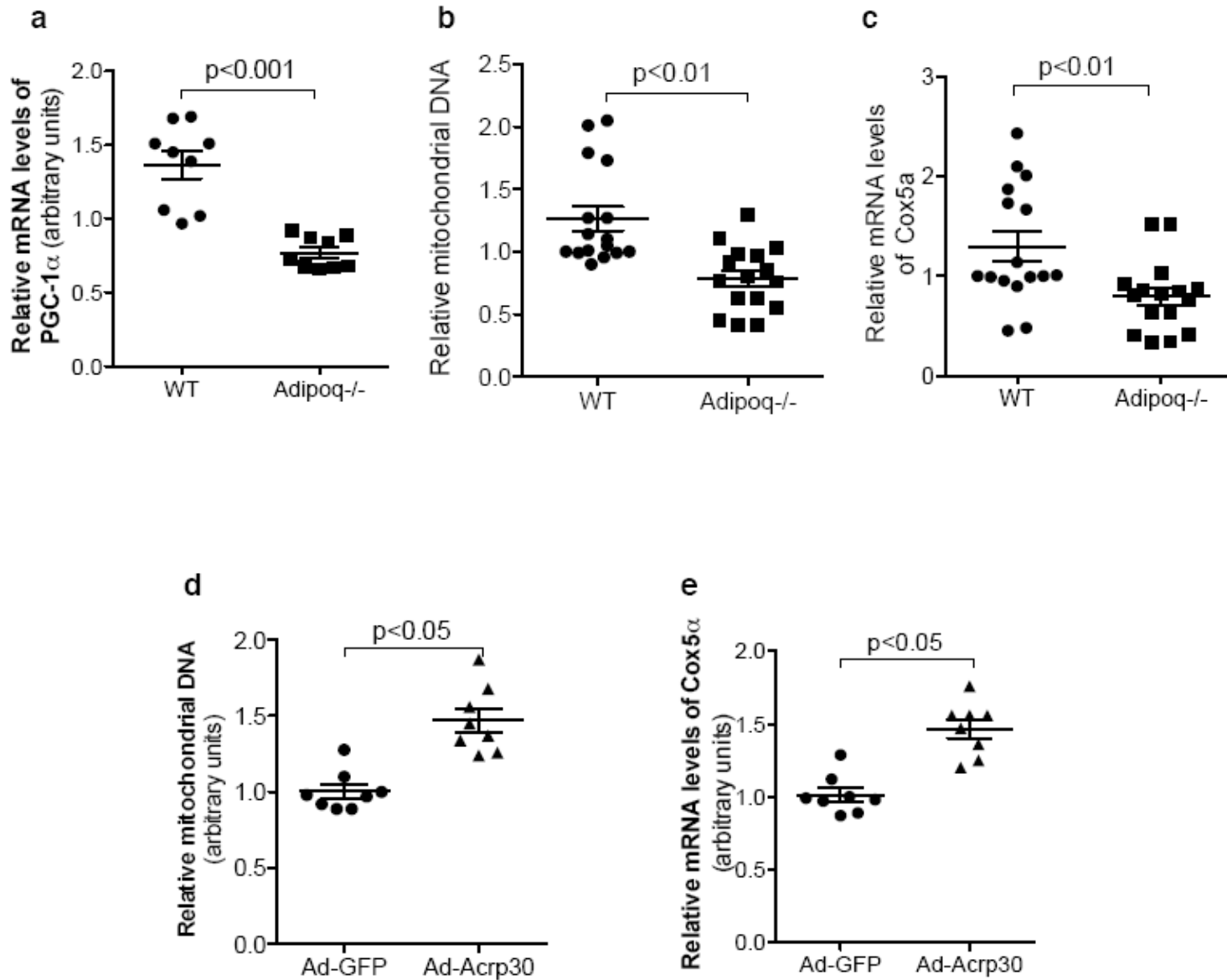
SUPPLEMENTARY DATA

Supplementary Figure 1. Adenovirus-mediated in vivo and in vitro adiponectin gene transduction. Purified adenoviruses encoding adiponectin (Ad-Acrp30) or GFP were injected into mice through the tail vein (a&b). Three days after injection, blood samples were collected. Total adiponectin (0.25 μ l of serum) and multimeric (0.5 μ l serum) adiponectin were measured by Western blotting under reducing (a) or non-reducing (b) conditions. For the setting of coculture (c), FAO cells were transduced with Ad-Acrp38 in medium supplemented with 0.5% BSA and without any FBS. Total adiponectin levels from 1 or 3 μ l of medium were compared with 1 μ l C57BL/6 mouse serum (c). For the multimeric adiponectin assay (b), lanes 1-3 were from serum of WT mice, lanes 4-7 were from serum of Ad-Acrp30 transduced mice, lanes 8&9 were from medium from Ad-Acrp30-transduced FAO cells. The mobilities of molecular weight markers are indicated in kDa on the left side of panels a and b.



