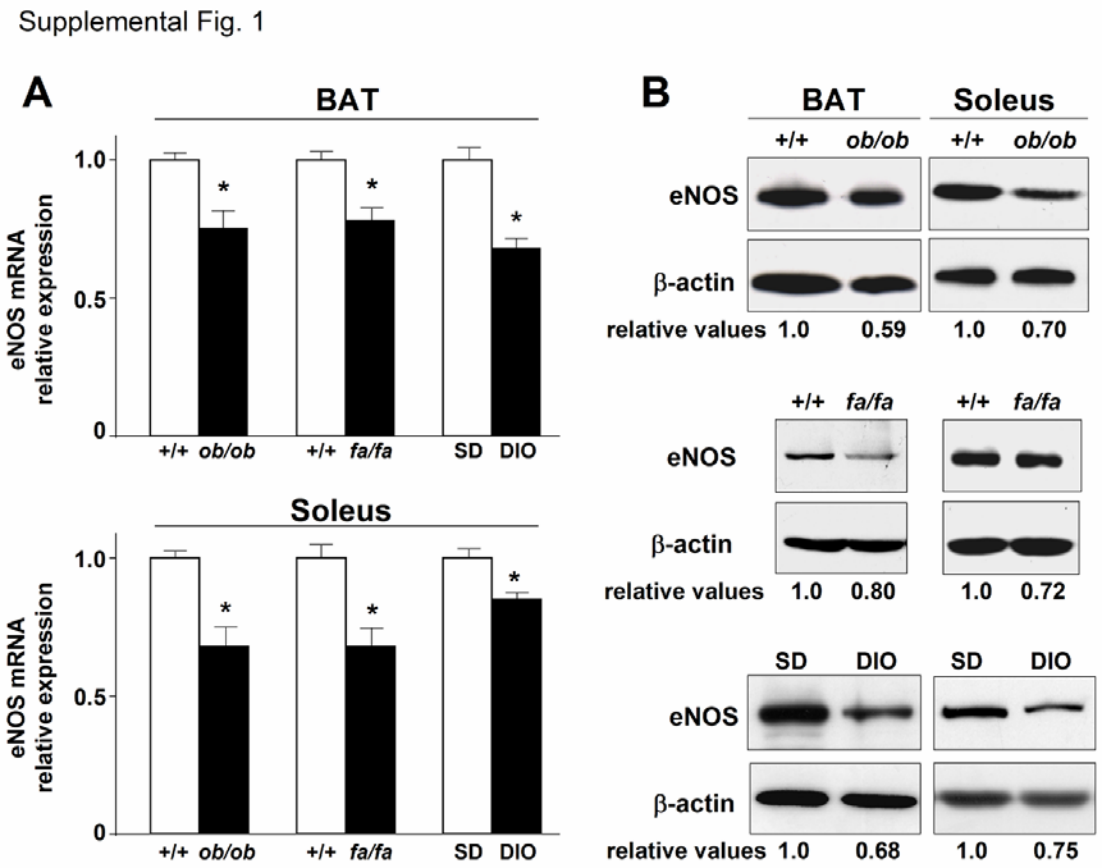
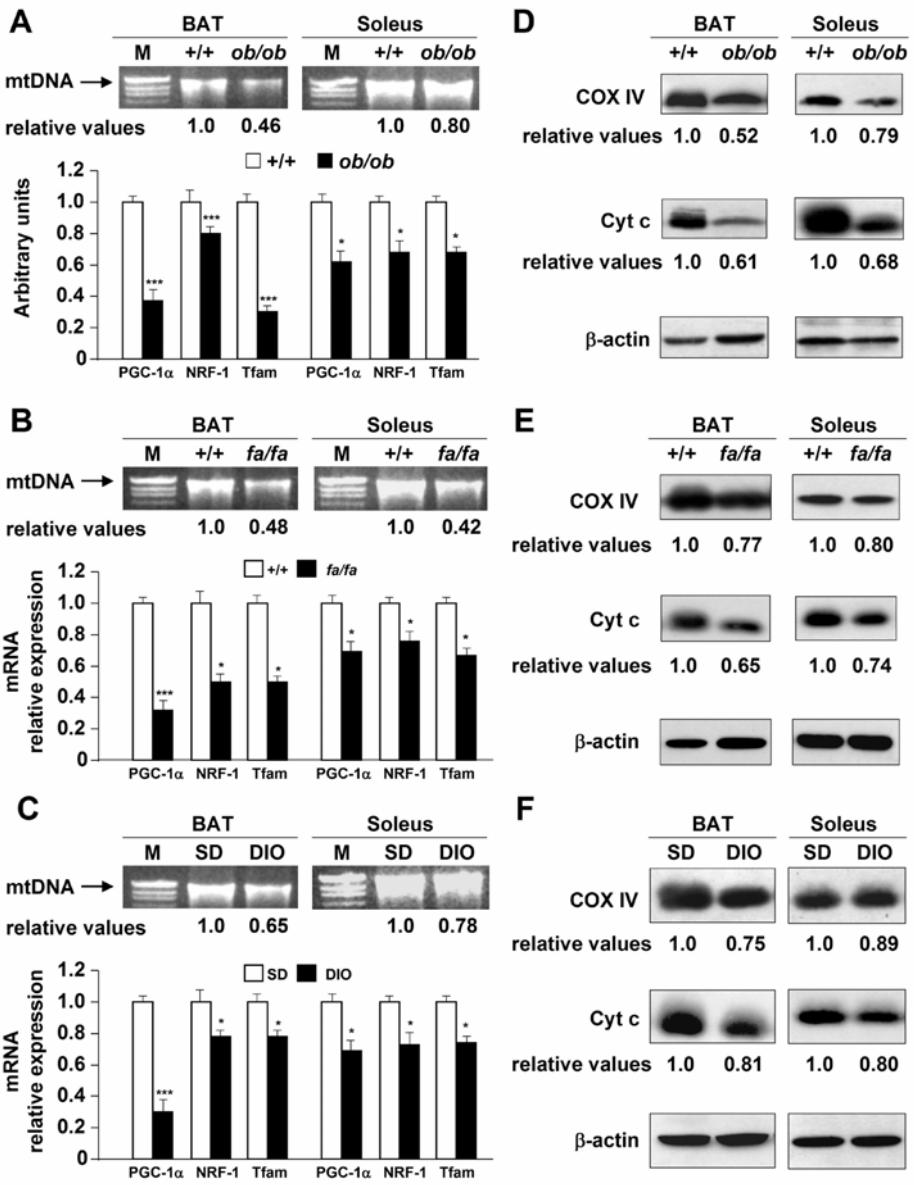
Supplemental Fig. 1



