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Supplemental information

Integrated genomic and proteomic analyses identify stimulus-dependent molecular changes associated with distinct modes of skeletal muscle atrophy

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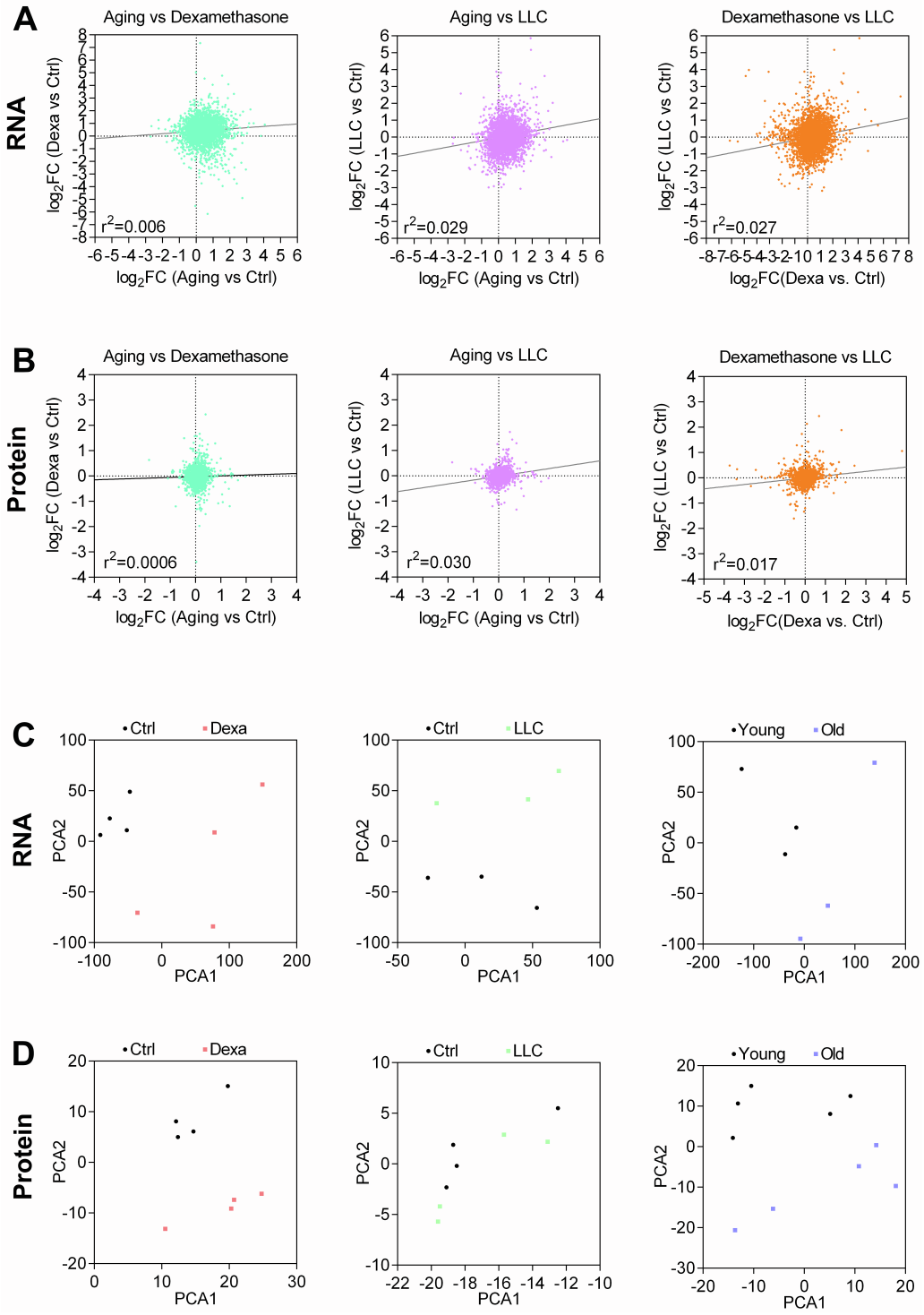
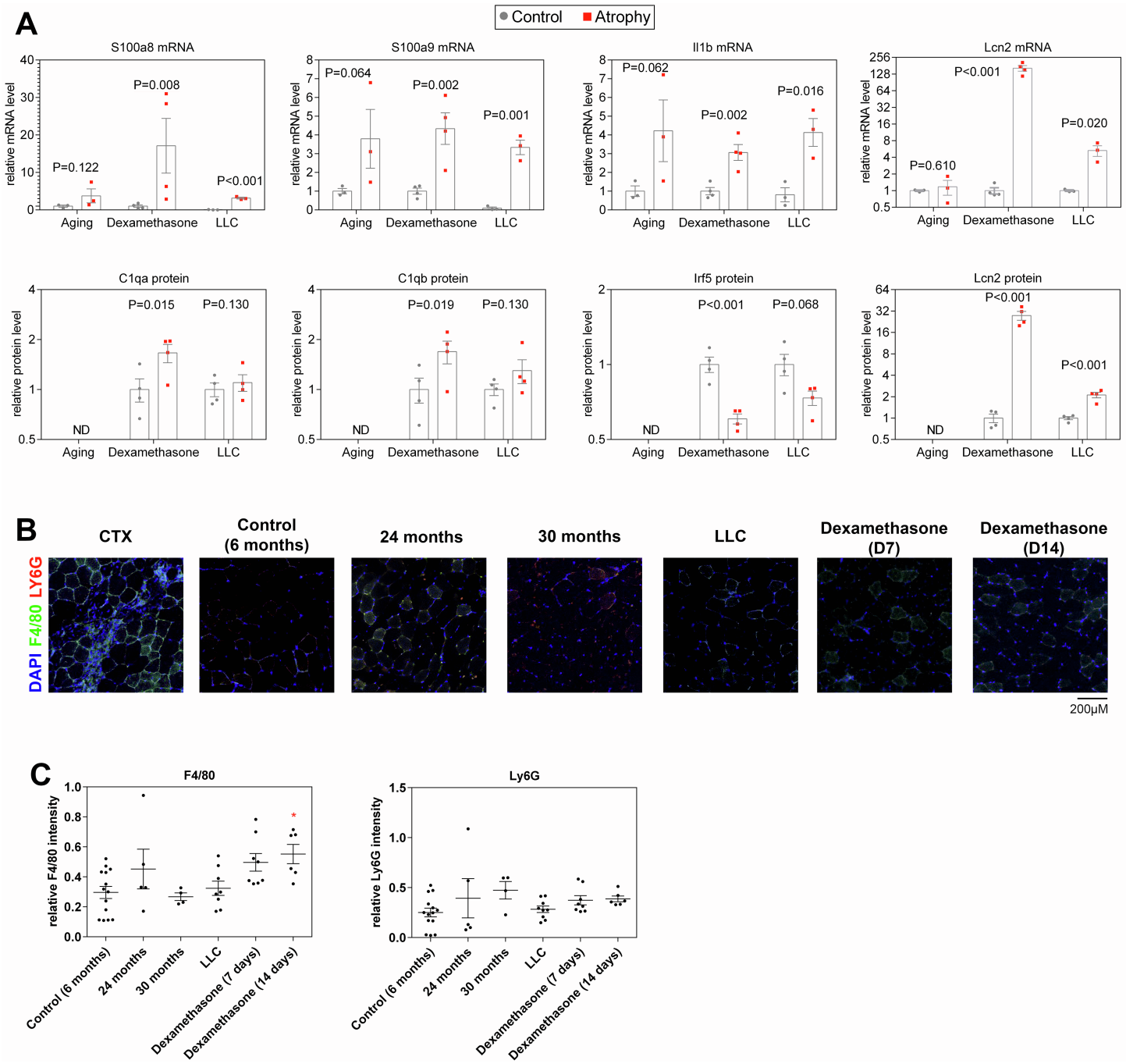
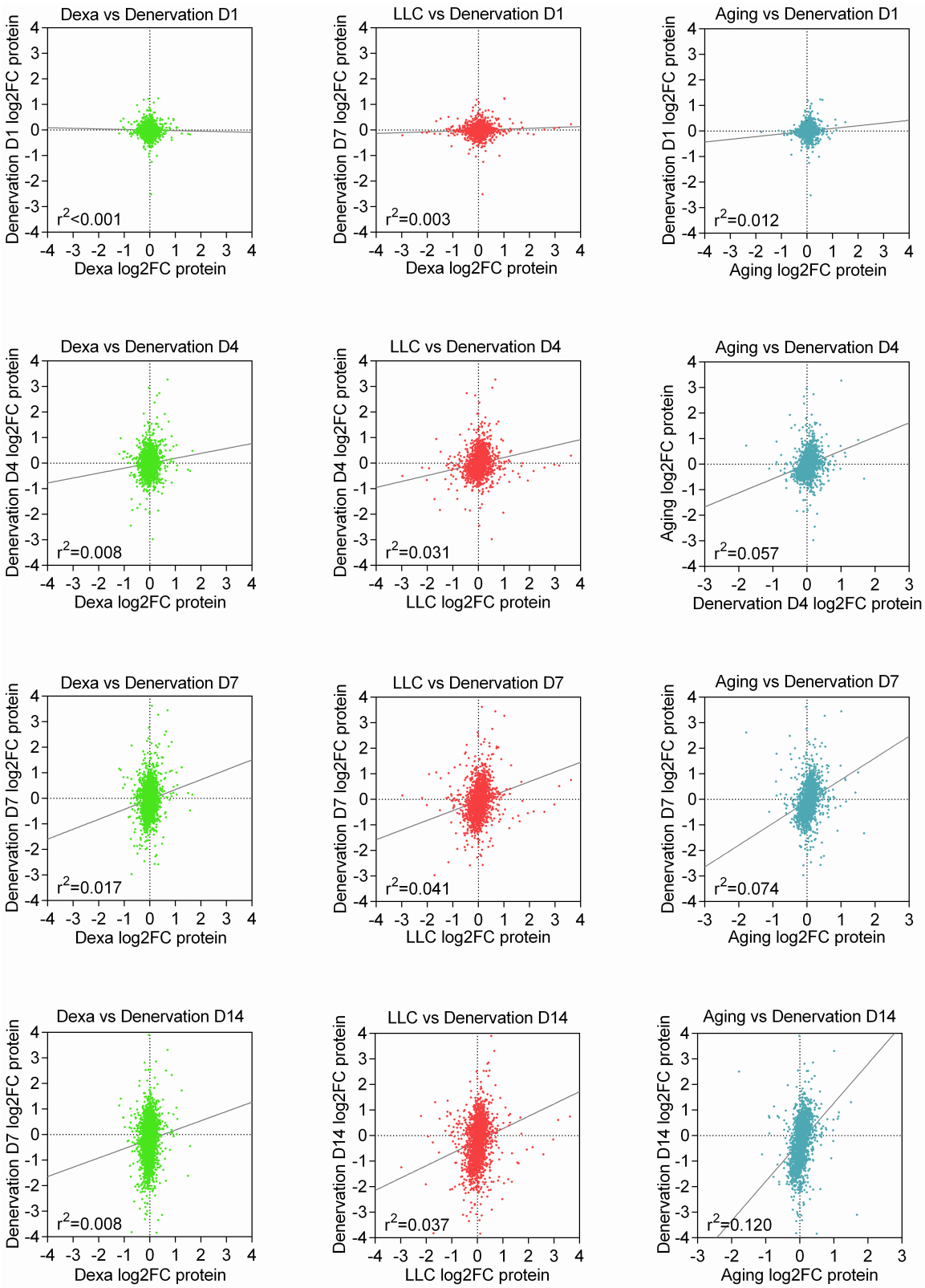
Supplemental Figures



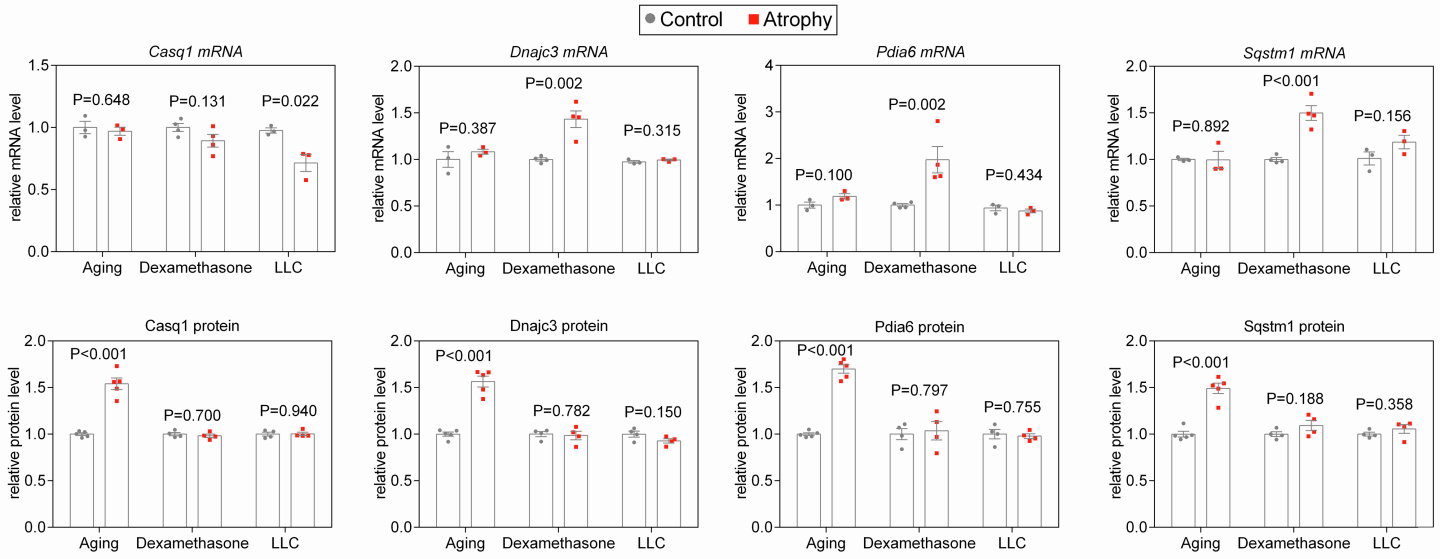
Supplemental Figure S1, Related to Figures 2-4. Comparison of RNA-seq and mass spectrometry datasets, and