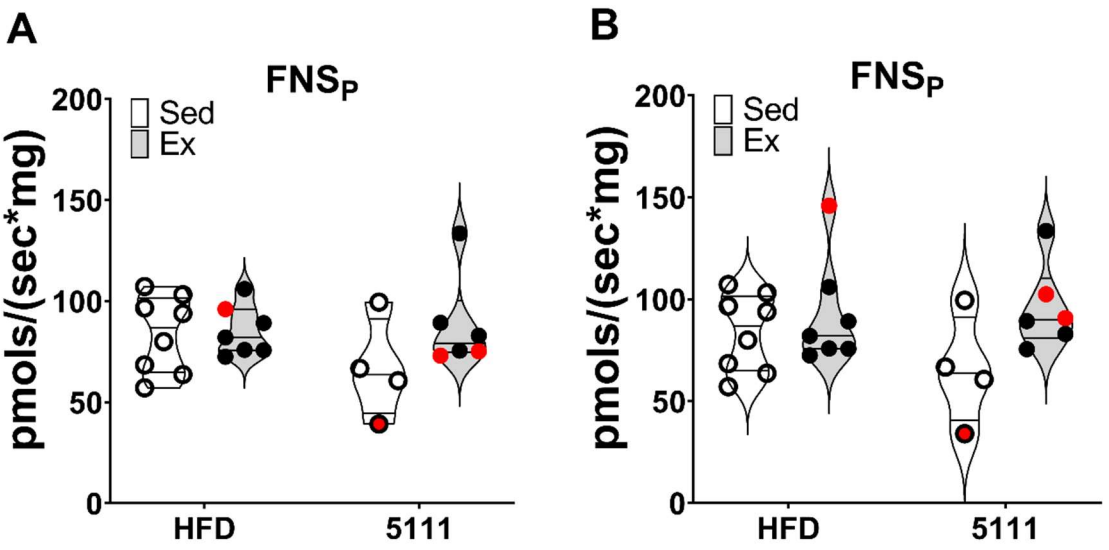

Supplemental Figure 2. OXPHOS permeabilized skeletal muscle fibers. Panels A and B present Violin Plots showing exploratory assessment of skeletal muscle oxidative capacity measured in permeabilized extensor digitorum longus (EDL) muscle fibers measured in C57BL/6 mice that were started on a 45% high fat diet (HFD) without access to a running wheel (Sed) for 8 weeks at ~4 weeks of age. The mice were then randomly allocated to one of four groups; HFD no running wheel (HFD Sed), HFD with running wheel access (HFD Ex), HFD with 1% PMI-5011 with no running wheel (5011 Sed), or the 5011 HFD with running wheel access (5011 Ex). Skeletal muscle oxidative capacity was measured in the presence of 50 µM palmitoyl carnitine, 2 mM malate, 5 mM pyruvate, 10 mM succinate, and 7.5 mM ADP (FNS_P). Data points highlighted in red had one duplicate with relatively low response following the addition of pyruvate. Panel A includes both duplicate measurements for the fibers that had a low pyruvate response duplicate, while Panel B excludes the low responding duplicate.

Supplemental Figure 2 Methods

In an exploratory analysis, we assessed mitochondrial respiratory function in the extensor digitorum longus (EDL) muscles. The EDL collected from a convenience sample of the mice that were euthanatized for the collection of tissues as described in this study. The EDL muscles were placed in ice-cold relaxation and preservation buffer (BIOPs) [1]. Subsequently, the EDL muscle were further dissected on ice under stereo-microscopy to acquire small fiber bundles (~1-3 mg per bundle). The fiber bundles were placed in fresh ice-cold BIOPS buffer with 50 ug/mL of saponin and placed on an orbital shaker for 30-min to chemically permeabilize the plasma membrane. The fiber bundles were then washed in ice-cold MiR05 for ~15 min. The fiber bundles were then blotted dry for 40s on Whatman paper and weighed on an analytical balance (Mettler Toledo) to the nearest 0.01 mg. The fiber bundles were placed into two independent oxygraph chambers with Mi05 supplemented with ~ 15 mM phosphocreatine and 25 µM blebbistatin (myosin ATPase inhibitor) for duplicate measurements. The respiratory measurements were made with O_2 concentrations between ~425 to 200 nM O_2. Specifically, the LEAK respiration was measure at ~200 nM O_2 and OXPHOS measurements were made at ~400-250 nM O_2. In the absence of ADP, 50 µM palmitoyl carnitine and 2 mM malate were added to stimulate fatty acid linked LEAK respiration (F_L). Next, a high concentration of ADP (5 mM) was added to stimulate F-linked oxidative capacity F_P. Subsequently, 10 µM cytochrome c was added to access mitochondrial membrane integrity (F_{CytC}). Next, 5 mM pyruvate was also added to stimulate N-linked oxidative capacity (FN_P). Then, 10 mM succinate was added to stimulate S-linked oxidative capacity (FNS_P) and an additional bolus of ADP to increase total concentration to 7.5 mM. An uncoupling agent, Carbonyl

cyanide m-chlorophenyl hydrazone (CCCP), was next titrated at ~0.5 µM per step to assess the electron transfer capacity (FNS_E). Lastly, 2.5 mM antimycin A was added to measure residual oxygen consumption. The O_2 flux (JO_2) in the permeabilized muscle fibers was acquired and calculated using DatLab 7.0 software (Oroboros Instruments), after correcting for residual oxygen consumption and normalizing per mg wet-weight (pmol/s/mg). All measurements were corrected for daily room air calibrations as well as instrumental background and zero calibrations. Note: After adding the second bolus of ADP after adding succinate we observed a consistent increase in respiration rates, thus for the purpose of the present exploratory analysis, we will focus on the FNS_P measured at 7.5 mM ADP. One set of animals were excluded due to a titration errors. One sample only had enough sample to run as a singlet, three duplicate samples were excluded due to poor sample quality (e.g., high cytochrome C response, unstable readings). Finally, four of the samples had one of the duplicate measurements that showed relatively low response following the addition of pyruvate, which could reflect sub-optimal permeabilization. Thus, we analyzed the samples with and without including the low pyruvate responding duplicate measurements. Due in part to the nature of the convenience sampling and the imbalance in the sample size between groups these studies should be viewed as exploratory and no formal statistical analyses were performed.

Supplemental Figure 2. Panels A and B present Violin Plots showing exploratory assessment of skeletal muscle oxidative capacity measured in permeabilized extensor digitorum longus (EDL) muscle fibers measured in C57BL/6 mice that were started on a 45% high fat diet (HFD) without access to a running wheel (Sed) for 8 weeks at ~4 weeks of age. The mice were then randomly allocated to one of four groups; HFD no running wheel (HFD Sed), HFD with running wheel access (HFD Ex), HFD with 1% PMI-5011 with no running wheel (5011 Sed), or the 5011 HFD with running wheel access (5011 Ex). Skeletal muscle oxidative capacity was measured in the presence of 50 μM palmitoyl carnitine, 2 mM malate, 5 mM pyruvate, 10 mM succinate, and 7.5 mM ADP (FNS_P). Data points highlighted in red had one duplicate with relatively low response following the addition of pyruvate. Panel A includes both duplicate measurements for the fibers that had a low pyruvate response duplicate, while Panel B excludes the low responding duplicate.

1. Pesta D, Gnaiger E: **High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle.** *Methods Mol Biol* 2012, **810:**25-58.