
Figures and figure supplements

Quantitative proteomic analysis of skeletal muscles from wild-type and transgenic mice carrying recessive *Ryr1* mutations linked to congenital myopathies

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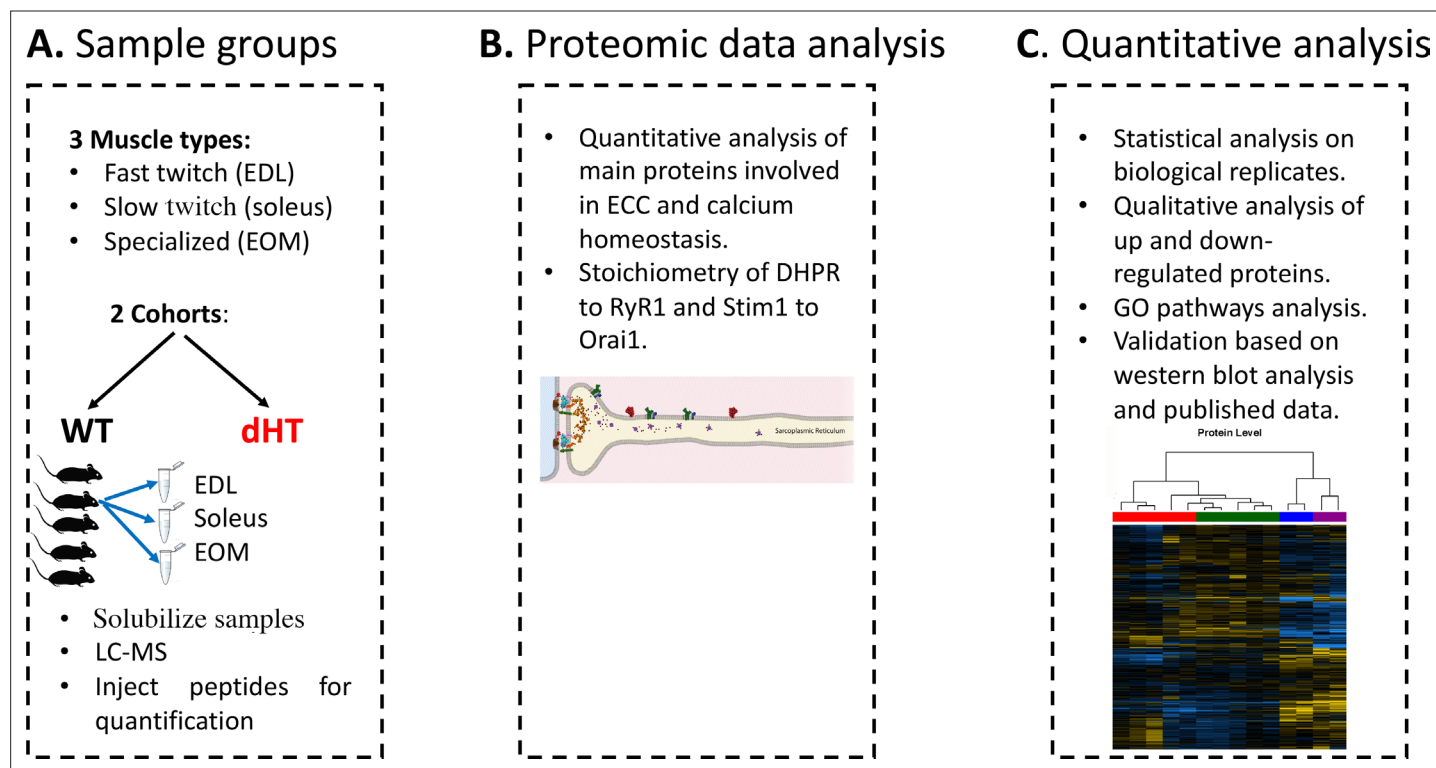


Figure 1. Schematic overview of the workflow. **(A)** Skeletal muscles from 12 weeks old WT (5 mice) and dHT littermates (5 mice) were isolated and flash frozen. Three different types of muscles were isolated per mouse, namely EDL, soleus and EOMs. On the day of the experiment, muscles were solubilized and processed for LC-MS. **(B)** For absolute protein quantification, synthetic peptides of RyR1, Cav1.1, Stim1 and Orai1 were used. **(C)** Protein content in different muscle types and in the different mouse genotypes were analyzed and compared.

