Supporting Information

Jing et al. 10.1073/pnas.1111308108

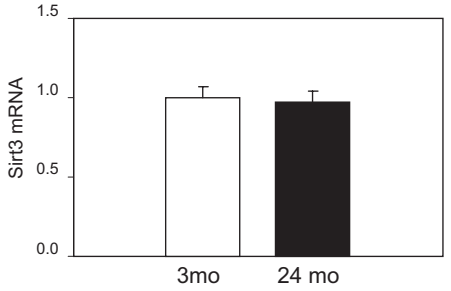

Fig. S1. Unchanged Sirt3 expression in aging. Skeletal muscle from hind limbs of young (3 mo) and old (24 mo) mice were collected, extracted, and subjected to quantitative real-time PCR for Sirt3 expression.

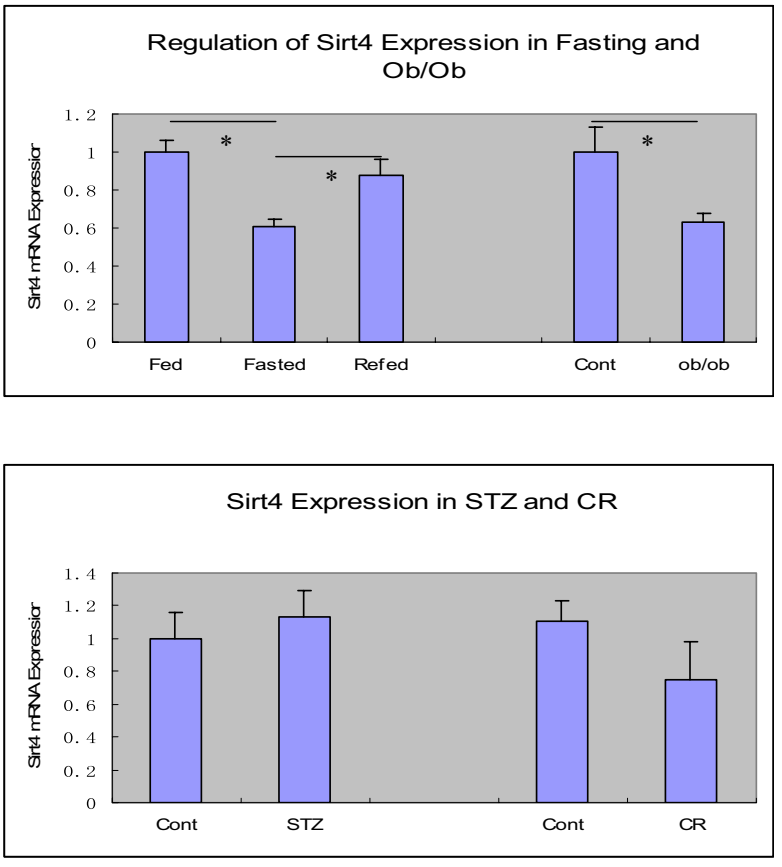

Fig. S2. Sirt4 mRNA expression in different rodent models. Sirt4 mRNA is significantly regulated in fasting, refeeding, and *ob/ob* skeletal muscles (*Upper*). Muscle Sirt4 mRNA expression is unchanged in streptozotocin-induced diabetes and caloric restriction (*Lower*).

