

METHOD
State 2 (basal respiration) = mean of 3 basal state OCR measurements
State 3 (ADP-dependent respiration) = OCR after ADP injection
State 4 = OCR after Oligomycin M injection (inhibition of ATP synthase)
Respiratory Control Index (RCI) = State 3/State 4

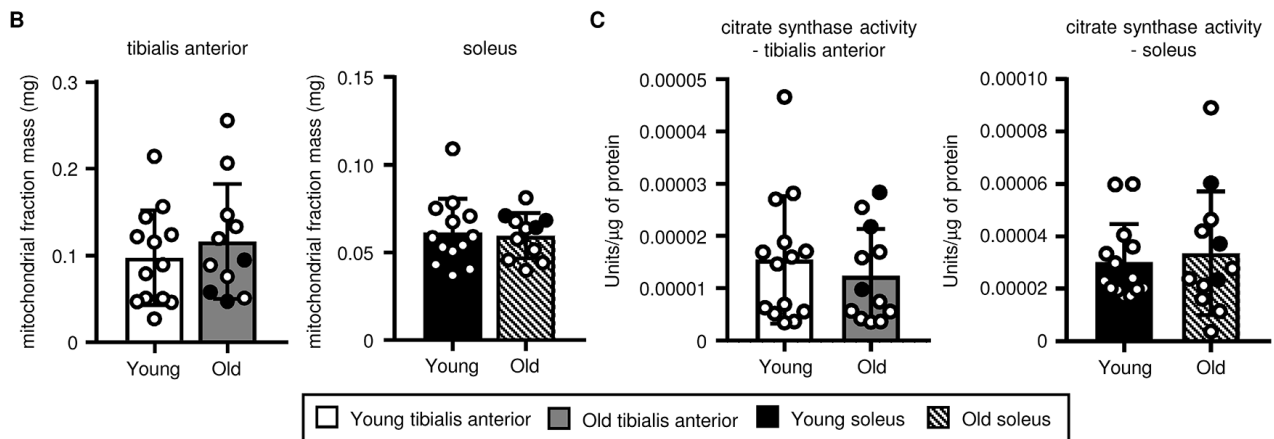


Figure S4. Respirometry analysis and assessment of mitochondrial aging. Seahorse assays were performed to assess mitochondrial function. (A) Experimental set up for respirometry assessment using pyruvate, succinate or palmitoylcarnitine as substrates. OCR: oxygen consumption rate; OligoM: oligomycin; AntiM-A: antimycinA. State 2 (basal), State 3 (ADP-dependent), and State 4 (oligomycin-insensitive) respiration, and the Respiratory Control Index (RCI) were calculated as indicated. (B) The mass of the mitochondrial fractions isolated from the whole tibialis anterior and soleus muscles of young (3 mo, n=13) and old (28-29 mo, n=11) mice was calculated using a DC assay. (C) The citrate synthase activity per microgram of the mitochondrial fraction from the tibialis anterior and soleus muscles of young (3 mo, n=13) and old (28-29 mo, n=12) mice was evaluated using an assay kit. Congenic CD45.2 and CD45.1 old mice were used (b-c; young mice were all CD45.2): white dots = CD45.2, black dots = CD45.1.