



Figure S5. Respiriometry analysis and assessment of mitochondrial aging. (A-F) State 4 (oligomycin-insensitive) mitochondrial respiration normalized to either the protein content of the mitochondrial fraction (A-B) or citrate synthase activity/ μg of protein (C-D), and the Respiratory Control Index (RCI = State 3/State 4 ratio, which is the same using both normalization methods; (E-F) were assessed in the tibialis anterior and soleus muscles of young (3 mo, $n=13$) and old (28-29 mo, $n=11$) mice. Congenic CD45.2 and CD45.1 old mice were used (young mice were all CD45.2): white dots = CD45.2, black dots = CD45.1. (G) Quantitative PCR was used to assess mtDNA mutation. A long fragment covering a mtDNA locus that is highly susceptible to mutation was amplified and quantified to assess intact mtDNA (left). Total mtDNA was evaluated by amplifying a short fragment of mtDNA in an un-mutated locus (middle). The long/short fragment ratio (fraction of mtDNA that is intact) was then calculated (right). * $p \leq 0.05$, ** $p \leq 0.01$