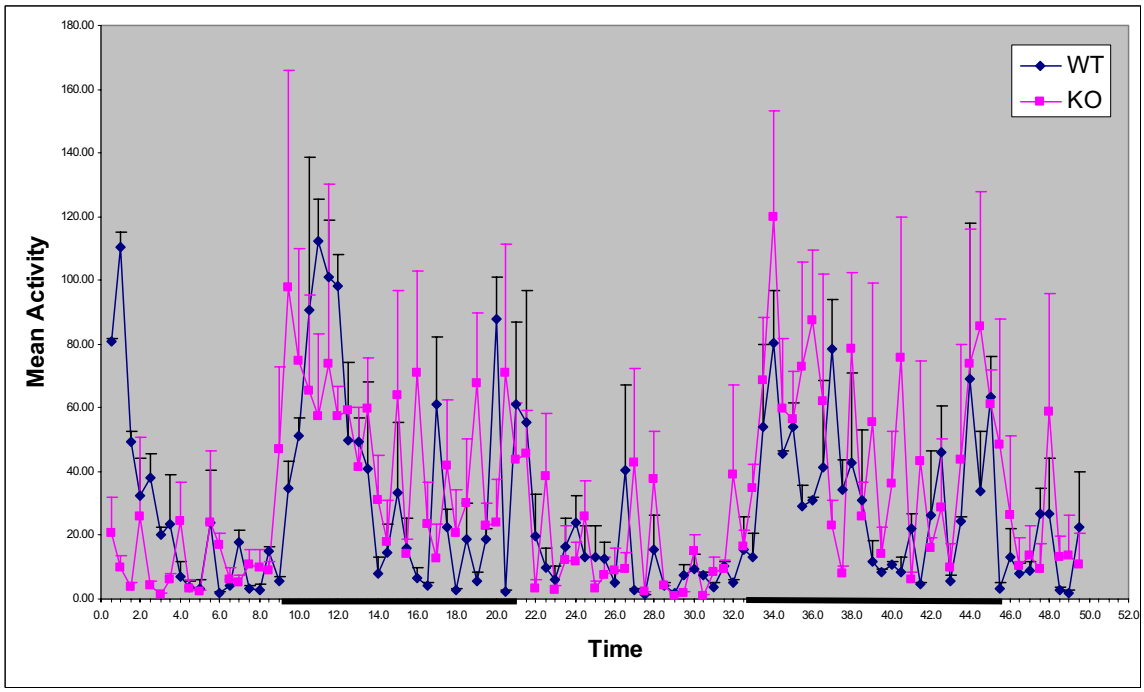

Fig. S3. Unchanged voluntary activity in Sirt3 KO mice. The voluntary activities of 16-wk-old male WT and KO mice were measured with the Comprehensive Lab Animal Monitoring System over a period of 48 h, and no difference was observed in either light or dark cycles between genotypes.

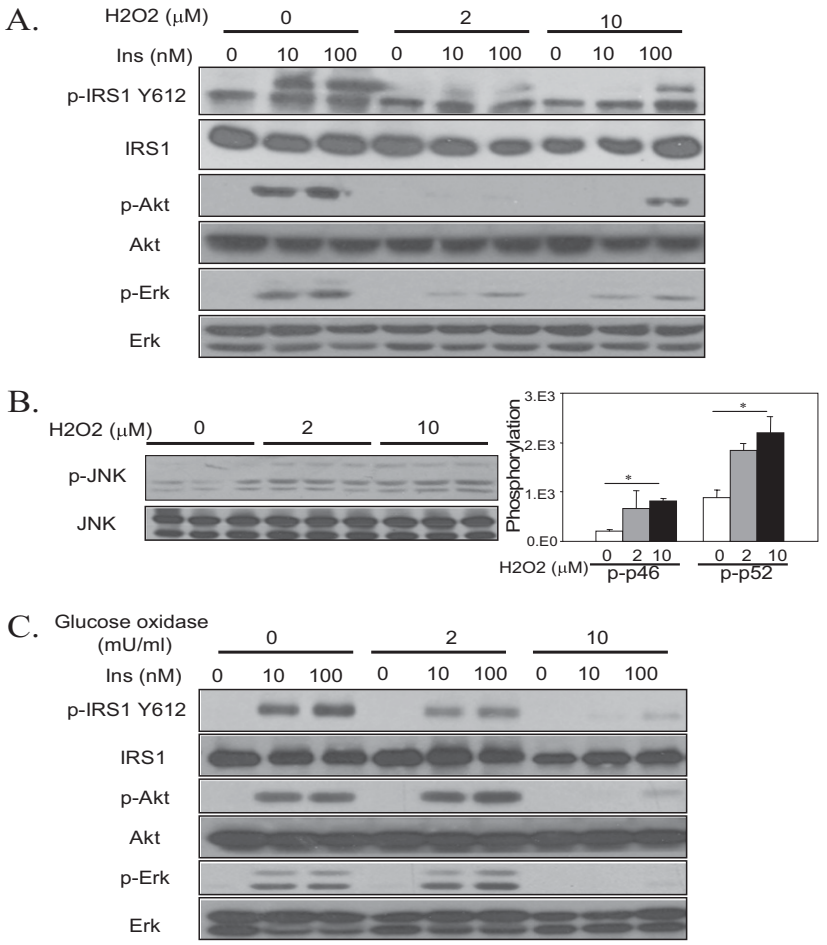

Fig. S4. H_2O_2-induced insulin resistance in C2C12 myoblasts. (*A*) Wild-type C2C12 cells were cultured with different concentrations of H_2O_2 in growth media for 12 h. Cells were serum-deprived for 2 h and then stimulated with different concentrations of insulin. The lysates were collected and subjected to Western blotting with different antibodies against components of insulin signaling. (*B*) Cells were cultured with different concentrations of H_2O_2 in growth media for 12 h, and lysates were collected and subjected to Western blotting for basal JNK phosphorylation and total JNK expression. Autoradiobiography was quantified with ImageJ software and Student's *t* test was performed. (*C*) Wild-type C2C12 cells were cultured with low-glucose growth media containing different concentrations of glucose oxidase for 6 h. After 2 h of serum deprivation, cell lysates were collect and subjected to Western blotting analysis.