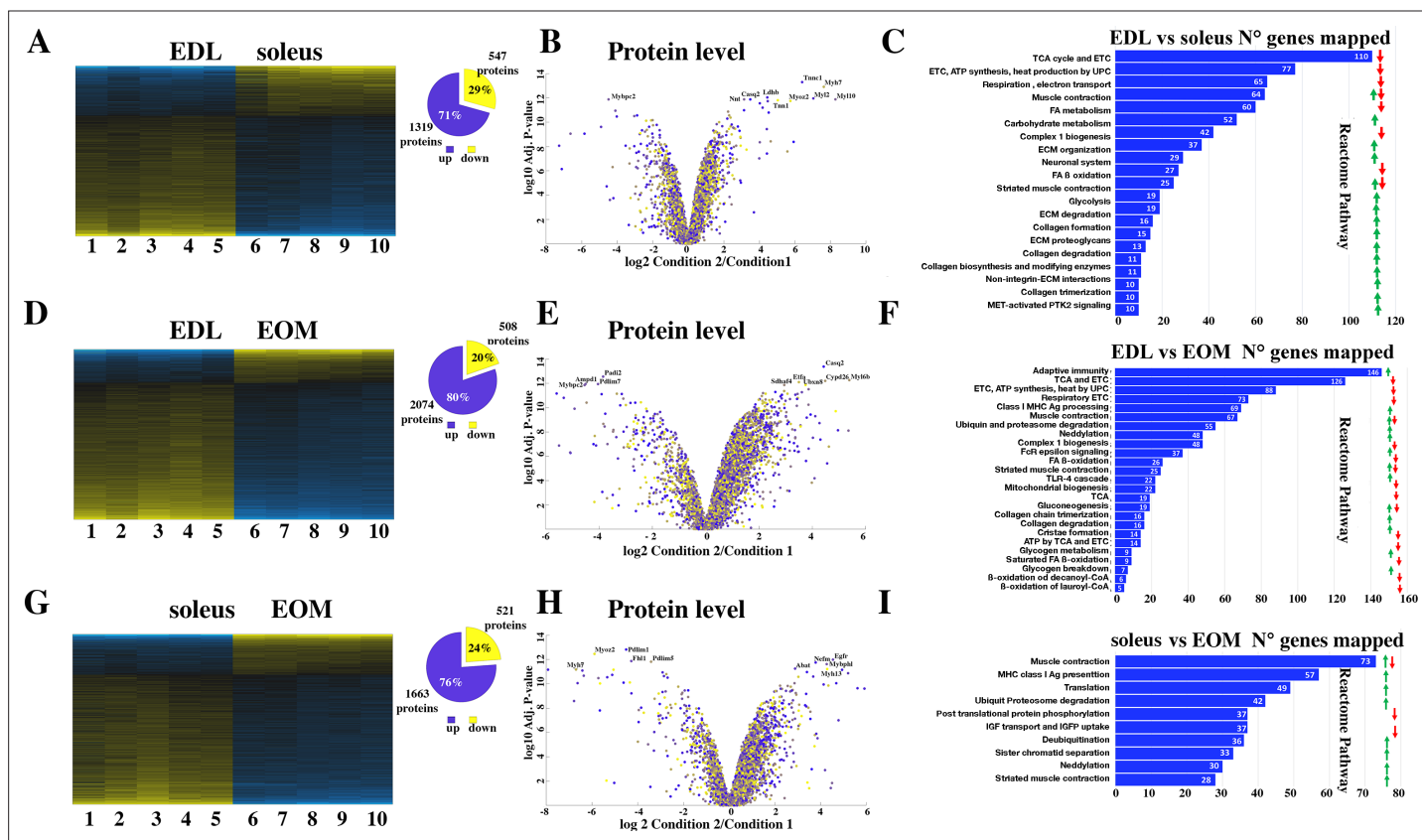


Figure 2. Proteomic analysis of EDL, soleus and EOM muscles from WT mice confirms the significant difference in content of proteins involved in the TCA cycle and electron transport chain, fatty acid metabolism and muscle contraction. **(A)** Hierarchically clustered heatmaps of the relative abundance of proteins in EDL (columns 1–5) and soleus muscles (columns 6–10) from five mice. Blue blocks represent proteins which are increased in content, yellow blocks proteins which are decreased in content in EDL versus soleus muscles. Right pie chart shows overall number of increased (blue) and decreased (yellow) proteins. Areas are relative to their numbers. **(B)** Volcano plot of a total of 1866 quantified proteins which showed significant increased (blue) and decreased (yellow) content. The horizontal coordinate is the difference multiple (logarithmic transformation at the base of 2), and the vertical coordinate is the significant difference p value (logarithmic transformation at the base of 10). The proteins showing major change in content are abbreviated. Soleus: condition 2; EDL: condition 1 **(C)** Reactome pathway analysis showing major pathways which differ between EDL and soleus muscles. **(D)** Hierarchically clustered heatmaps of the relative abundance of proteins in EDL (columns 1–5) and EOM muscles (columns 6–10) from five mice. Blue blocks represent proteins which are increased in content, yellow blocks proteins which are decreased in content in EDL versus EOM muscles. Right pie chart shows overall number of increased (blue) and decreased (yellow) proteins. Areas are relative to their numbers. **(E)** Volcano plot of a total of 1866 