Supplementary information for Deshmukh et al. "Deep muscle proteome…"

Figure legends

Figure S1 Reproducibility of proteomic data. A - Density plot showing that 95% of the peptides that were identified by matching lie in a 0.4 min match time window (blue color). The remaining 5% identification lie in less than 0.5 min match time window. **B -** Comparison of triplicate analysis of skeletal muscle and C2C12 myotubes shows high correlation between replicates. Pearson correlation (r) values are displayed for individual graph.

Figure S2 Proteins identified by matching. A- Histogram showing that the identification of low abundant proteins is rescued by match between runs features (Blue: total identified, orange: proteins identified by matching). **B** – S curve displaying examples of low abundant regulatory proteins identified by matching.

Figure S3 Composition of branched chain amino acid (BCAA) in mouse skeletal muscle. Weighted abundance of branched chain amino acids in skeletal muscle, C2C12 myotubes **(A)** and other mouse tissues **(B).**

Figure S4 Percent abundances of proteins in C2C12 myotubes and skeletal muscle. Percent protein abundance of contractile proteins **(A)** and protein categories associated with cellular compartment (GOCC) and molecular function (GOMF) **(B).**

Figure S5 Western blot analysis Western blot analysis for the proteins involved in glucose metabolism. Top panel (A) represents the images. Blots are quantified and normalized to GAPDH expression. Values (B) are represented in arbitrary units ± standard deviation of mean.

Figure S6 Protein identification and quantitation using 'deamidation of Asn' as a variable modification. Distribution and % occurrence of deamidated peptides in muscle (A) and C2C12 (B) cells. Number of proteins and peptide identified with/without using 'deamidation of Asn' in muscle (C) and C2C12 cells

Figure S7 MSMS spectra for the single peptide based identification Images displays fragments assignments and masses detected for MSMS spectrum for the single peptide based identification.

Supplemental Table S1

Table contains list of identified proteins in skeletal muscle and C2C12 cells after enabling match between runs feature in MaxQuant.

Supplemental Table S2

Table contains list of identified proteins in skeletal muscle and C2C12 myotubes without enabling match between runs feature in MaxQuant.

Supplemental Table S3

Cumulative and ranked protein abundance for all identified proteins in skeletal muscle and C2C12 myotubes.

Supplemental Table S4

Table displays the list of significantly different proteins between skeletal muscle and C2C12 myotubes.

Supplemental Table S5

Table represents the list of the significantly enriched protein annotations for cluster 1 and cluster 2.

Supplemental Table S6

2D annotation scores and annotation categories for skeletal muscle and C2C12 myotubes.

Supplemental Table S7

Absolute abundance for protein involved in insulin, AMPK mediated signaling pathways and members of different metabolic pathways.

Supplemental Table S8

Details of single peptide based identification. The table includes peptide sequence identified, precursor m/z, charge, score, intensities and associated proteins groups.

Supplemental Table S9

This table contains a list of identified proteins in skeletal muscle and C2C12 cells with 'deamidation of Asn' as a variable modification. The additional worksheet named 'modificationspecificpeptides' contains all details of modified and unmodified peptides.

Perseus workflows (Version 1.3.10.0)

Perseus workflow is provided for figures which were generated in Perseus software. Since the Perseus software is constantly under development, we recommend readers to download the most recent version of Perseus http://www.perseus-framework.org.

Readers can also learn more about the Perseus workflow on YouTube:

https://www.youtube.com/watch?v=jHgifeg-aCU

https://www.youtube.com/watch?v=-cgG5yujMug

Functional analysis of proteome difference between muscle and C2C12 cells (Figure 2)

1. Load protein group txt files using generic upload tab ((Generic upload – load)
2. Filter reverse and contaminants (Processing – filter – reverse – contaminant)
3. Log transform the LFQ values (Processing – basic – transform)
4. Add annotations (Processing – annotation columns – add annotations – GOCC, GOBP, GOMF, KEGG)
5. Create groups (Processing – annotation rows –categorical annotation rows- Expt 1,2,3 – Muscle, Expt 3,4,5 – C2C12)
6. Filter valid values (Processing – filter-valid values – at least 2 in at least one group)
7. Imputation (Processing – imputation – replace missing values by valid distribution)
8. Perform two sample t test (Processing – test-two sample t test – FDR 0.05)

9. Filter significantly different proteins (Processing – filter – significant)
10. Clustering (Processing –normalization – z score) – (Analysis – clustering – hierarchical clustering-row cluster 2 –show enrichment FDR 0.04)
11. Export images (Click in pdf symbol – save images)

2D annotation enrichment

1. Load protein group txt files using generic upload tab (Generic upload – load)
2. Filter reverse and contaminants (Processing – filter – reverse – contaminant)
3. Log transform the LFQ values (Processing – basic – transform)
4. Add annotations (Processing – annotation columns – add annotations – GOCC, GOBP, GOMF, KEGG)
5. Create groups (Processing – annotation rows –categorical annotation rows- Expt 1,2,3 – Muscle, Expt 3,4,5 – C2C12)
6. Filter valid values (Processing – filter-valid values – at least 2 in at least one group)
7. Imputation (Processing – imputation – replace missing values by valid distribution)
8. Average groups (Processing – annotation rows – groups – average groups - median)
9. 2D annotations (Processing – annotation columns – 2D annotation – column 1 muscle – column 2 C2C12)
10. Scatter plot (Analysis – basic visualization – scatter plot – muscle – C2C12)
11. Export image (Click in pdf symbol – save image)

Density plot (Supplementary figure 1 A)

1. Load the evidence.txt file generated by MaxQuant (Generic upload – load – match time difference-retention time)

2. Filter evidences with MULTI-MATCH (Processing – filter – Type – MULTI-MATCH)
3. Click on density estimation (Load parameter 1 – match time difference – parameter 2 – retention time)
4. Above step will generate two new columns 1 – column with density 2 – Excluded fraction
5. Scatter plot (Analysis – match time difference – retention time – excluded fraction)
6. Export density plot (Click in pdf symbol – save image)

Scatter plots (Supplementary figure 1 B)

1. Load protein group txt files using generic upload tab.
2. Filter reverse and contaminants (Processing – filter – reverse – contaminant)
3. Log transform the LFQ values (Processing – basic – transform)
4. Multi-scatter plot (Analysis – visualization – multiscatter plot)
5. Get Pearson correlation and Export image (Click in pdf symbol – save image)