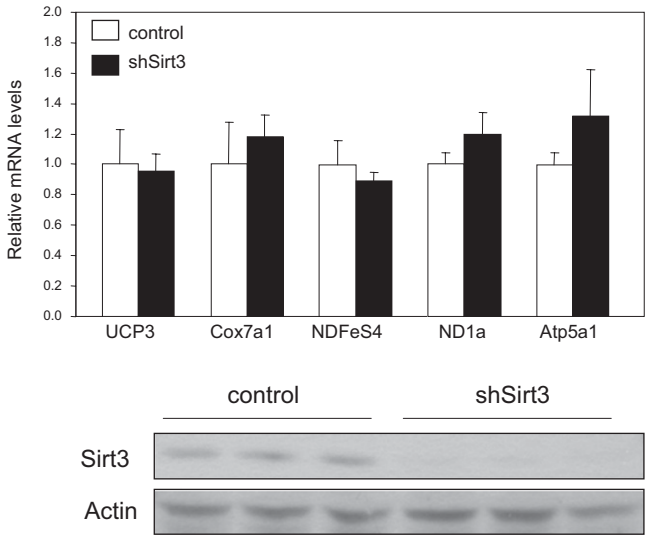

Fig. S5. Mitochondrial gene expression is unchanged in Sirt3 knockdown C2C12 myoblasts. Control and Sirt3 knockdown cells were harvested and processed for total RNA extraction using a Qiagen RNeasy mini kit, and cDNA was synthesized using an ABI RT-PCR synthesis kit. Quantitative real-time PCR was performed using primers targeting different mitochondrial genes. Student's *t* tests were performed for significance between control and Sirt3 knockdown samples. Western blotting showed a 90% knockdown of Sirt3 protein in C2C12 cells.

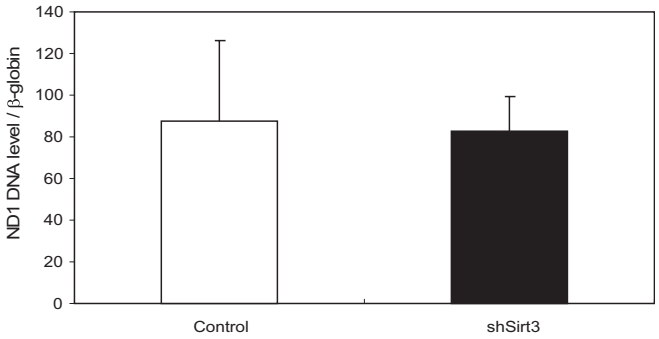

Fig. S6. Mitochondrial-encoded ND1 DNA level is unchanged in Sirt3 knockdown C2C12 myoblasts. Control and Sirt3 knockdown cells were harvested and processed for total DNA extraction using a Qiagen DNeasy kit. ND1 DNA level was measured using quantitative real-time PCR and normalized with nuclear β-globin DNA. Student's *t* tests were performed for significance between control and Sirt3 knockdown samples ($P = 0.9192$).

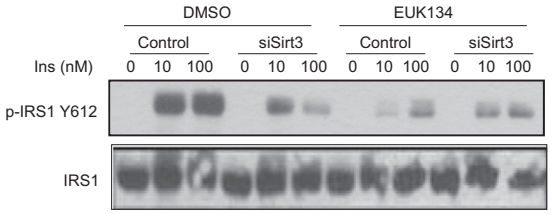

Fig. S7. Antioxidant treatments abolished the insulin-signaling difference between control and Sirt3 knockdown C2C12 cells. Both control and Sirt3 knockdown cells were treated with either control reagent (DMSO) or EU.K.134 for 16 h and then cells were stimulated with different dosages of insulin after 2 h of serum starvation. Lysates were harvested after a 5-min insulin treatment and subjected to Western blotting with antibody against phosphorylated IRS-1 tyrosine 612 and total IRS-1 protein.