



**Supplementary Figure 1** Mitochondrial volume density and mitochondrial gene expression in skeletal muscle and BAT

(A) Transmission electron microscopy (TEM) (Förster et al., 2002) of the soleus muscle from SOD2-overexpressing (hSOD2) and wild-type (Wt) mice. The size bar represents 0.4  $\mu\text{m}$ . (B) Quantification of subsarcolemmal mitochondrial volume density (Weibel et al., 1979) in the soleus muscle of wild-type (gray bars) or SOD2-overexpressing PAC662D1 mice (black bars) acclimated to 25°C or to 4°C for 11 weeks (n = 4 - 6). (C) Relative levels of mtDNA (Ekstrand et al., 2004), mtRNA, Tfam protein, cytochrome *c* protein (Cyto *c*) and cytochrome *c* oxidase subunit II protein (COXII) in muscle of wild-type (gray bars) or SOD2-overexpressing (black bars) mice acclimated to 25°C (n = 4 - 6). (D) Total UCP1 content in BAT of wild-type (Wt) and SOD2-overexpressing (hSOD2) female mice acclimated to 25°C or to 4°C for 11 weeks. UCP1 levels are expressed as arbitrary units (AU) per left interscapular BAT lobe; the values obtained for wild-type mice at 25°C were set to 100 AU. (E) Relative levels of mtDNA, PGC-1 $\alpha$  mRNA and PPAR $\gamma$  mRNA in the right interscapular BAT lobe of wild-type (gray bars) or SOD2-overexpressing (black bars) mice acclimated to 4°C for 11 weeks. The values are expressed in % of the levels in wild-type mice (n = 4 - 6). Primary antibodies were diluted as follows: anti-COXII (Ekstrand et al., 2004) 1:5000, anti-mouse-TfAM 1:3000 (Ekstrand et al., 2004), anti-cytochrome *c* (Santa Cruz) 1:1000, anti-actin (Santa Cruz) 1:500, anti-UCP1 (prepared in rabbit from the C-terminal decapeptide of mouse UCP1) 1:5000, anti-COXII (Houstek et al., 1988) 1:1000 and anti-PGC-1 $\alpha$  (Calbiochem) 1:1000, respectively. Secondary horseradish peroxidase-conjugated antibodies were used at a dilution of 1:2000.