quantified proteins which showed significant increased (blue) and decreased (yellow) content. The horizontal coordinate is the difference multiple (logarithmic transformation at the base of 2), and the vertical coordinate is the significant difference p value (logarithmic transformation at the base of 10). The proteins showing major change in content are abbreviated. EOM: condition 2; EDL: condition 1 **(F)** Reactome pathway analysis showing major pathways which differ between EDL and EOM muscles. **(G)** Hierarchically clustered heatmaps of the relative abundance of proteins in soleus muscles (columns 1–5) and EOM (columns 6–10) from five mice. Blue blocks represent proteins which are increased in content, yellow blocks proteins which are decreased in content in soleus muscles versus EOM. Right pie chart shows overall number of increased (blue) and decreased (yellow) proteins. Areas are relative to their numbers. **(H)** Volcano plot of a total of 1866 quantified proteins which showed significant increased (blue) and decreased (yellow) content. The horizontal coordinate is the difference multiple (logarithmic transformation at the base of 2), and the vertical coordinate is the significant difference p value (logarithmic transformation at the base of 10). The proteins showing major change in content are abbreviated. EOM: condition 2; soleus: condition 1 **(I)** Reactome pathway analysis showing major pathways which differ between soleus and EOM muscles. A q-value of equal or less than 0.05 was used to filter significant changes prior to the pathway analyses. An additional filter was applied to the Heatmaps and Piecharts and only proteins showing a significant change ≥ 0.2 fold are included.

