

Supporting Information

A Complete Workflow for High Throughput Human Single Skeletal Muscle Fiber Proteomics

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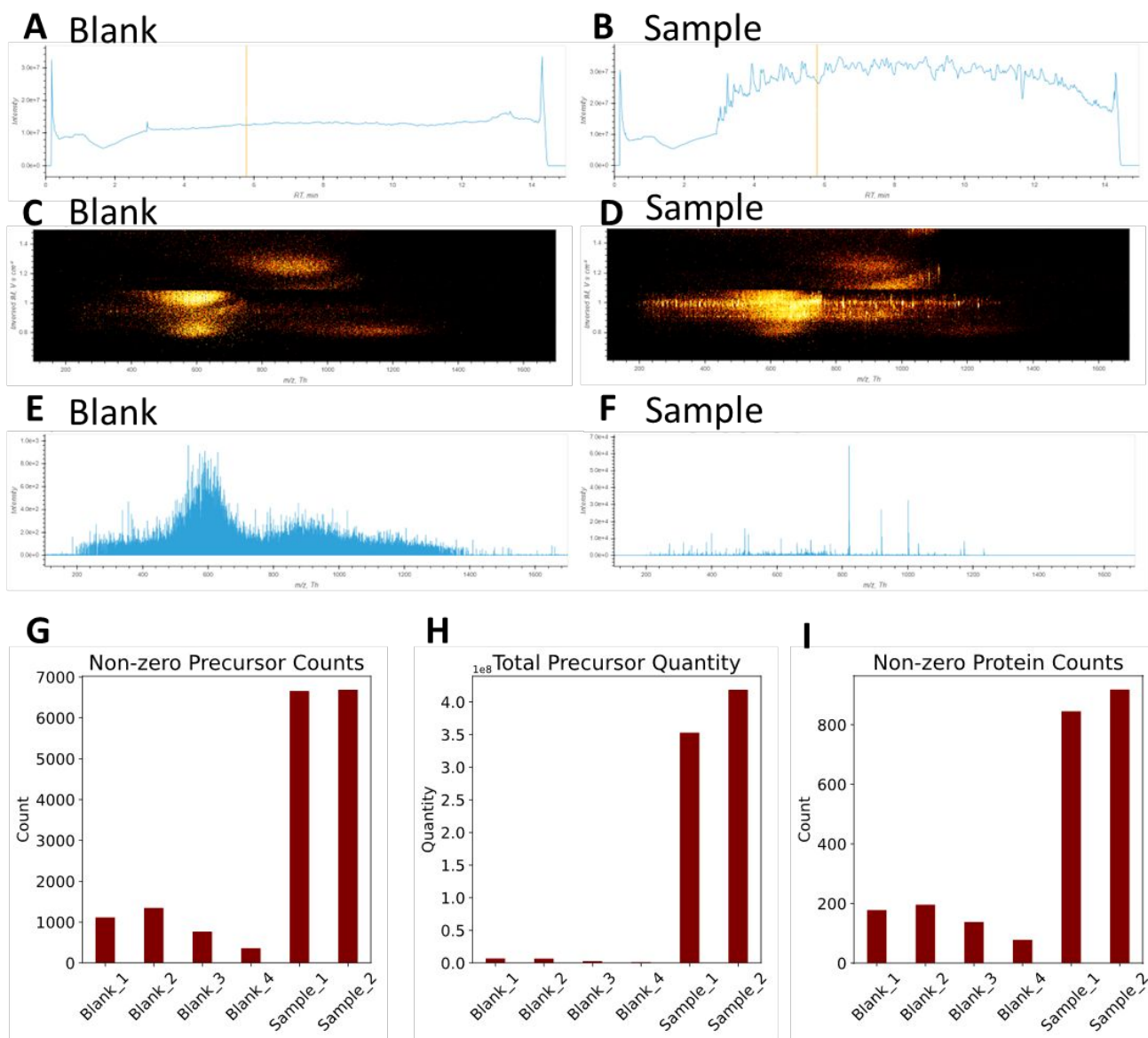


Figure S1 Quality Control Metrics. (A) Total ion chromatogram (TIC) for a blank well. (B) TIC for a sample well. (C) Trapped Ion Mobility Spectrometry (TIMS) heatmap for a blank well. (D) TIMS heatmap for a sample well. (E) Mass spectrum (MS) for a blank well. (F) MS for a sample well. (G) Counts of non-zero precursors in blank compared to sample wells. (H) Summed quantities of precursors in blank compared to sample wells. (I) Counts of non-zero proteins in blank compared to sample wells.

