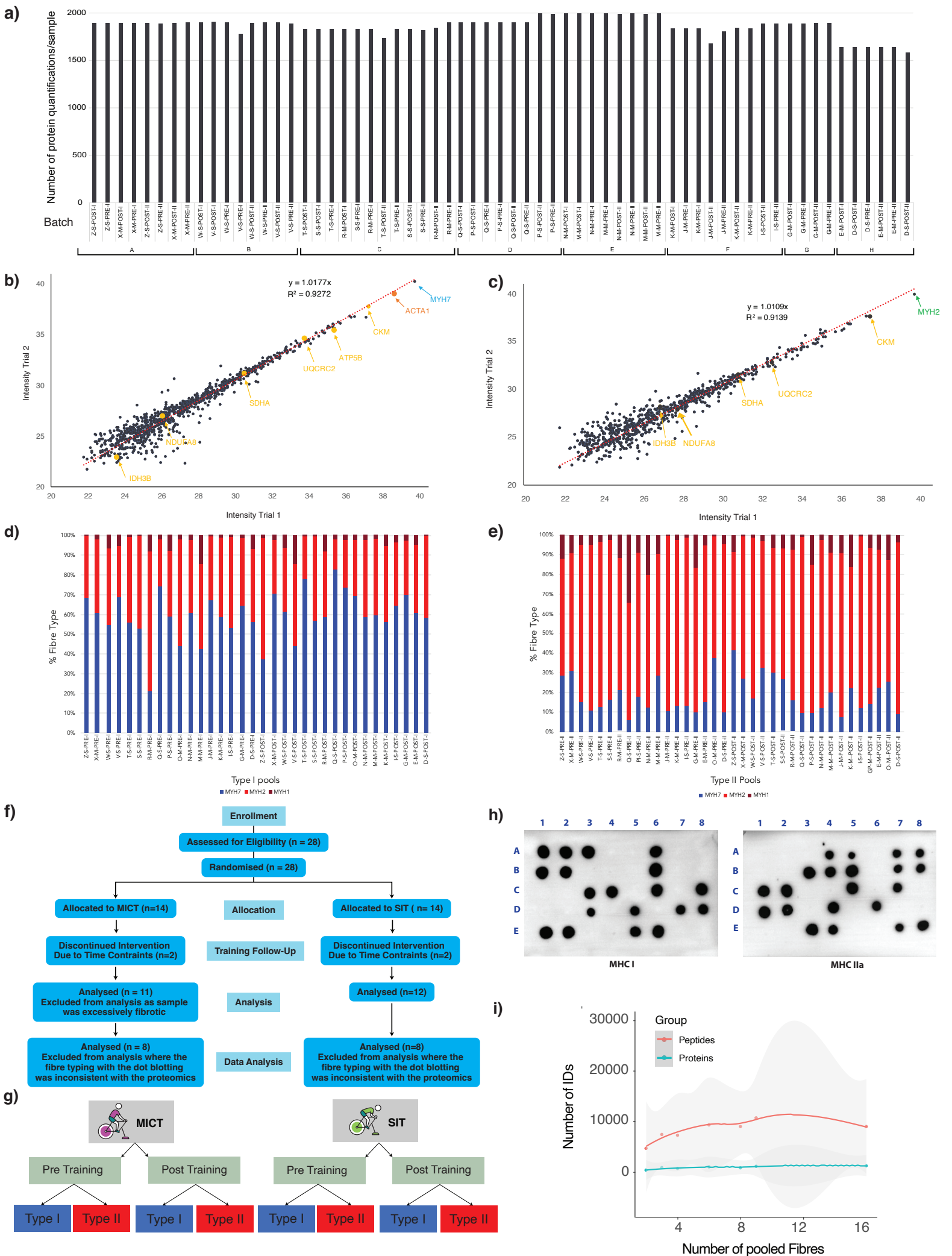
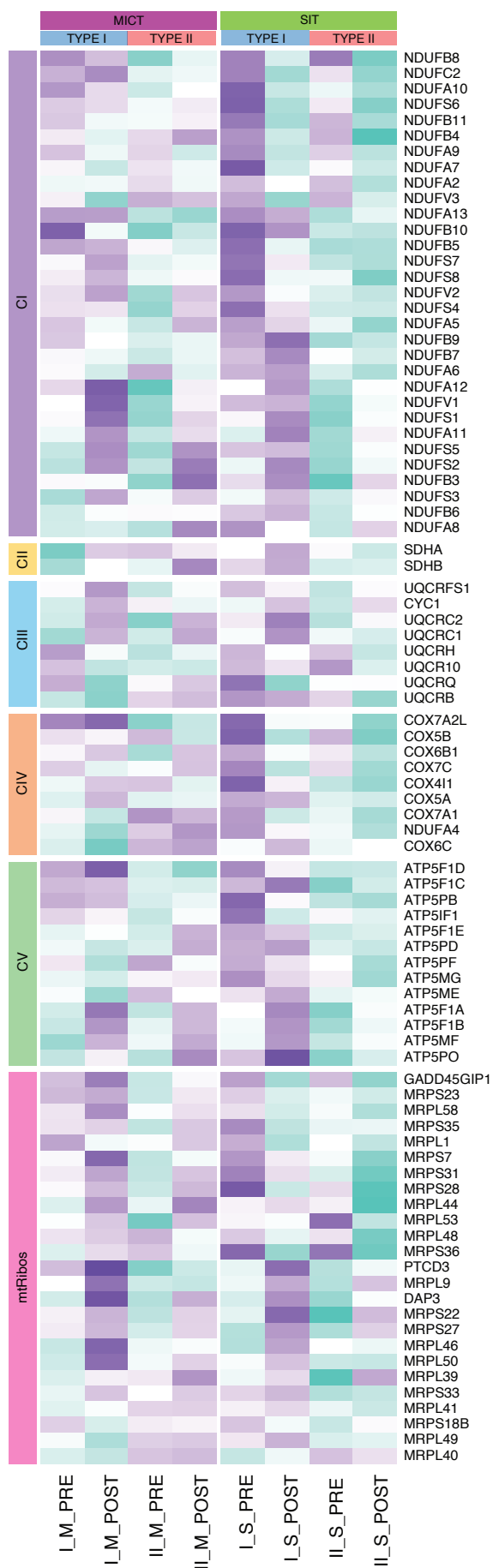
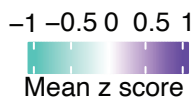