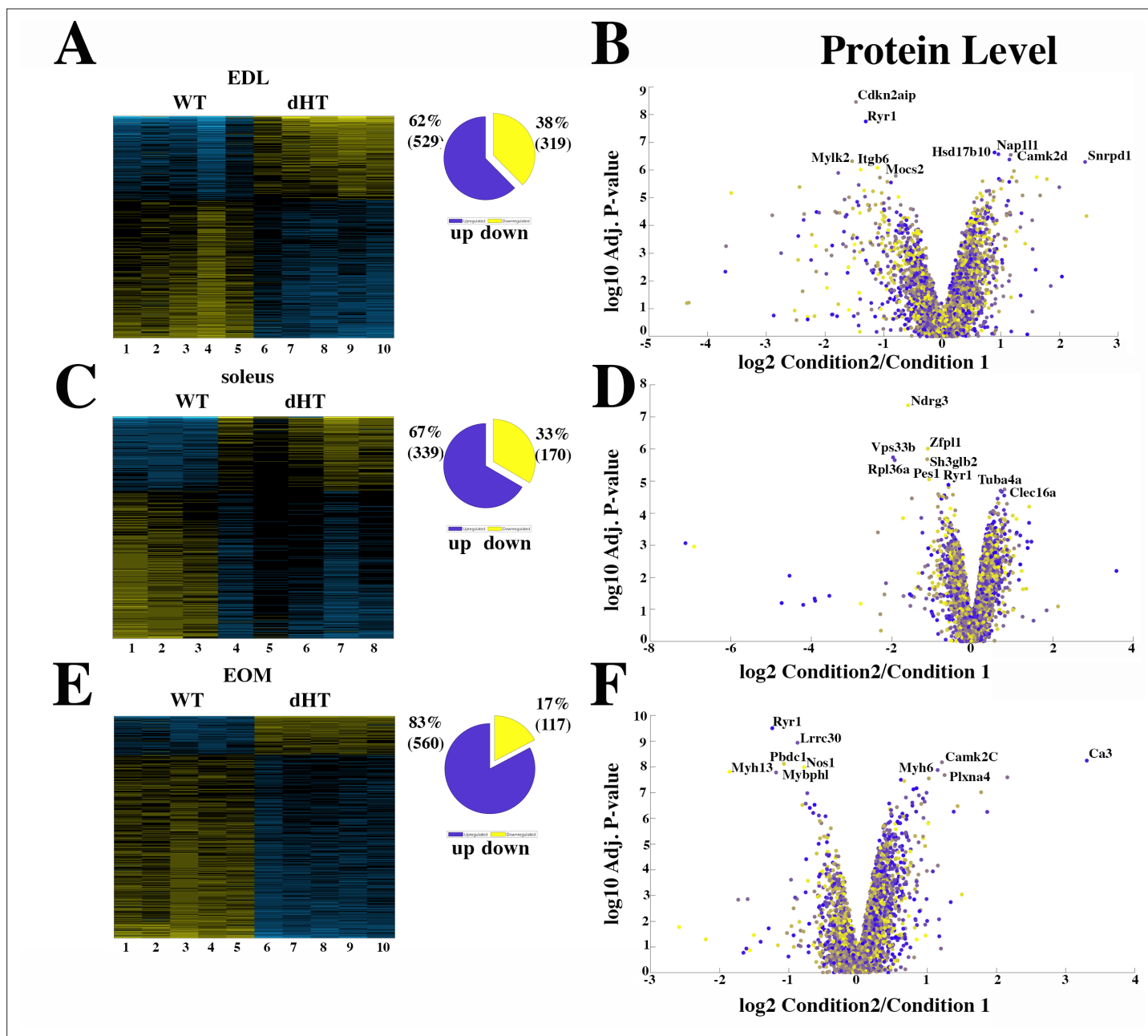


Figure 3. Proteomic analysis comparison of muscles from dHT and WT mice. (**A**, **C** and **E**) Hierarchically clustered heatmaps of the relative abundance of proteins in EDL (**A**), soleus muscles (**C**) and EOMs (**E**) from three to five mice. Blue blocks represent proteins which are increased in content, yellow blocks proteins which are decreased in content in WT (columns 1–5 in **A** and **E**; 1–3 in **C**) versus dHT (5–10 in **A** and **E**; 4–8 in **C**). Right pie chart shows overall number of increased (purple) and decreased (yellow) proteins. Areas are relative to their numbers. (**B**, **D** and **F**) Volcano plots of total quantified proteins showing significant increased (blue) and decreased (yellow) content in dHT (condition 2) versus WT (condition 1) EDL (**B**), soleus (**D**) and EOMs (**F**). The horizontal coordinate is the difference multiple (logarithmic transformation at the base of 2), and the vertical coordinate is the significant difference p value (logarithmic transformation at the base of 10). The proteins showing major change in content are abbreviated. A q-value of equal or less than 0.05 was used to filter significant changes prior to the pathway analyses. An additional filter was applied to the Heatmaps and Piecharts and only proteins showing a significant change ≥ 0.2 -fold are included.