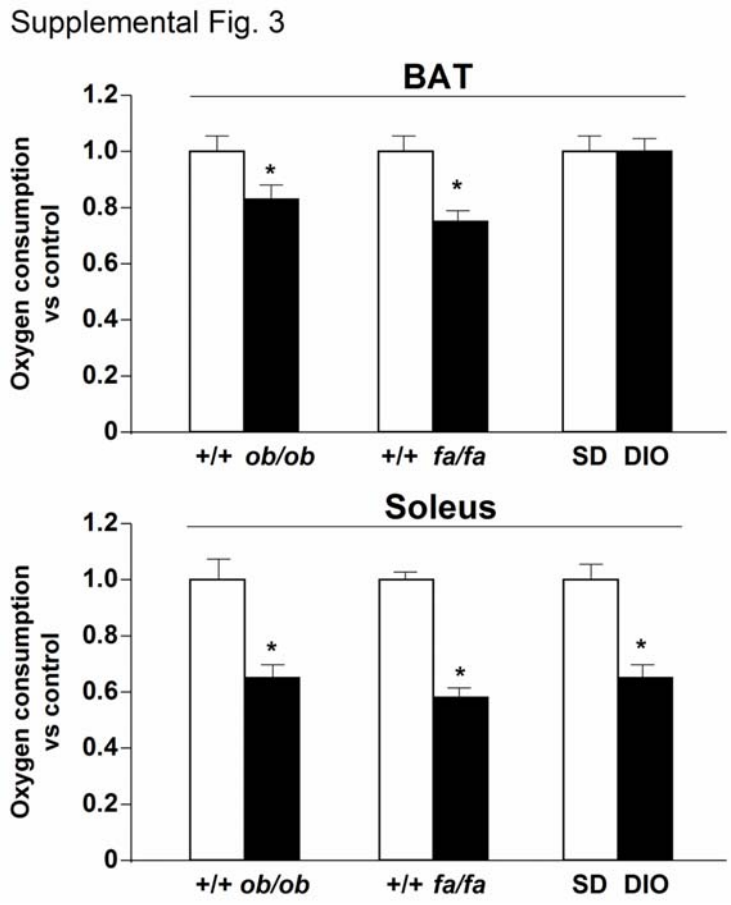
eNOS expression is reduced in BAT and soleus muscle from obese animals. (**A**) eNOS mRNA levels, measured by means of quantitative RT-PCR in brown adipose tissue (BAT) and soleus muscle of genetically obese mice (*ob*/*ob*) and rats (*fa*/*fa*) and of environmentally obese mice (DIO, diet-induced obesity) compared to respective controls (+/+, wild type; SD, standard diet fed animals). The cycle number at which the various transcripts were detectable was compared to that of β-actin as an internal control, and expressed as arbitrary units versus values in control animals taken as 1.0 (n = 5 experiments). *P < 0.05. (**B**) eNOS protein was detected by immunoblotting (one experiment representative of five reproducible ones) in BAT and soleus muscle of *ob*/*ob* mice, *fa*/*fa* rats and DIO mice compared to respective controls. The numbers below the blots show the relative values from the densitometric analysis, referred to β-actin levels, when control measurements per group are given a value of 1.0.

