

Supplementary Figures

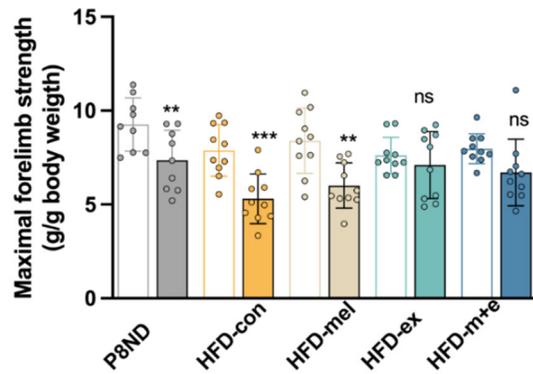


Figure S1. Comparison of maximal forelimb strength between before (open bars; 6 months) and after (filled bars; 8 months) intervention. Data are mean \pm SD, $n = 9-10$; ** $p < 0.01$, *** $p < 0.001$ vs. before intervention by paired t -test. ns, not significant.

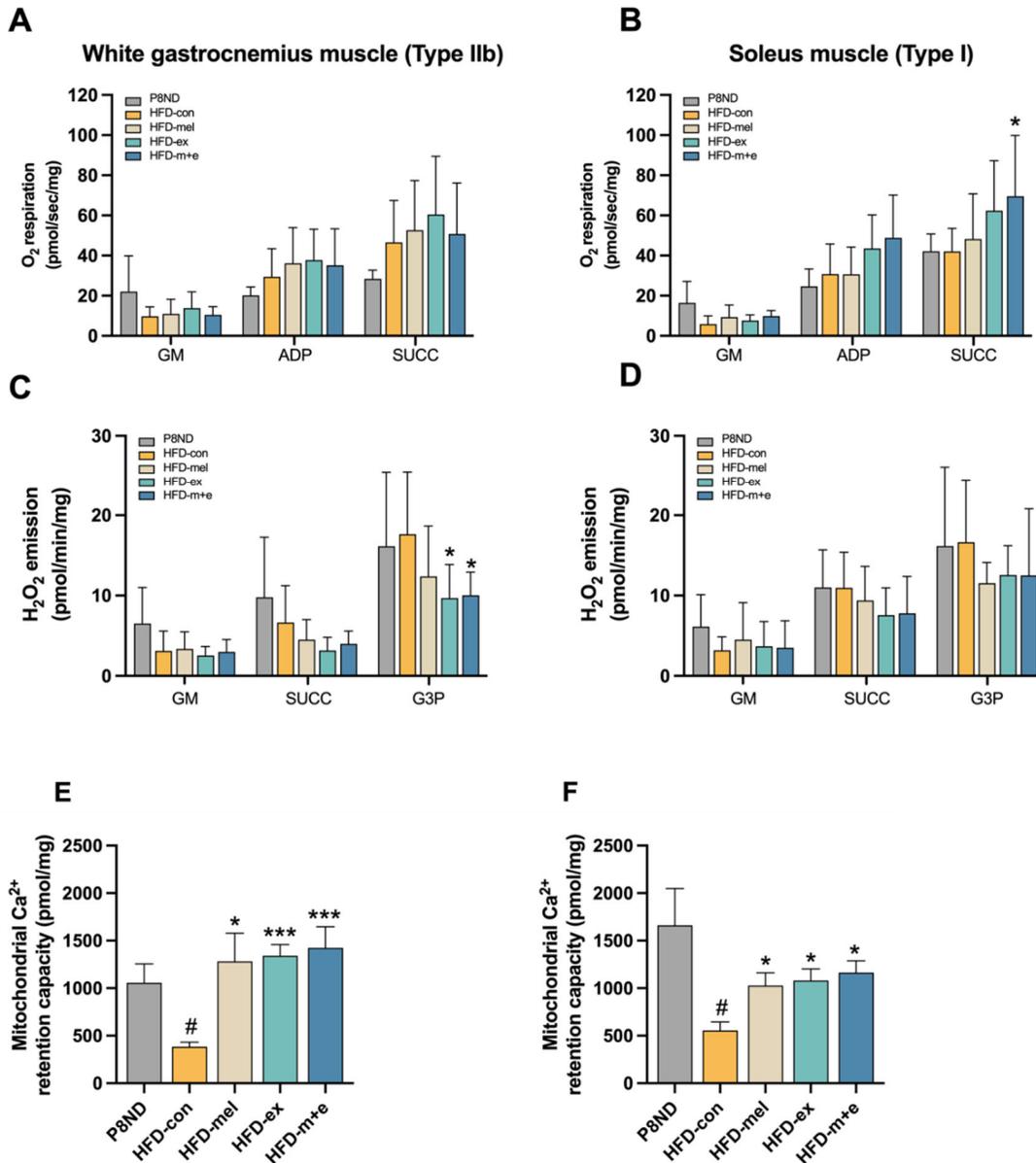


Figure S2. Effects of melatonin and/or exercise training on mitochondrial function. Mitochondrial function for (A,B) O₂ respiration, (C,D) H₂O₂ emission, and (E,F) calcium retention capacity were measured in white gastrocnemius (A,C,E) and soleus muscle (B,D,F), respectively (n = 9–10). Data presents mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001 compared with HFD-con and # p < 0.05 versus P8ND group by unpaired t-test. GM, glutamate/malate; ADP, adenosine diphosphate; SUCC, succinate; G3P, glycerol-3-phosphate.

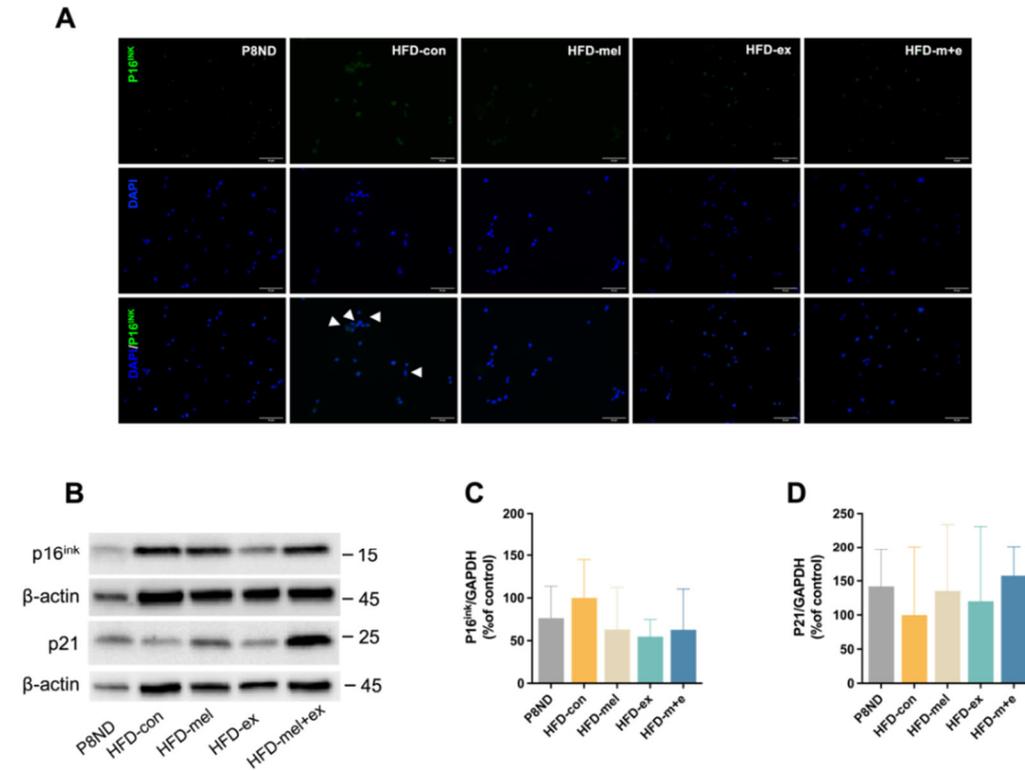


Figure S3. Expression of cellular senescence markers, p16^{ink} in SC-derived primary myoblasts. **(A)** Representative immunofluorescence shows p16^{ink} (green) and nucleus (DAPI; blue), and **(B)** immunoblots for p16^{ink} and p21 protein expression. **(C,D)** Density analyses of **(C)** p16^{ink} and **(D)** p21 protein expression are shown. Data are mean \pm SD, n = 3–5. Scale bar represents 50 μ m. White arrowheads indicate p16^{ink} positive cells.