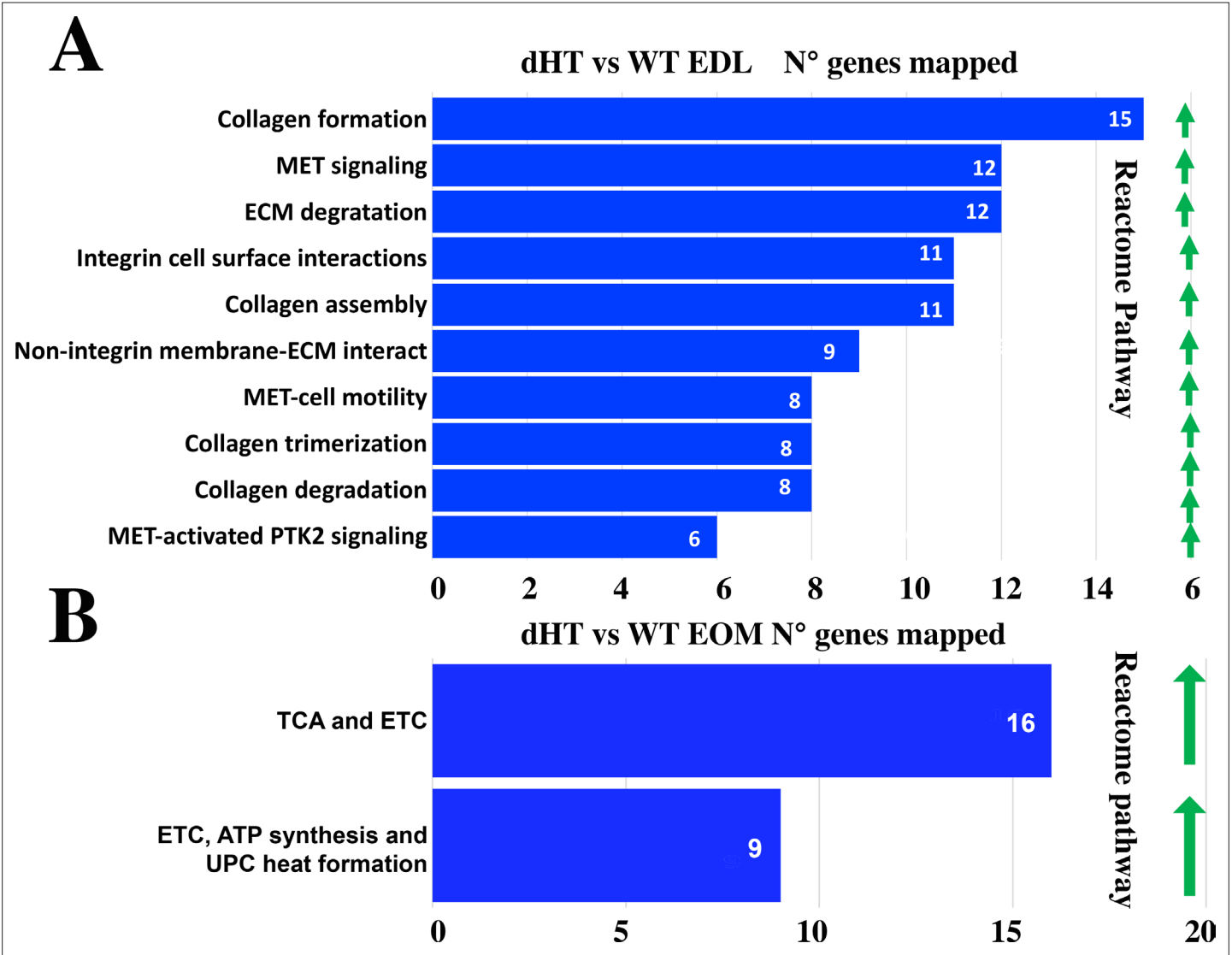


Figure 3—figure supplement 1. Reactome pathway analysis showing major pathways which differ between EDL muscles (A) and EOM muscles (B) in dHT versus WT mice. A q-value of equal or less than 0.05 and showing a significant change ≥ 0.2 fold was used to filter significant changes prior to the pathway analyses.