principal component analysis (PCA). (A) Comparison of RNA sequencing datasets indicates limited correlation between transcriptional changes induced by aging, dexamethasone, and LLC cancer. (B) Comparison of quantitative mass spectrometry datasets indicates limited correlation between protein changes induced by aging, dexamethasone, and LLC cancer. (C-D) Principal component analysis (PCA) of RNA sequencing and proteomics data. The PCA plots for each condition demonstrate the reproducibility of data within individual samples of a specific condition clustering together, indicating that the overall mRNA (C) and protein (D) profiles under a given condition are similar to each other and not due to random biological variation.



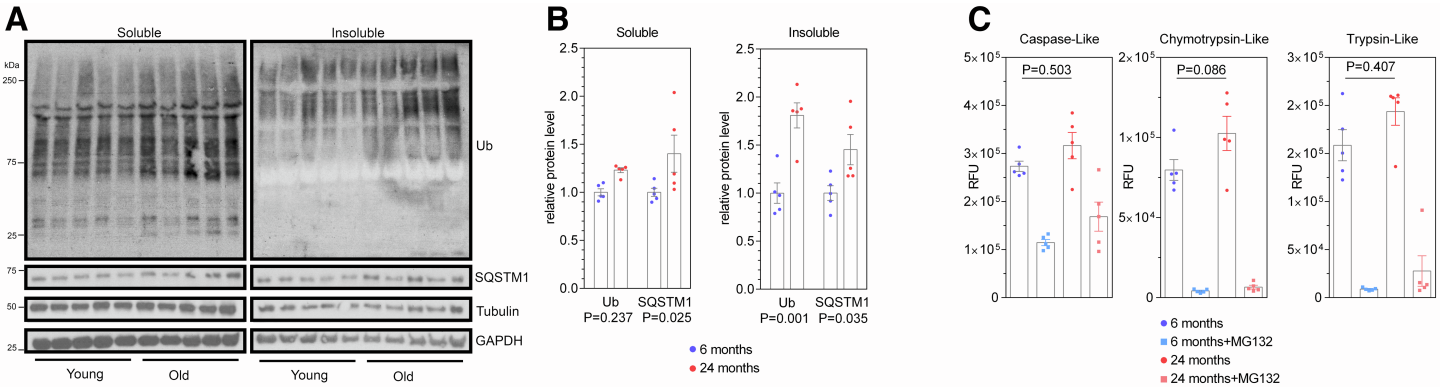
Supplemental Figure S2, Related to Figures 2-3. Inflammatory markers are upregulated in multiple modes of muscle atrophy. (A) RNA sequencing data indicates that inflammatory markers are upregulated in multiple modes of muscle atrophy. (B) Immunostaining for neutrophil and macrophage markers, Ly6g and F4/80 respectively, indicates no major infiltration of these inflammatory cells to muscles undergoing wasting with aging, dexamethasone, or LLC cancer cachexia, especially when compared to the large influx of inflammatory cells that occurs with muscle injury induced by cardiotoxin (CTX). (C) Quantitation of immunofluorescent staining for F4/80 and Ly6G only shows a significant increase in F4/80 immunoreactivity after 14 days of dexamethasone treatment, which suggests increased macrophage influx. Altogether muscle wasting is characterized by inflammatory changes with little influx of inflammatory cells. N(biological replicates) are indicated.