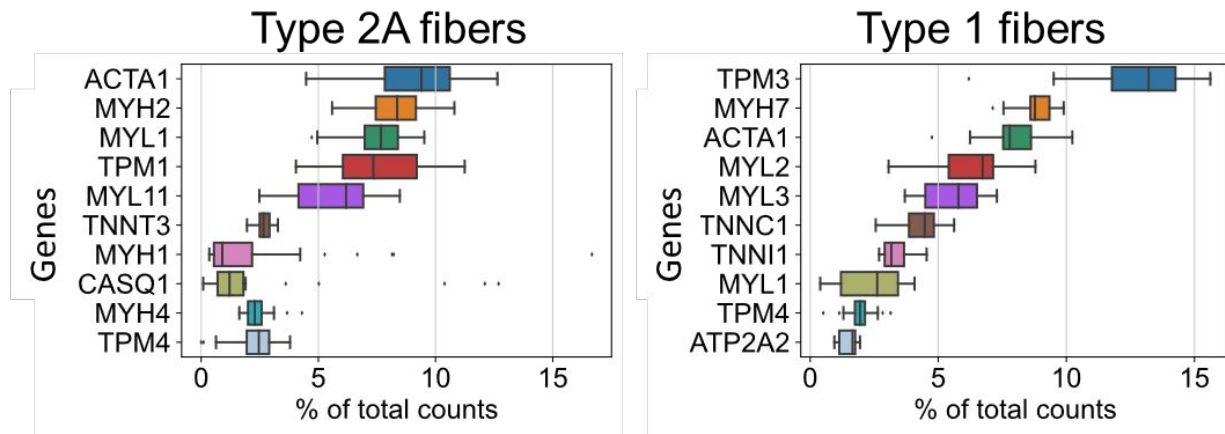


Figure S2. Ten most abundant proteins when quantities are computed by MaxLFQ. Top ten proteins with highest percent of total counts in type 1 (left) and type 2A (right) fibers using MaxLFQ quantification. MYL11 is interchangeable with MYL1 and MLC2-fast.