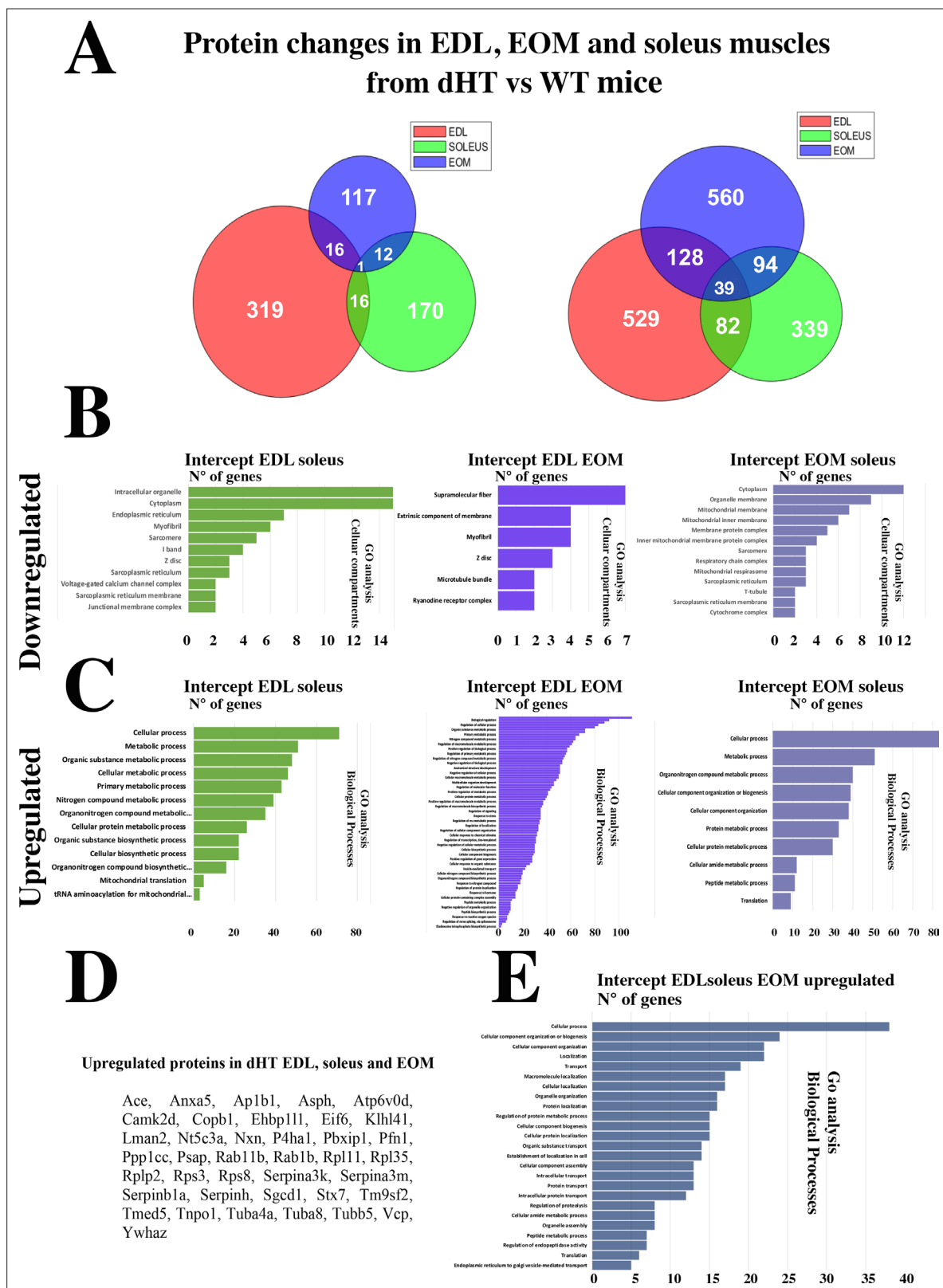


Figure 4. Changes in protein content in EDL, soleus and EOM between dHT vs WT mice. **(A)** Venn diagram showing significantly decreased proteins (left) and increased proteins (right) in the three muscle types. **(B)** GO biological process analysis of common proteins that are downregulated and **(C)** upregulated in muscle from dHT mice. Left panels, common proteins showing significant changes in content in both EDL and soleus muscles. Central panels, common proteins showing significant changes in content in EDL and EOMs; right panels, common proteins showing significant changes in content in EOM and soleus muscles.

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in content in EOM and soleus muscles. **(D)** List of the 39 proteins whose content is increased in EDL, soleus and EOMs in dHT mice. **(E)** GO analysis annotated to Biological processes of the 39 proteins that are increased in muscles from dHT mice.