Supplemental Fig. 2



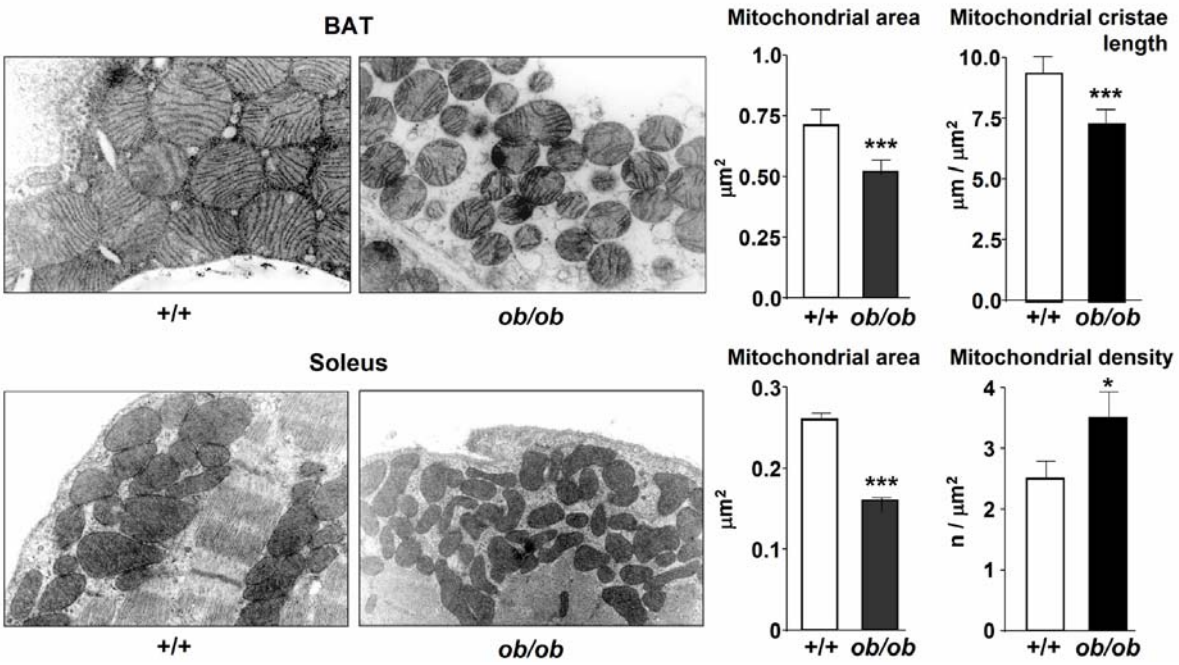
Mitochondrial biogenesis is reduced in BAT and soleus muscle of *ob*/*ob* and DIO mice and in *fa*/*fa* rats. (**A**), (**B**) and (**C**) PGC-1α, NRF-1, and Tfam mRNA, analyzed by means of quantitative RT-PCR with gene-specific oligonucleotide probes in obese and control animals. The cycle number at which the various transcripts were detectable was compared to that of β-actin as an internal control, and expressed as arbitrary units versus values in control animals taken as 1.0 (n = 5 experiments). ***P < 0.01, *P < 0.05. (Inset) mtDNA [one experiment representative of five gels (n = 5 animals per group); the numbers show the relative amounts from the densitometric analysis when control measurements per group are given a value of 1.0; M, DNA marker]. (**D**), (**E**) and (**F**) COX IV and Cyt c proteins were detected by immunoblot analysis (one experiment representative of five reproducible ones). The numbers below the blots show the relative values from the densitometric analysis, referred to β-actin levels, when control measurements per group are given a value of 1.0.