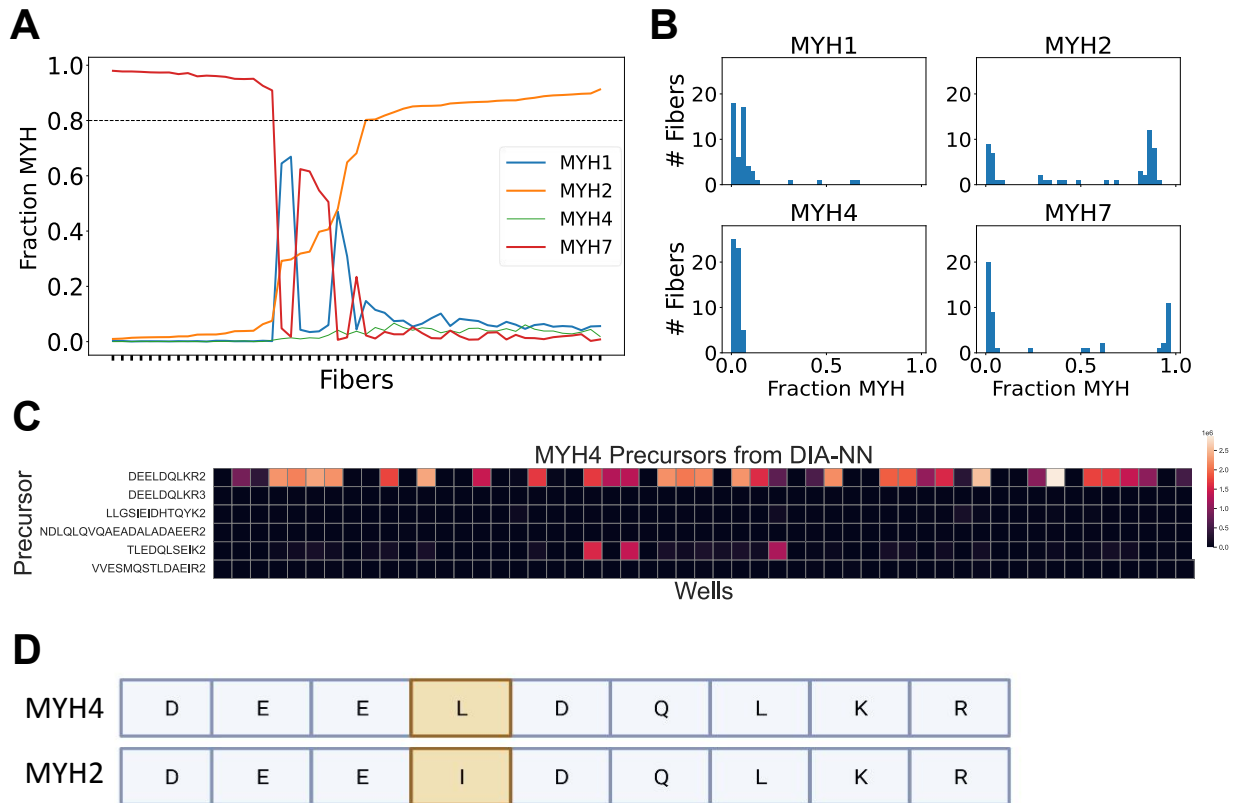


Figure S3. MYH4 peptide overlap with MYH2. (A) Fraction of MYH subtypes (sorted by MYH2 from low to high) using protein quantities from iBAQ prior to removing MYH4 peptide with overlap with MYH2. (B) Histogram showing MYH fractions per fiber using protein quantities from iBAQ prior to removing MYH4 peptide with overlap with MYH2. (C) Precursors mapping to MYH4 with one peptide driving the abundance of MYH4. Peptide charge is given at the end of each sequence. (D) BLAST results for DEELDQKR²⁺ peptide indicate identical sequence to MYH2 except for substitution of I for L.