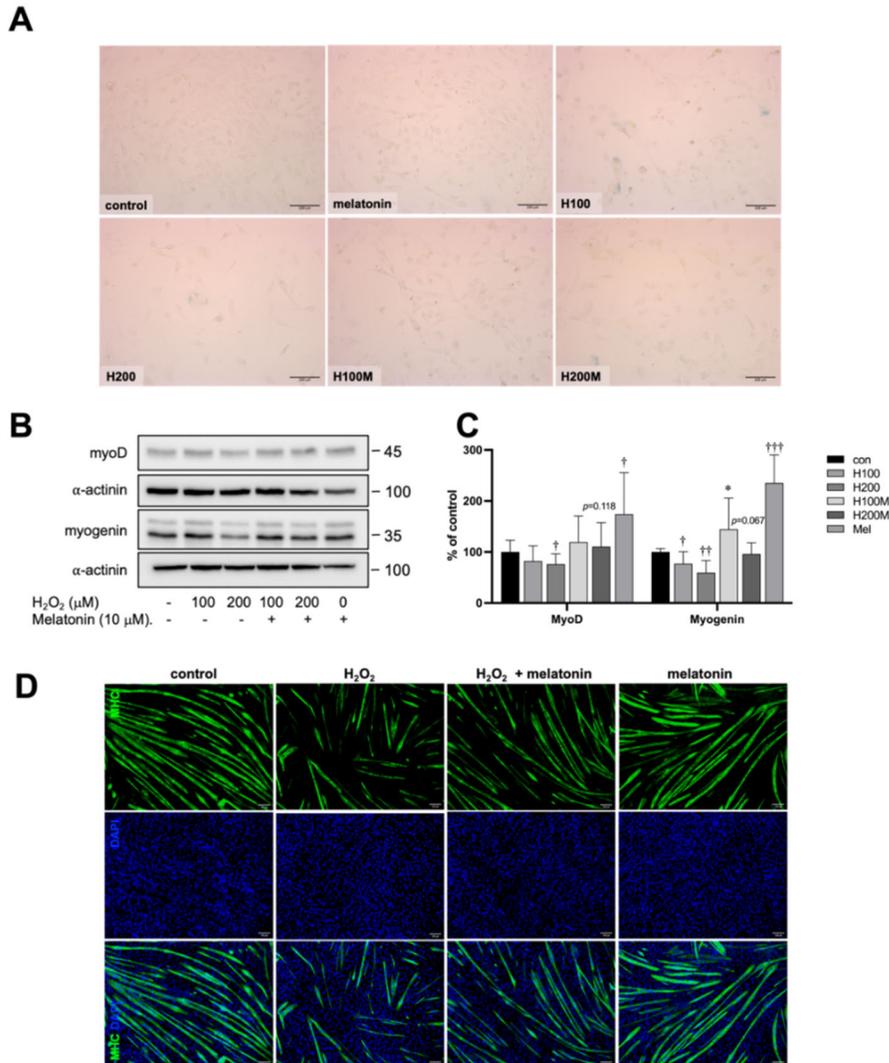


Figure S4. Effect of melatonin on myoblast senescence and deterioration of myotube differentiation by H₂O₂. **(A)** Representative image of SA-β-gal staining of myoblast and **(B)** immunoblots for myoD and myogenin in C2C12 myoblasts with or without treatment of H₂O₂ and/or melatonin are shown. **(C)** Bar graph indicates the density analysis of band intensities from immunoblots. The data are expressed as mean ± SD of 3 independent experiments. † $p < 0.05$, †† $p < 0.01$, and ††† $p < 0.001$ vs. untreated control, and * $p < 0.05$ or marginal significant p -values for H₂O₂+melatonin treatment vs. H100 by unpaired t test. Con, vehicle-treated control; H100, cells treated with H₂O₂ at 100 μM; H200, cells treated with H₂O₂ at 200 μM; H100M, cells treated with H₂O₂ at 100 μM and melatonin (10 μM); H200M, cells treated with H₂O₂ at 200 μM and melatonin; Mel, cells treated with melatonin

only. **(D)** Representative immunofluorescent image using anti-MHC antibody followed by fluorescent staining with species-specific Alexa Fluor 488 conjugated antibody (green) and DAPI (blue) nucleus staining is shown. Scale bars represent 100 μm .