Supplemental Fig. 3



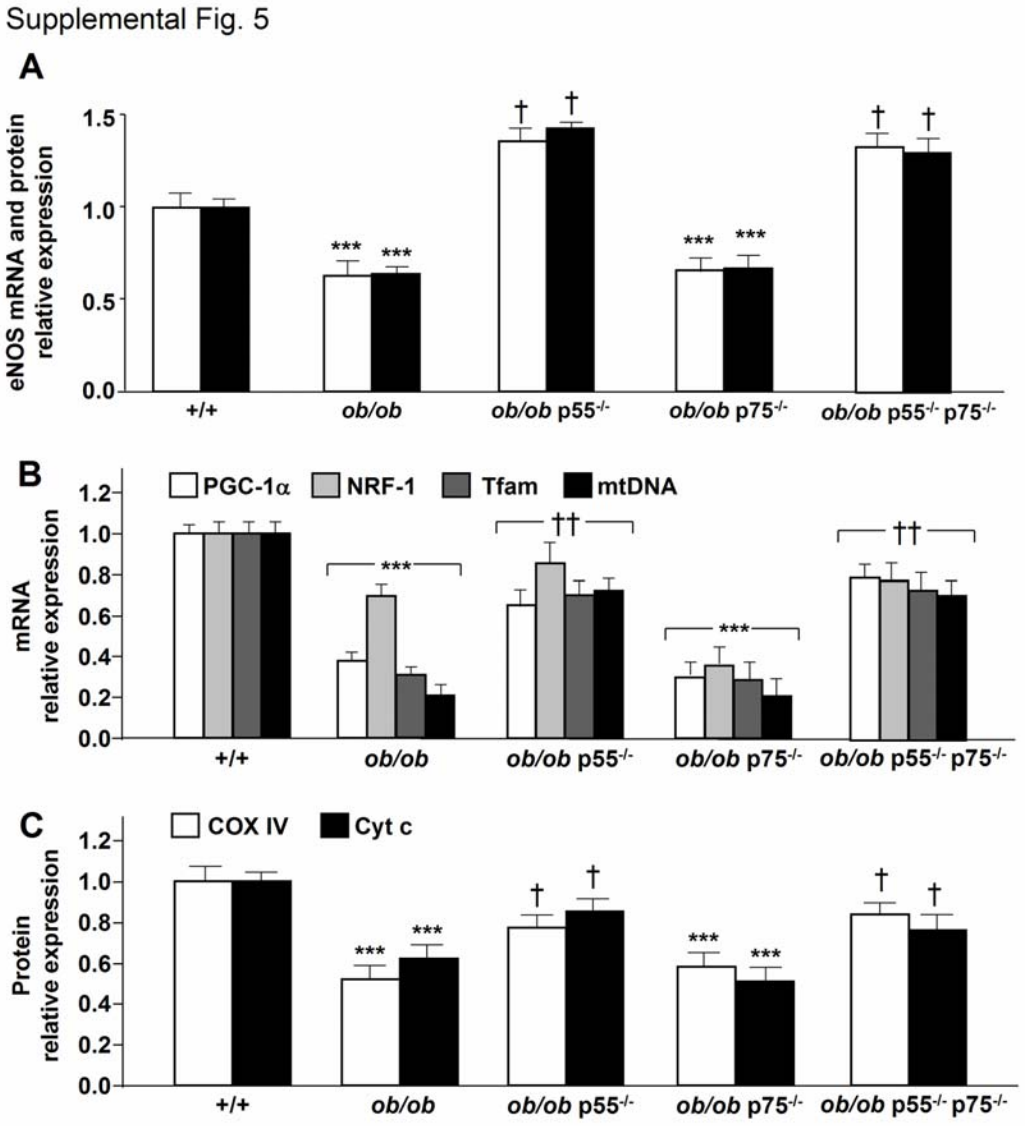
Oxygen consumption by BAT and soleus muscle of obese and control animals was measured in a gas-tight chamber using an O_2 electrode. Oxygen consumption values were normalized to the tissue protein content (*n* = 3 experiments). *$P < 0.05$ *vs*. controls taken as 1.0.