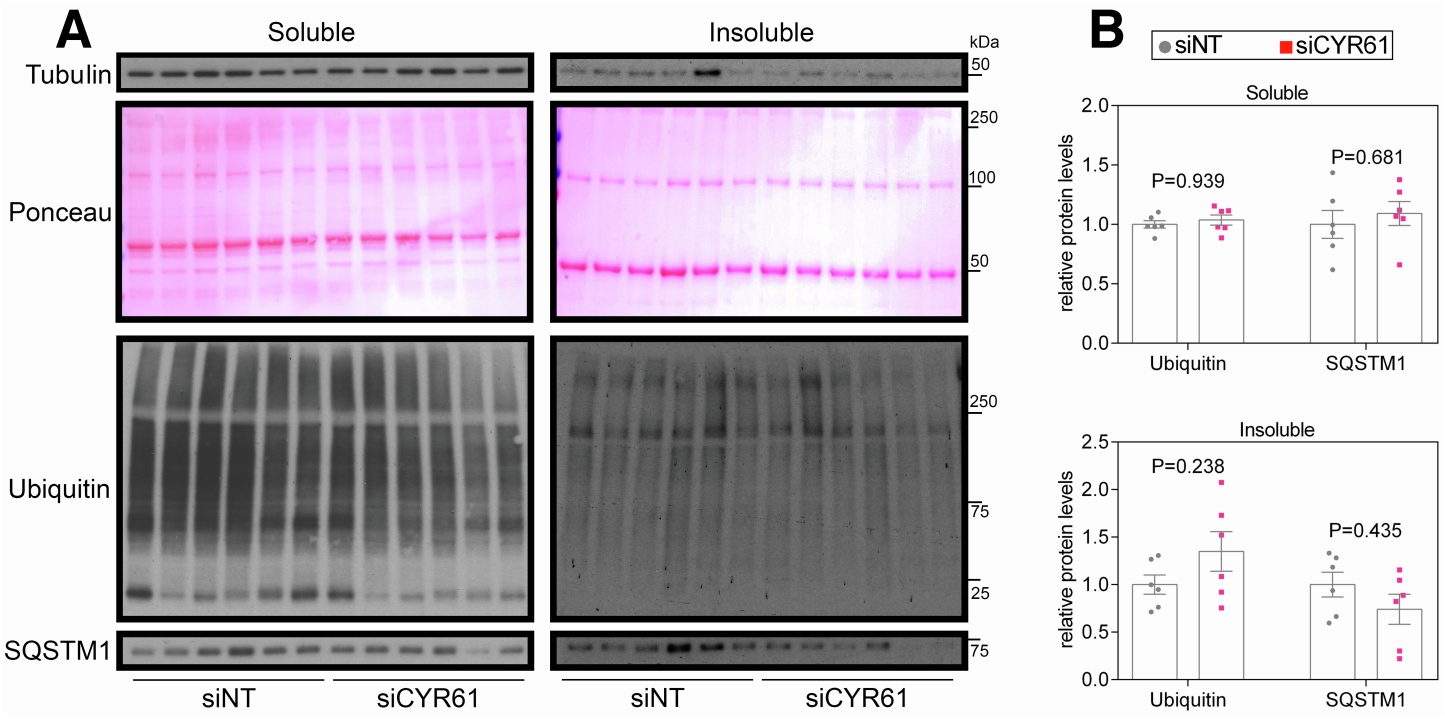
Supplemental Figure S3, Related to Figure 3. Cross-comparison of muscle proteomic changes induced by aging, cancer cachexia, dexamethasone, and denervation. Comparison of our proteomic datasets for muscle wasting induced by aging, dexamethasone, and LLC cancer cachexia with a publicly-available proteomic dataset for denervation-induced muscle wasting induced over a 14-day period (Lang et al., *Dis. Model. Mech.* 2017, PMID: 28546288). Limited correlation is found between denervation and other modes of muscle wasting.