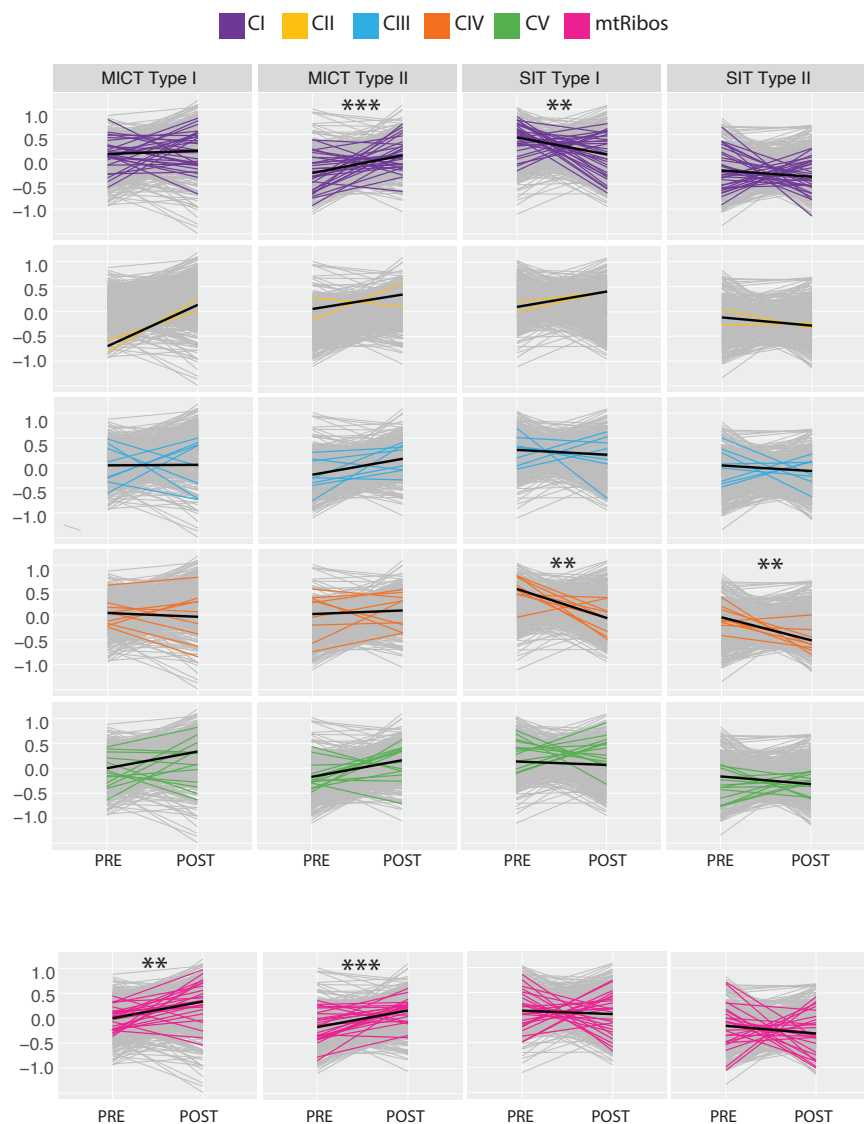
Supplementary Figure 1. Methodological approach to identify proteins in pools of single fibres.