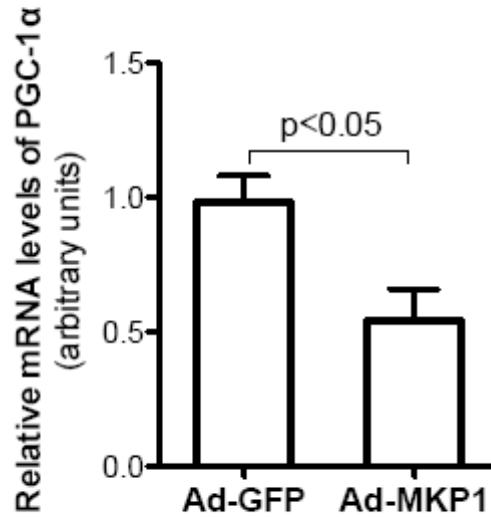
SUPPLEMENTARY DATA

Supplementary Figure 2. Decreased mitochondrial biogenesis in skeletal muscle of adiponectin knockout mice (a-c). The gastrocnemius muscle samples were collected from 8-10 week old male WT and Adipoq^{-/-} mice. mRNA levels of PGC-1 α and Cox5 α and mitochondrial DNA content were measured using real-time PCR. Adiponectin treatment increased mitochondrial biogenesis in C2C12 myotubes (d&e). Differentiated C2C12 myotubes were cocultured overnight with Ad-Acrp30 transduced FAO cells. Mitochondrial DNA and mRNA levels of Cox5 α were measure by real-time PCR. Data are expressed as mean \pm SEM.

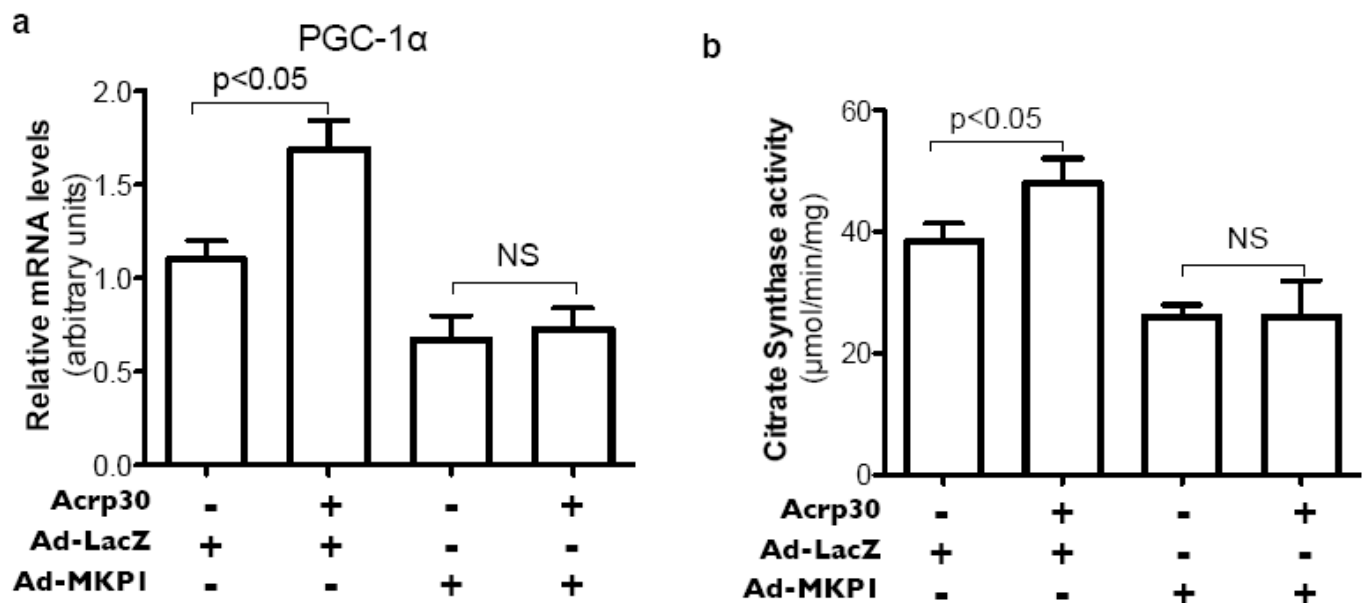


SUPPLEMENTARY DATA

Supplementary Figure 3. MKP1 overexpression decreased PGC-1 α mRNA in C2C12 myotubes. mRNA sample were prepared from Ad-MKP1 and Ad-GFP-transduced C2C12 myotubes. PGC-1 α mRNA levels were determined by real-time PCR. n=8, data are expressed as mean \pm SEM.



Supplementary Figure 4. Adiponectin does not increase PGC-1 α mRNA and citrate synthase activity in C2C12 myotubes overexpressing MKP1. Differentiated C2C12 myotubes were transduced with adenovirus vectors encoding MKP1 or LacZ, then treated with adiponectin overnight using the co-culture system. PGC-1 α mRNA levels were measured using real-time PCR (a). Citrate synthase activity was measuring from lysed cells. n=6, data are expressed as mean \pm SEM.



SUPPLEMENTARY DATA

Supplementary Figure 5. Adiponectin increases PGC-1 α activity and reduces PGC-1 α Ser570 phosphorylation. Cidea promoter-directed luciferase constructs were transiently transfected into C2C12 cells with pcDNA-PGC-1 α , pcDNA-PGC-1 α 3A or pcDNA-p38. 24 h after transfection, the indicated cells were incubated in the presence of conditioned medium containing adiponectin secreted from Ad-Acrp30-transduced FAO cells for 12h. Luciferase activities were normalized to co-transfected LacZ internal control. (b), differentiated C2C12 myotubes were treated with Acrp30-enriched conditioned medium overnight. (c), blood adiponectin levels were elevated after transduction with Ad-Acrp30 in WT mice. Skeletal muscle tissues were collected 3 days after virus injection. Phosphorylation and total protein levels were measured by Western blotting.