Supplemental Fig. 4



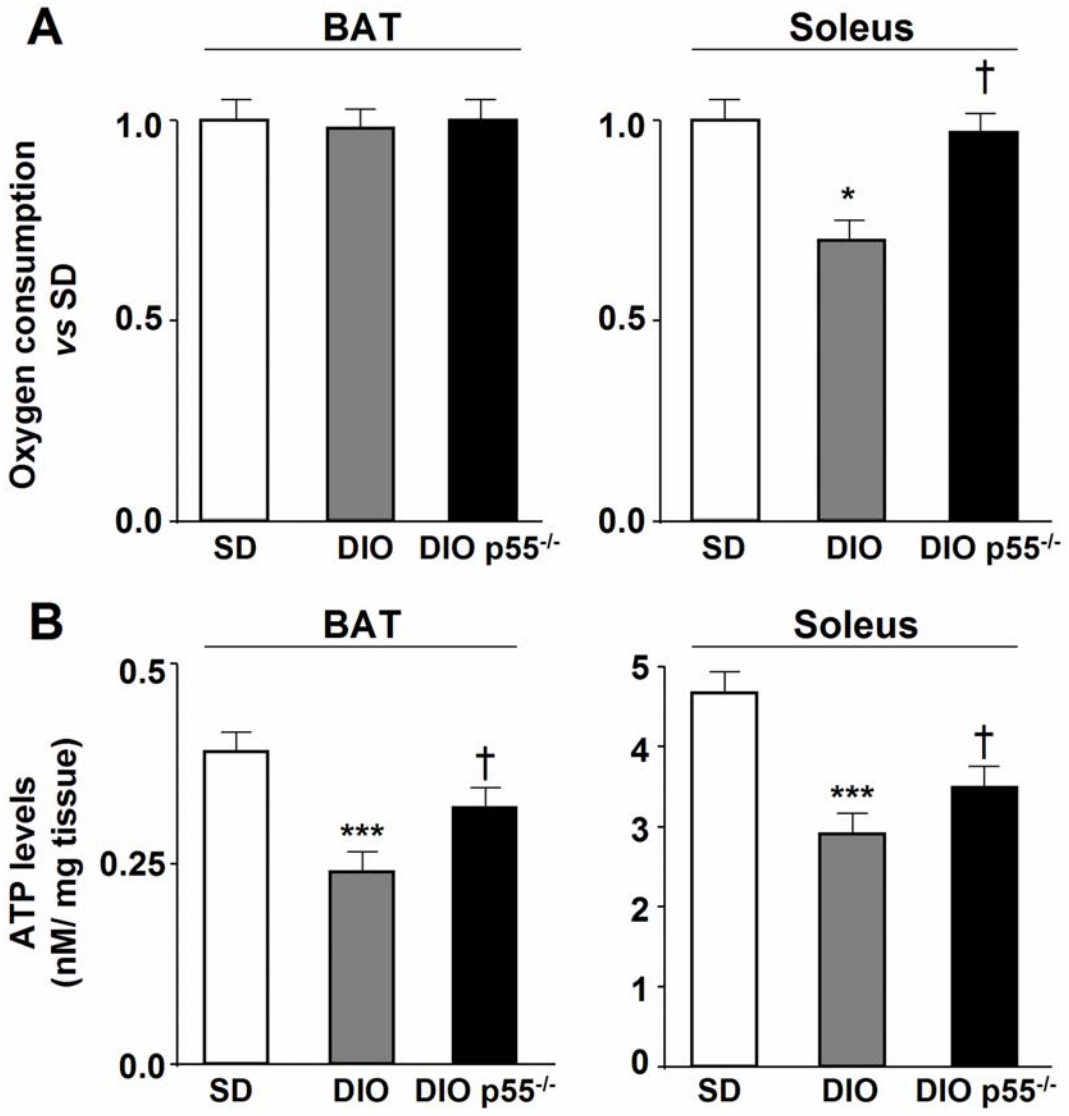
Mitochondrial pattern is altered in BAT and soleus muscle of *ob*/*ob* mice. Both BAT and soleus muscle from control (+/+) or obese (*ob*/*ob*) mice were processed to perform stereological analysis of mitochondria. The tissues were fixed and sectioned randomly and prepared for electron microscopy. Representative electron micrographs are shown on the left. Fifty photographs at a magnification of x 11,500 were taken, and the area and density of more than 1,000 mitochondria per tissue were determined. The results are reported on the right as the means ± SEM (n = 3 animals per group). ***$P < 0.01$, *$P < 0.05$.