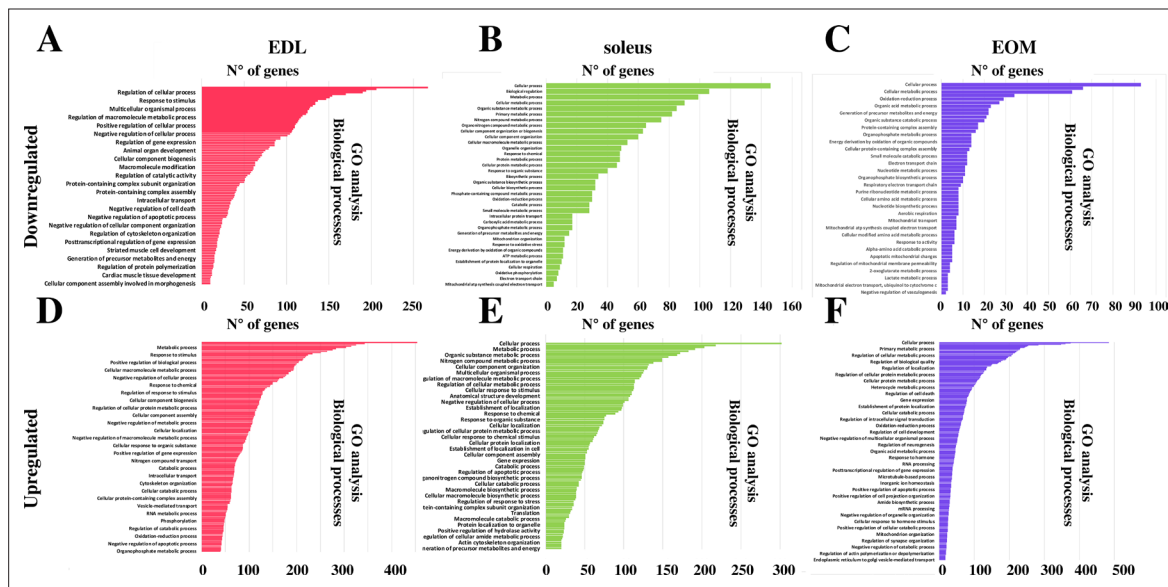


Figure 4—figure supplement 1. Gene Ontology annotated to Biological process genes showing significant differences in content between muscles from dHT and WT mice. (A, B) and (C) Downregulated genes and (D, E) and (F) upregulated genes in EDL (A and C), soleus (B and E) and EOM (C and F) muscles. The N° of genes annotated to each category is indicated on the Y-axis. Only proteins showing a q-value equal to or less than 0.05 and showing a significant change ≥ 0.2 fold were included in the pathway analyses.

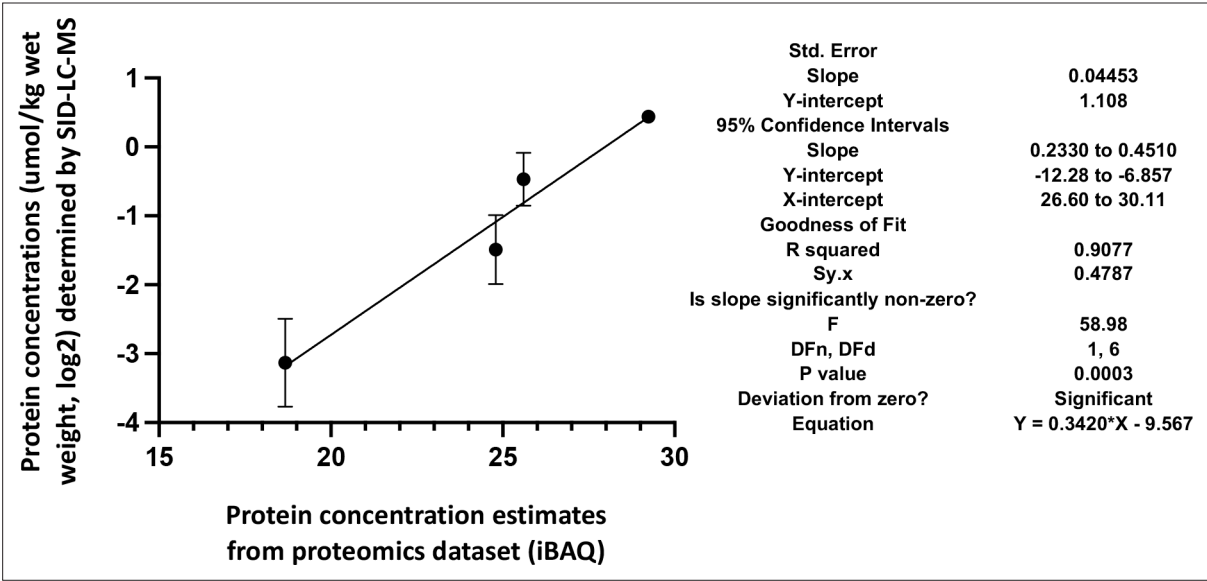


Figure 5. Correlation of the actual cellular abundances of four selected proteins (in $\mu\text{mol/kg}$ wet weight) determined by PRM/SID ($n=2$) and the iBAQ values ($n=5$) determined by label-free/TMT quantification (both in logarithmic scale, base 2) from the global proteomics discovery dataset for EDL samples. Error bars are indicated for the y-axis, but for the x-axis, due to their low scale (range from 0.058 to 0.086), they are not shown by the software PRISM, GraphPad Software, (v9). The simple linear regression results obtained by PRISM GraphPad Software, (v9) are shown on the right.

