

Supplemental materials and methods

Dihydroethidium (DHE) staining

Muscle superoxide levels were analyzed by DHE staining (Nacalai Tesque, Inc., Kyoto, Japan). Briefly, frozen sections (6 μ m) were dried and incubated in 10 μ M DHE solution at room temperature for 30 min.

Measurement of oxygen consumption rate (OCR)

The OCR of the muscle fibers isolated from the gastrocnemius was measured using an O2k-FluoRespirometer (Berthold Japan, Tokyo, Japan) according to the manufacturer's instructions.

EUK-134 treatment in vivo

EUK-134 (Axon MEDCHEM, Groningen, the Netherlands) dissolved in PBS (0.25 mg/ml) was intraperitoneally injected into muscle-*Sod2*^{-/-} mice at a dose of 35 mg/kg body weight. During the treadmill task, all mice were habituated to running for several days until their endurance time stabilized. The drug was administered 24 h before the experiment. The treadmill task was performed 24 h after injection.

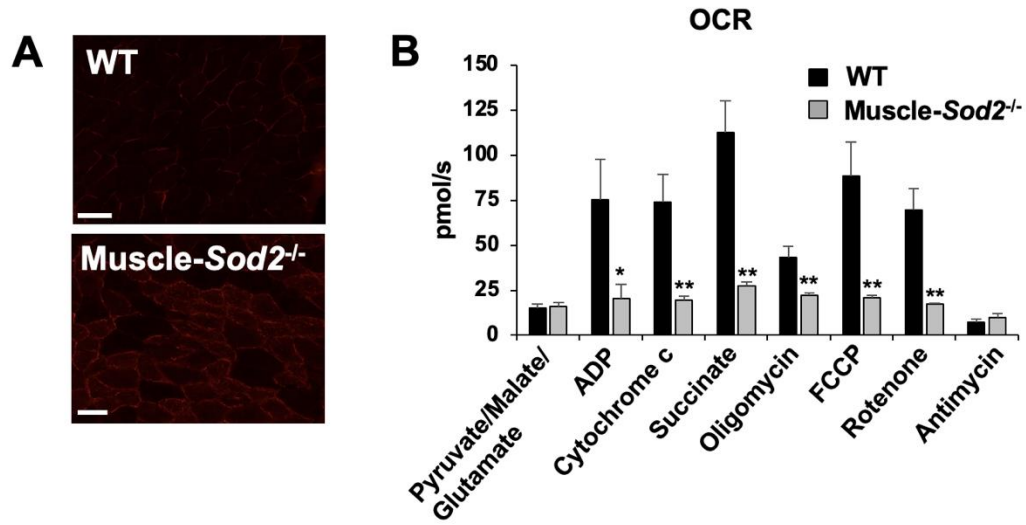


Figure S1. Mitochondrial superoxide generation and respiration in muscle-*Sod2*^{-/-} mice. (A) Mitochondrial superoxide staining by DHE (dihydroethidium) of the gastrocnemius muscle of WT and muscle-*Sod2*^{-/-} male mice at 5-6 months of age. Scale bars represent 50 μ m. (B) Oxygen consumption rate (OCR) of muscle fiber isolated from the gastrocnemius of WT and muscle-*Sod2*^{-/-} male mice (n = 3). Data are shown as the mean \pm SD; * p < 0.05, ** p < 0.01.

