

Acute exercise rewires the proteomic landscape of human immune cells

Supplementary information

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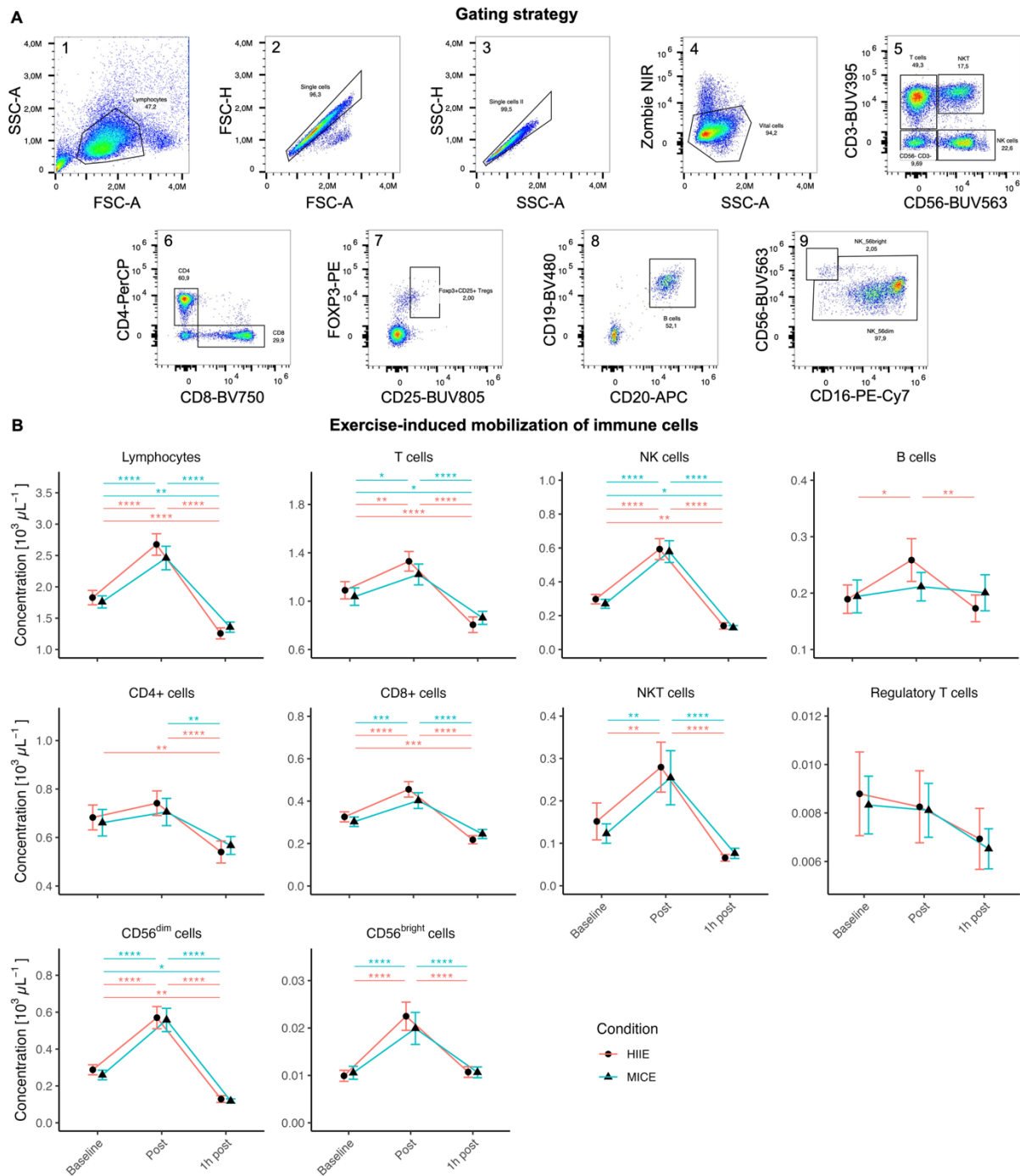


Figure S1 Immune cell mobilization is independent of exercise intensity

(A) Gating strategy applied to PBMCs. Gated immune cell populations included vital lymphocytes, T cells ($\text{CD}3^+\text{CD}56^-$) either as $\text{CD}8^+$ T cells ($\text{CD}3^+\text{CD}56^-\text{CD}8^+\text{CD}4^-$) or $\text{CD}4^+$ T cells ($\text{CD}3^+\text{CD}56^-\text{CD}8^-\text{CD}4^+$), natural killer (NK) T cells ($\text{CD}3^+\text{CD}56^+$), regulatory T cells ($\text{CD}3^+\text{CD}56^-\text{CD}8^-\text{CD}4^+\text{CD}25^+\text{Foxp}3^+$), NK cells ($\text{CD}3^-\text{CD}56^+$) either as $\text{NK}^{\text{bright}}$ ($\text{CD}3^-\text{CD}56^+\text{CD}56^{\text{bright}}\text{CD}16^-$) or NK^{dim} ($\text{CD}3^-\text{CD}56^+\text{CD}56^{\text{dim}}\text{CD}16^+$), and B cells ($\text{CD}3^-\text{CD}56^-\text{CD}19^+\text{CD}20^+$).