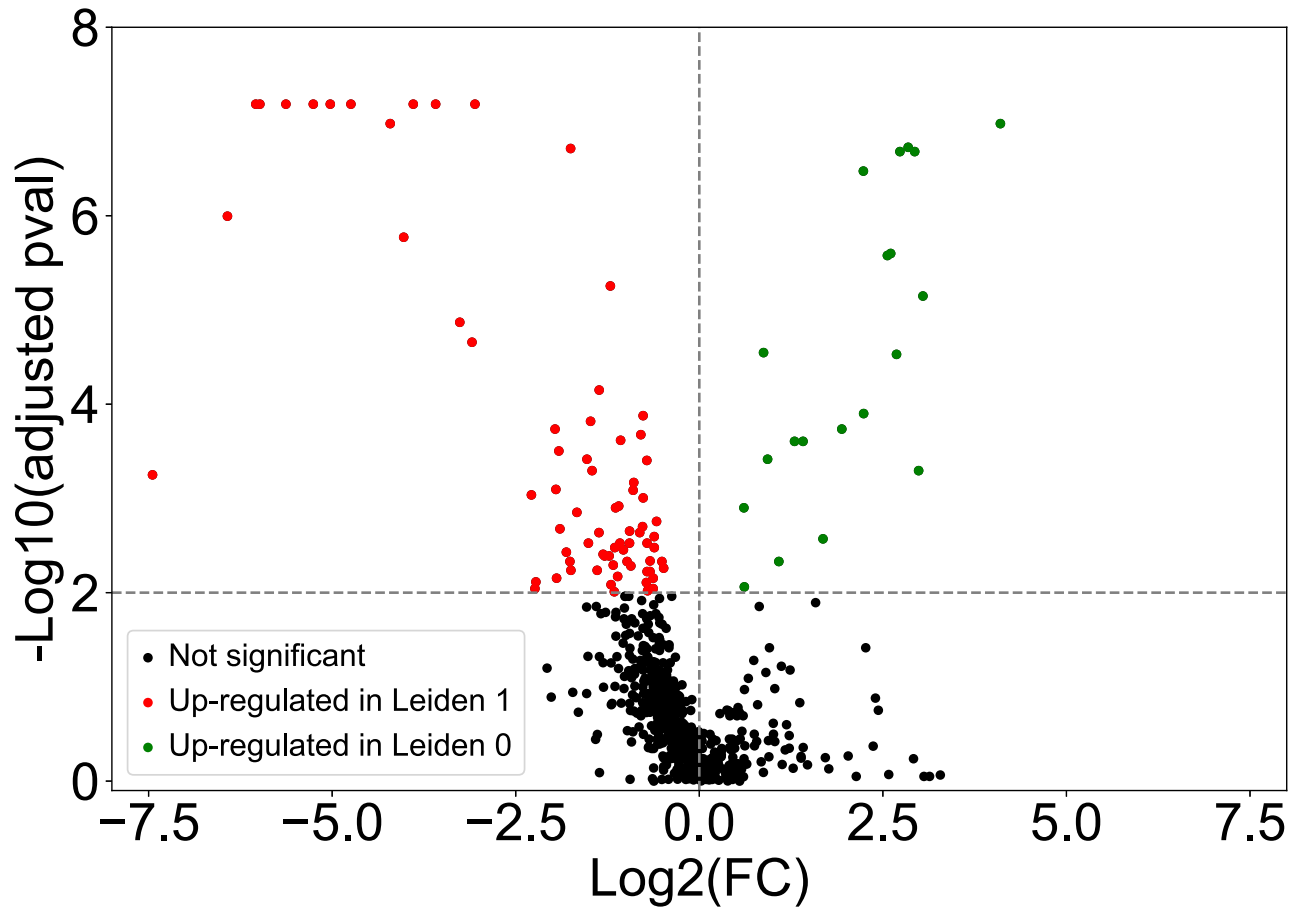


Figure S4. Volcano plot depicting all proteins. Proteins above $\log_{10}(\text{adjusted p-value})=2$ (equivalent to B-H adjusted p-value below 0.01) are statistically different between Leiden cluster 0 and 1. Ninety-four proteins were defined as significantly different according to the adjusted p-value cutoff of < 0.01 (see **Table S4** for $\log_2\text{FC}$ and adjusted p-values for each protein).

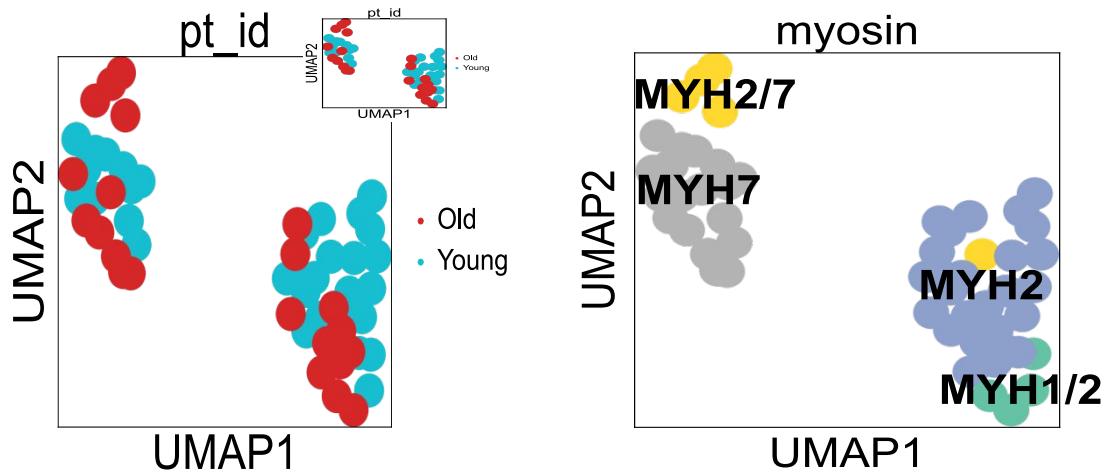


Figure S5. UMAP distribution of old and young fibers. UMAP colored by young and old fibers (left) in the same layout as Figure 4B (right).