Supplemental Fig. 5



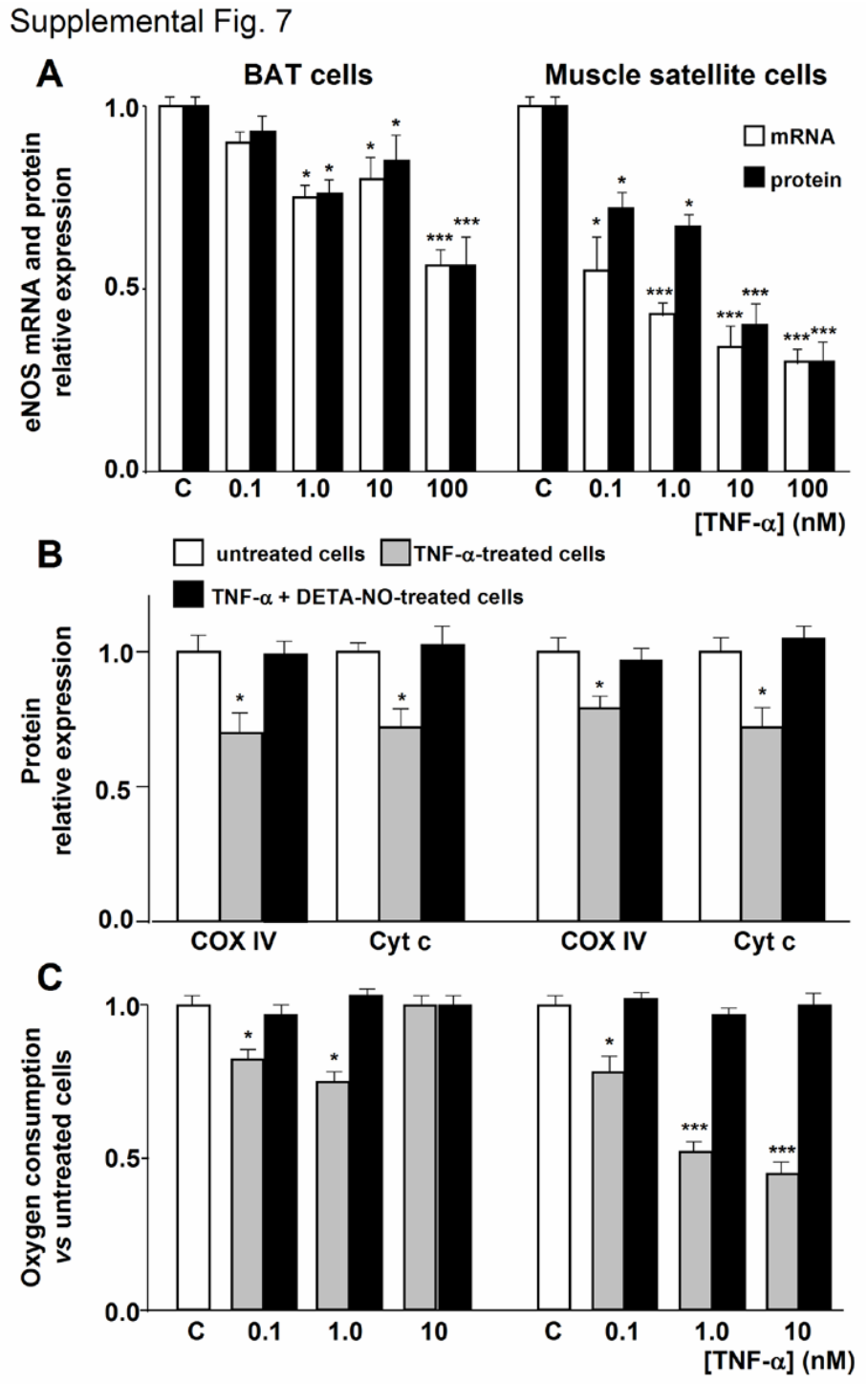
TNF-α signaling deficiency partly restores mitochondrial biogenesis in obesity. (**A**) eNOS mRNA and protein analysed by quantitative RT-PCR or immunoblotting, respectively, in BAT of control (+/+, SD), *ob*/*ob* (wild-type at TNF-α receptor loci) and *ob*/*ob* with TNF-α signaling deficiency (*ob*/*ob*−p55$^{-/-}$, *ob*/*ob*−p75$^{-/-}$ and *ob*/*ob*−p55$^{-/-}$p75$^{-/-}$) mice. The relative values are shown as means ± SEM (*n* = 3 animals per group) when control measurements (+/+) are given a value of 1.0. ***P < 0.01 and †P < 0.05 *vs.* wild-type animals. (**B**) PGC-1α, NRF-1, and Tfam mRNA, analyzed by means of quantitative RT-PCR with gene-specific oligonucleotide probes in BAT of wild-type, obese and obese with TNF-α signaling deficiency mice. The cycle number at which the various transcripts were detectable was compared to that of β-actin as an internal control, and expressed as arbitrary units. ***P < 0.01 *vs.* wild-type animals taken as 1.0 (*n* = 3 animals per group), ††P < 0.05 *vs. ob*/*ob* mice. (**C**) COX IV and Cyt c protein levels were detected by densitometric analysis of immunoblots in BAT of wild-type, obese and obese with TNF-α signaling deficiency mice. ***P < 0.01 *vs.* wild-type animals taken as 1.0 (*n* = 3 animals per group), †P < 0.05 *vs. ob*/*ob* mice.

Supplemental Fig. 6



TNF-α signaling deficiency partly restores mitochondrial biogenesis in obesity. (**A**) Oxygen consumption and (**B**) ATP levels in BAT and soleus muscle of either DIO or DIO−p55$^{-/-}$ mice compared to control (SD) mice. *n* = 3-4 animals per group, three independent experiments. ***P < 0.01 *vs*. controls (SD), †P < 0.05 *vs*. DIO mice.

Supplemental Fig. 7



Mitochondrial biogenesis is down-regulated by TNF-α and restored by NO-donor in BAT and muscle satellite cells. (**A**) eNOS mRNA and protein, analyzed by quantitative RT-PCR and immunoblotting, respectively, in untreated cells (C, control) or after exposure to TNF-α at different doses for 2 days. (**B**) COX IV and Cyt c protein levels and (**C**) oxygen consumption were measured in untreated BAT and muscle satellite cells and after treatment with 1 nM (**B**) or different doses (**C**) of TNF-α alone or in combination with 50 μM DETA-NO for 2 days. The relative values are shown as means ± SEM (*n* = 4 different experiments), when control measurements are given a value of 1.0. ***$P < 0.01$, *$P < 0.05$ *vs*. untreated cells.