

## Supplementary Figure Legends

### Supplementary Figure 1

A) Fusion index of C2C12 cells at collection time points. Significance was tested using one-way ANOVA to compare means.  $n=3$ .  $****<0.0001$ . Middle line corresponds to mean  $\pm$ SD.

B) Frequency distribution of MyHC positive C2C12 diameter measurements between Dex 24 hr and 72 hr. Values are indicated as % of total.  $n=3$ .

C) PCA plot of bulk mRNA-seq using raw count intensity with the DESeq2 package.  $n=4$ .

D) and E) RT-qPCR measurements (D) of indicated genes using the same cDNA samples submitted for sequencing, plotted in TPM (E).  $n=4$  per time point. Significance was tested using one-way ANOVA and Tukey's post-hoc test. Non-significant results have been omitted for legibility unless deemed relevant.  $*<0.05$ ,  $**<0.01$ ,  $***<0.001$ ,  $****<0.0001$ .  $n=4$ . Middle line corresponds to mean  $\pm$ SD.

F) Volcano plot of transcripts at Dex  $\diamond$  Ctrl 24 hr. Grey dashed line indicates adj.p-value =0.05. Genes plotted further outside of the x- and y-axis limits are indicated with arrows on respective edges.

G) Venn diagram of significantly up/downregulated genes in Dex  $\diamond$  Ctrl 24 hr and 72 hr.

H) TMT-MS measured protein levels of genes undergoing DEG and DAS. Values have been median-MAD normalized and tested for significance using two-way ANOVA.  $*<0.05$ ,  $**<0.01$ ,  $****<0.0001$ .  $n=5$ . Middle line corresponds to mean  $\pm$ SD.

### Supplementary Figure 2

A) PCA plot of TMT-MS reporter intensity.  $n=5$ .

B) Z-score heat map of TMT-MS median-MAD normalized data highlighting ubiquitin (Ub) E1~E3 enzymes, DUBs and components of the proteasome complex as searched on the MGI database, with adj.p-value  $\leq 0.05$  in comparisons between Dex  $\diamond$  Ctrl 24 hr or 72 hr or Dex 24 hr  $\diamond$  Dex 72hr. None of the Ub E1 enzymes passed the significance filter. Each square represents a biological replicate.  $n=5$ .

### Supplementary Figure 3

Z-score heat map of median-MAD normalized protein levels associated with oxidative phosphorylation from RNA-seq (left,  $n=4$ ) and TMT-MS data (right,  $n=5$ ) which passed the adj.p-value  $\leq 0.05$  filter in comparisons of Dex  $\diamond$  Ctrl at 24 hr and 72 hr. Genes and proteins (labelled with gene names) were tested for significance using

a moderated *t*-test. RNA data has additional genes which were significant ( $\text{adj.p} \leq 0.05$ ) in MT  $\triangleright$  MB comparisons and protein has additional genes which were significant in Dex 24 hr vs Dex 72 hr comparisons. Each square represents a biological replicate measurement.

#### **Supplementary Figure 4**

A) Median-MAD normalized intensity of transcription factors responsible for mitochondrial gene expression. TMT-MS data were filtered for the following transcription factors: NRF-1, NRF-2, PPAR $\alpha$ , ERR $\alpha$ , Sp1, PGC-1 $\alpha$ , PGC-1 $\beta$ , and PRC (Scarpulla et al. 2008). Significance testing with two-way ANOVA. \* $<0.05$ , \*\* $<0.01$ , \*\*\* $<0.001$ , \*\*\*\* $<0.0001$ .  $n=5$ . Middle line corresponds to mean  $\pm$ SD.

B) and C) Z-score heat map of cytosolic large ribosomal subunits (C) and small ribosomal subunits (D from TMT-MS data.  $n=5$ . Each square represents a biological replicate measurement.

#### **Supplementary Figure 5**

Log2FoldChanges of RNA (x-axis,  $n=4$ ) plotted against those of proteins identified by TMT-MS (y-axis,  $n=5$ ) for MT $\triangleright$ MB.  $R^2$  values were calculated using linear regression modelling using base R. Where there are duplicated gene names indicated, our proteome study identified different Uniprot entry corresponding protein species.

#### **Supplementary Figure 6**

A) Venn diagram of significantly changing proteins (moderated *t*-test,  $\text{adj.p-value} \leq 0.05$ ) from TMT-MS data between this study, at the 24 hr time point and Hunt et al. (2021) dex data.

B) GO analysis of up (red, left) or down (blue, right)-regulated proteins specific to C2C12 dex atrophy model from this study. Analysis using STRING.

C) GO analysis of up (red, left) or down (blue, right)-regulated proteins specific to dex atrophy model from Hunt et al. (2021). Analysis using STRING.

#### **Supplementary Tables**

**Supplementary Table 1:** GSEA of RNA-Seq data for MT over MB and Dex over Ctrl 72 hr. Analysis performed using clusterProfiler.

**Supplementary Table 2:** Genes undergoing differential expression and DAS. DAS analysis performed using IsoformSwitchAnalyzeR.