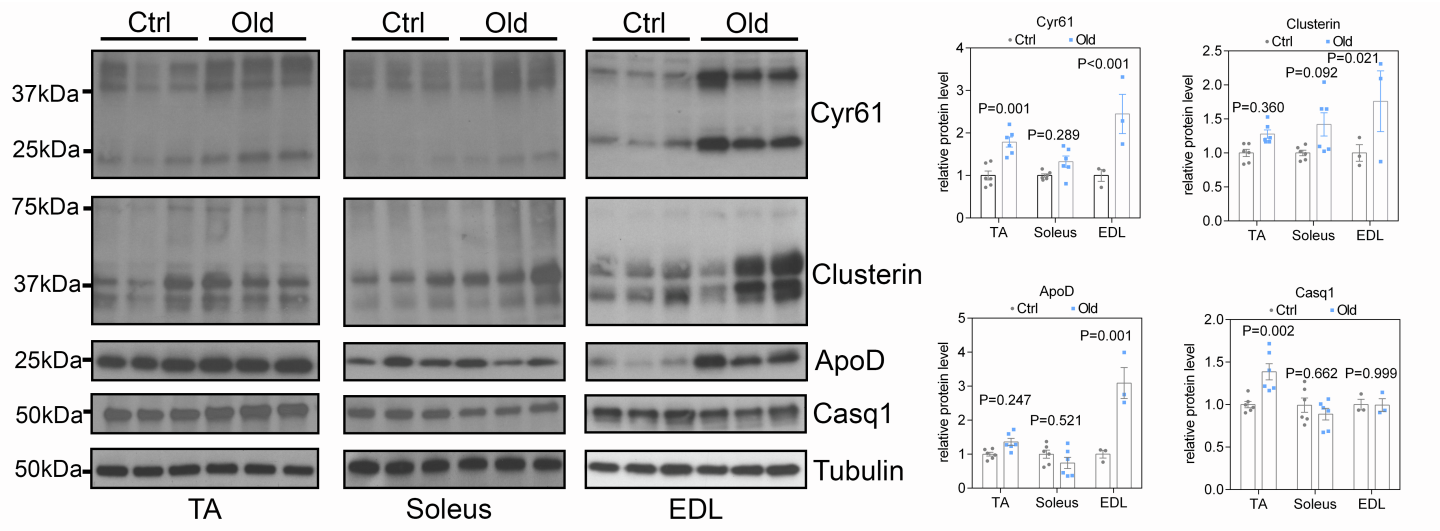
Supplemental Figure S4, Related to Figures 2-3. Age-upregulation of ER stress proteins. ER stress proteins are upregulated with aging but not with dexamethasone treatment and LLC cancer-induced cachexia. N(biological replicates) are indicated.



Supplemental Figure S5, Related to Figures 1 and 6. Loss of muscle protein quality control with aging. (A) Detergent-soluble and insoluble protein fractions from old muscles show accumulation of poly-ubiquitinated proteins and p62/SQSTM1 in old age. (B) Quantification of blots shown in (A). (C) Accumulation of poly-ubiquitinated proteins with aging is not due to reduced proteasome activity, as measured from tibialis anterior homogenates with proteolytic assays for caspase-like, chymotrypsin-like, and trypsin-like activities. Inhibition of proteasome activity with MG132 is included to demonstrate that the proteolytic activity measured indeed reflects proteasome activity. N(biological replicates) are indicated.



Supplemental Figure S6, Related to Figure 6. Cyr61 does not appear to regulate protein quality control with aging. (A) Western blot analysis of detergent-soluble and insoluble protein fractions from TA muscles of 30-month-old mice electroporated with Cyr61 siRNAs and control non-targeting (NT) siRNAs. (B) Cyr61 knockdown does not significantly affect the age-related accumulation of poly-ubiquitinated proteins and p62/SQSTM1 with aging. N(biological replicates) are indicated.



Supplemental Figure S7, Related to Figures 5-6. Validation and comparison of regulation of select atroproteins in the tibialis anterior (TA), soleus, extensor digitorum longus (EDL) skeletal muscles. Western blot for Cyr61, clusterin, ApoD and Casq1 proteins confirms that all these proteins are increased with old age (24 months compared to control 6 months) in the TA, as determined by TMT proteomics. However, some of these markers of TA sarcopenia are differently regulated in the soleus, a slow-twitching muscle resistant to atrophy in comparison to the fast-twitching TA. Specifically, Cyr61 and clusterin protein increase in the TA and soleus with aging, whereas ApoD and Casq1 protein increases in the TA but not in the soleus. Thus, the changes in some of the proteins identified in the TA proteomics may not occur in the soleus muscle and may underly the differential sensitivity to atrophic stimuli of fast versus slow-twitching muscles. N(biological replicates) are indicated.