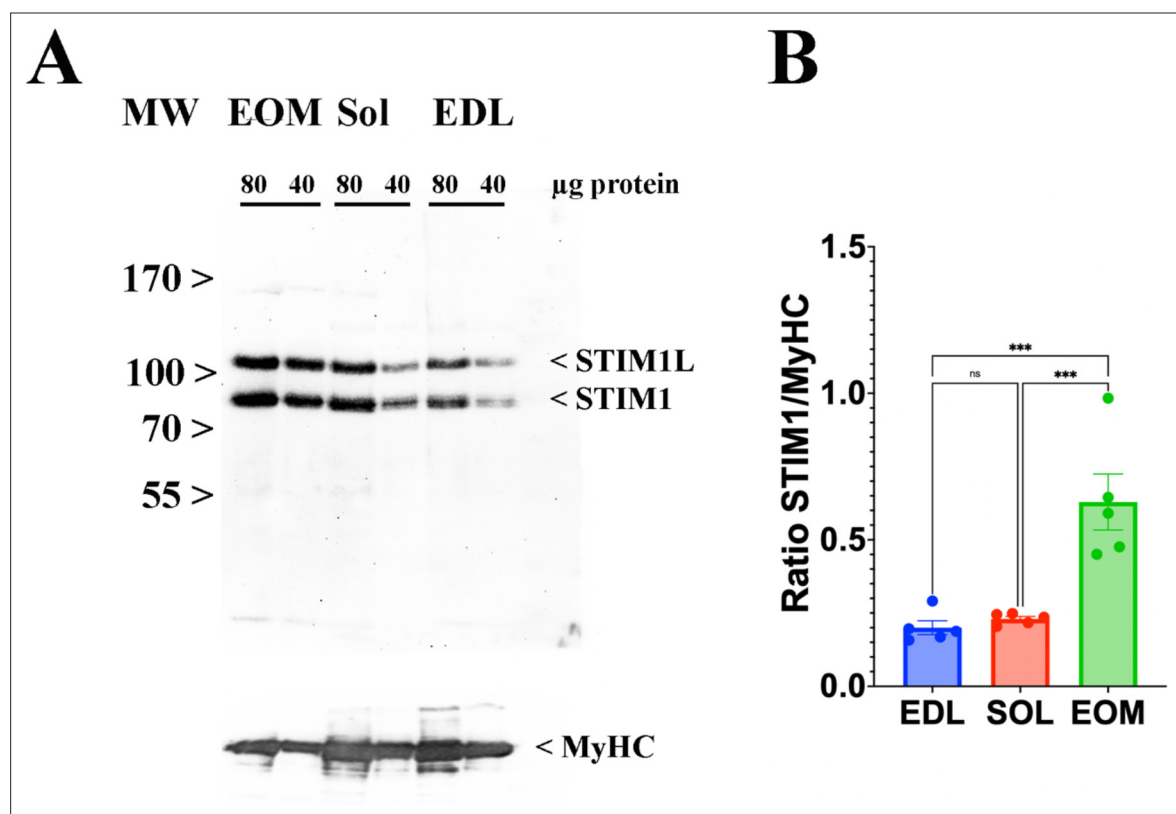


Figure 6. EOMs are enriched in Stim 1. **(A)** Representative western blots showing Stim1 and Stim1L immunopositive bands. Forty and eighty micrograms of total homogenates from EOM, soleus, and EDL muscles isolated from WT mice were loaded onto a 7.5% SDS PAGE. Proteins were blotted onto nitrocellulose, probed with an antibody recognizing Stim1 and Stim1L, followed by incubation with an anti-rabbit IgG HRP-linked antibody. Bands were visualized by chemiluminescence. Blots were subsequently stripped and probed with anti-MyHC (all) for loading normalization (bottom panel). **(B)** Relative content of Stim1 in the three muscle types examined. Each symbol represents the value of a single mouse. *** $p < 0.001$.