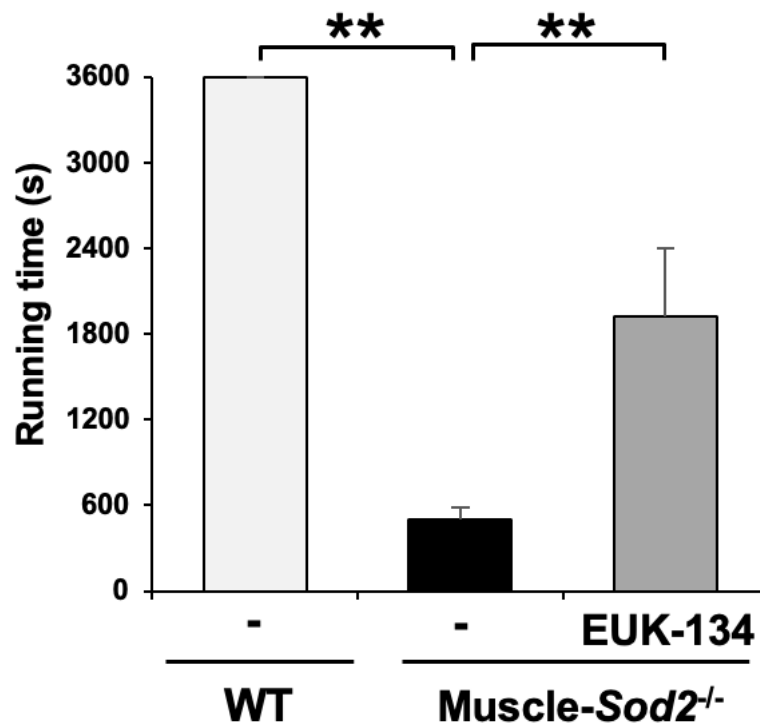


Figure S2. Treadmill task performance of EUK-134, an SOD mimetic, on muscle-*Sod2*^{-/-} mice. Treadmill task performance of WT and muscle-*Sod2*^{-/-} mice with or without administration of the antioxidant EUK-134 at 5 months of age (n = 5). Data are shown as the mean \pm SD; ** p < 0.01.

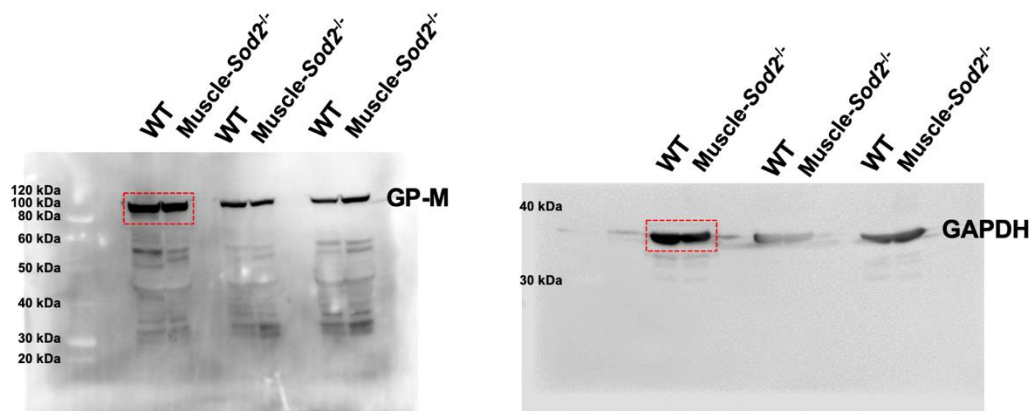
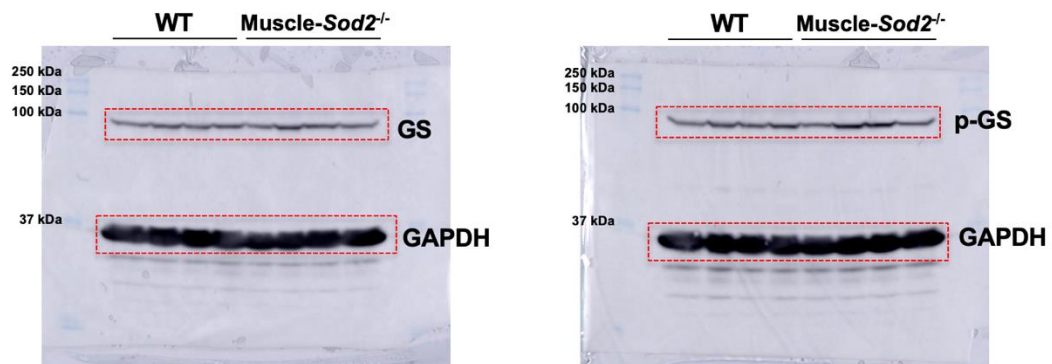
A**B**

Figure S3. Full blot images of western blotting. Full blot images of Figure 2A (A) and Figure 2C (B).