- a) The number of proteins quantified in the training study dataset by our proteomic workflow in all pooled single-fibre samples ($n = 64$). Samples are arranged according to TMT batches (A-H). Samples are labelled according to participant ID (Z to C), training type (S for SIT or M for MICT), biopsy time (PRE or POST training), and fibre type (I or II)).
- b) Reproducibility of protein intensities (intensity-based absolute quantification; IBAQ) for replicates of type I skeletal muscle fibre samples. Core mitochondrial proteins (OXPHOS – NDUFA8, SDHA, URCRC2, COX5B, ATP5B, IDH3B and CKM) are highlighted in yellow, in addition to sarcomeric proteins (MYH 7 - blue and ACTA1 - orange).
- c) Reproducibility of protein intensities (intensity-based absolute quantification; IBAQ) for replicates of type II skeletal muscle fibre samples. Core mitochondrial proteins (OXPHOS – NDUFA8, SDHA, URCRC2, COX5B, ATP5B, IDH3B and CKM) are highlighted in yellow, in addition to MYH2 (green).
- d) Relative abundance of myosin heavy chains (MYH) in type I samples ($n = 34$) pooled by dot blotting, according to the percentage of the MYH7 (blue = type I), MYH2 (red = type-IIa), MYH1 (brown = type-IIx), and MYH4 (purple = type IIb) protein isoforms detected.
- e) Relative abundance of myosin heavy chains (MYH) in type II samples ($n = 36$) pooled by dot blotting, according to the percentage of the MYH7 (blue = type I), MYH2 (red = type-IIa), MYH1 (brown = type-IIx), and MYH4 (purple = type IIb) protein isoforms detected.
- f) Study recruitment flow chart and final group sizes used within the study.
- g) Hierarchical visualisation of the statistical comparisons performed in this study.
- h) Fibre typing by dot blotting. This example shows membrane images for the identification of fibre types in a single participant. Type I fibres were identified via the presence of MHC I (left panel), and type II fibres were identified via the presence of MHC IIa (right panel).
- i) Depth of coverage in pooled single fibre samples ($n = 6$) for the number of proteins and peptides identified.

a)

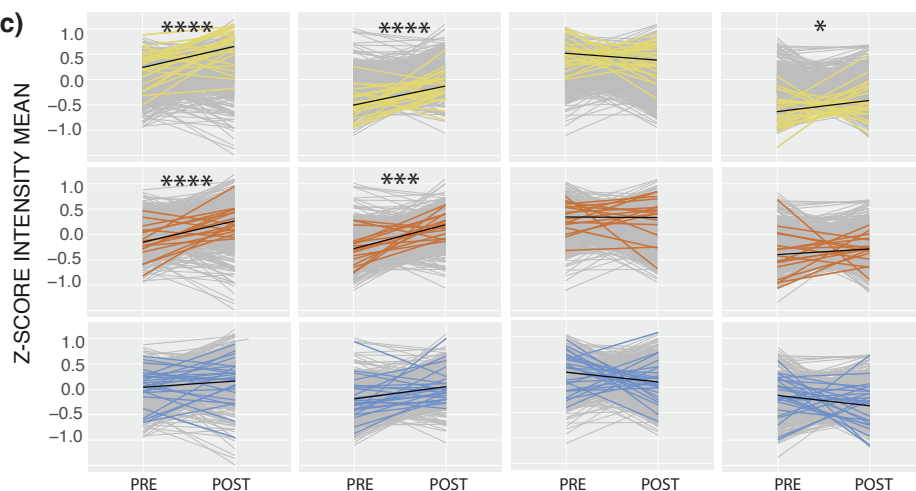


b)



FATTY ACID OXIDATION TCA CYCLE MITOCHONDRIAL DYNAMICS

c)



Supplemental Figure 2. Fibre-type differences in mitochondrial protein abundances on data not normalised for mitochondrial content.

- a)** Heatmap displaying z-score values of protein subunits of the OXPHOS complexes, as well as the mitochondrial ribosome, in each of the two training groups, moderate-intensity continuous training (MICT) or sprint interval training (SIT), in type I and type II fibre type pools pre- and post-training, without the application of mitochondrial normalisation.
- b)** Scaled profile plots without the application of mitochondrial normalisation showing changes in the relative abundance of the subunits in each of the oxidative phosphorylation (OXPHOS) complexes (CI to CV), as well as the mitochondrial ribosome, in type I and type II fibre types in response to both types of training (MICT and SIT). The mean of each group (all proteins identified in each pathway using MitoCarta 3.0) is indicated by the black line. Data are shown as Δ mean z-score. * indicates P value for significance (*: $P \leq 0.01$, **: $P \leq 0.001$, ***: $P \leq 0.0001$, ****: $P \leq 0.00001$) based on a paired t-test of the pre- to post-training values.
- c)** Scaled profile plots without the application of mitochondrial normalisation showing changes in the relative abundance of proteins involved in fatty acid oxidation, the tricarboxylic acid cycle, and mitochondrial dynamics (all proteins identified in pathway using MitoCarta 3.0) in type I and type II fibre types in response to both types of training (MICT and SIT). The mean of each group is indicated by the black line. Data are shown as Δ mean z-score. * indicates P value for significance (*: $P \leq 0.01$, **: $P \leq 0.001$, ***: $P \leq 0.0001$, ****: $P \leq 0.00001$) based on a paired t-test of the pre- to post-training values.