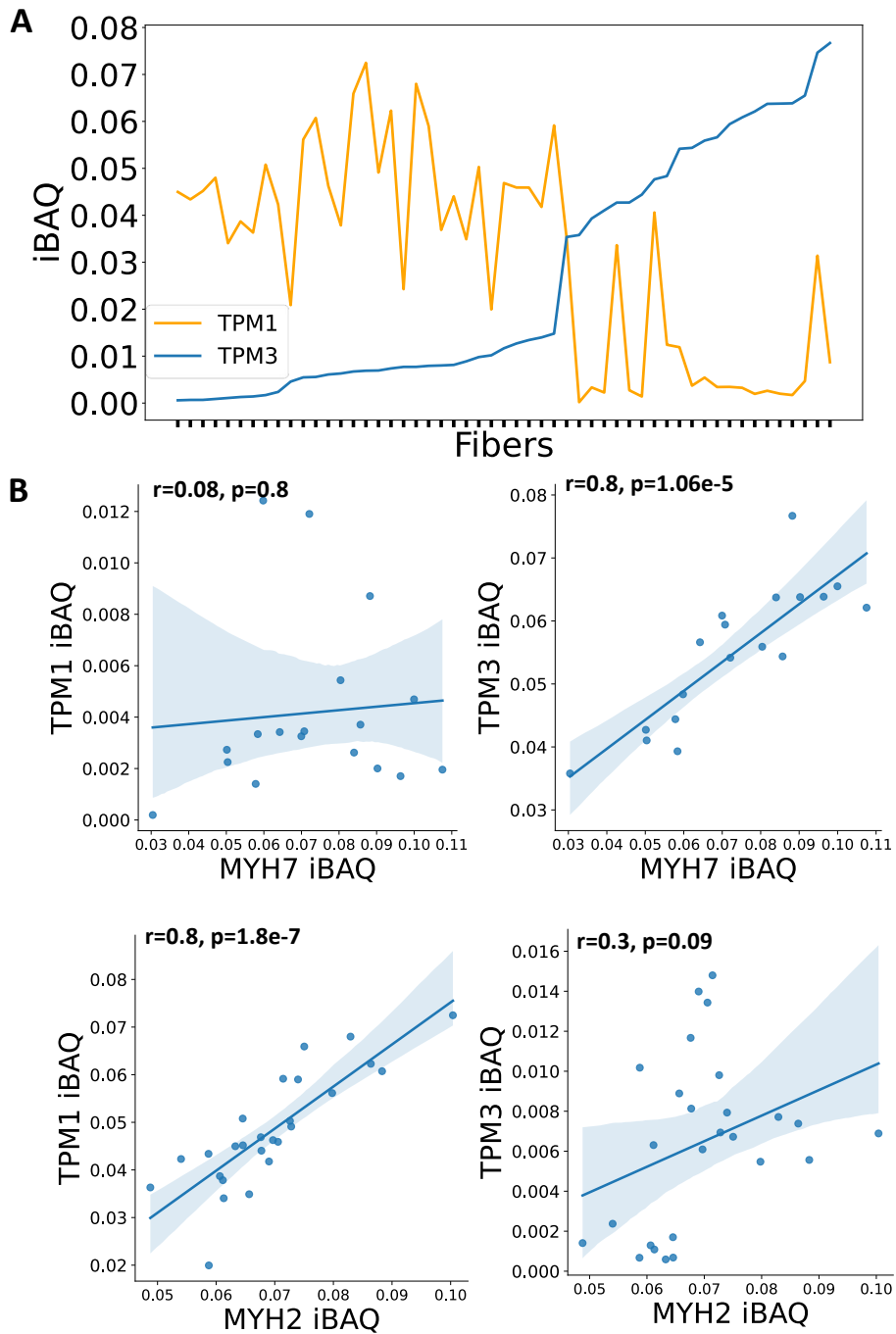


Figure S6. TPM1 and TPM3 quantities per fiber and correlation with fast and slow fibers. (A) iBAQ-calculated quantity of TPM1 and TPM3 by fiber sorted by TPM3 values from low to high. **(B)** Linear regression plots visualizing relationships between MYH2, MYH7 and TPM1, TPM3 and corresponding Pearson correlation coefficients (r) for each plot.