(B) Mobilization kinetics of total lymphocytes and lymphocyte subsets in response to high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MICE). Linear mixed models with subsequent analyses of variance and Bonferroni-corrected pairwise comparisons were applied. Data are represented as mean $\pm$ SEM ($N = 22$). Time effects: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

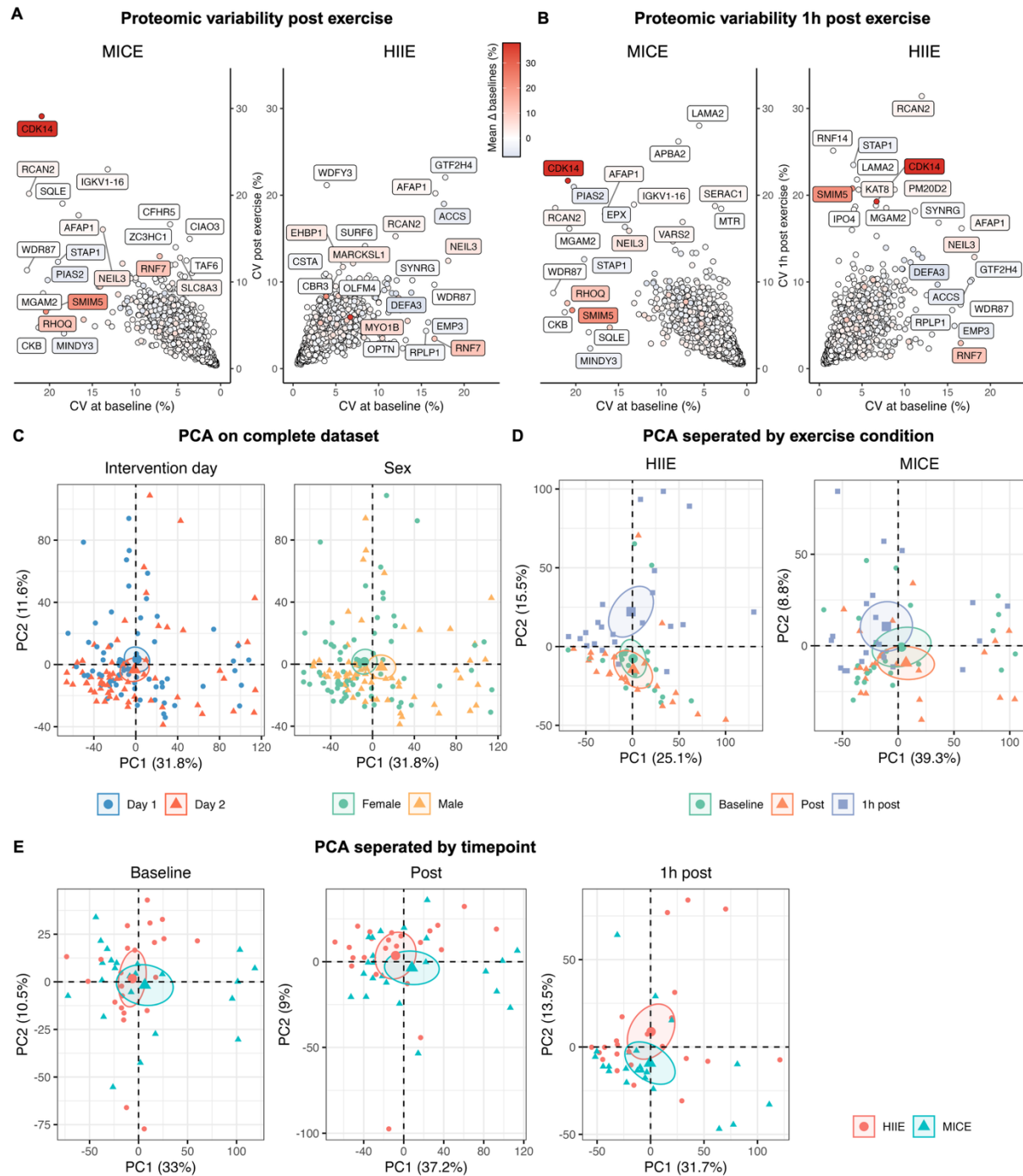


Figure S2 Proteomic variability and principal component analyses (PCA) separated by exercise condition and measurement timepoint

(A) Proteomic variability immediately after HIIE and MICE.

(B) Proteomic variability 1h after HIIE and MICE.

(C) PCA on complete dataset to evaluate the impact of intervention day and sex.

(D) PCA separated by exercise condition to evaluate the impact of measurement timepoint.

(E) PCA separated by measurement timepoint to evaluate the impact of exercise condition.

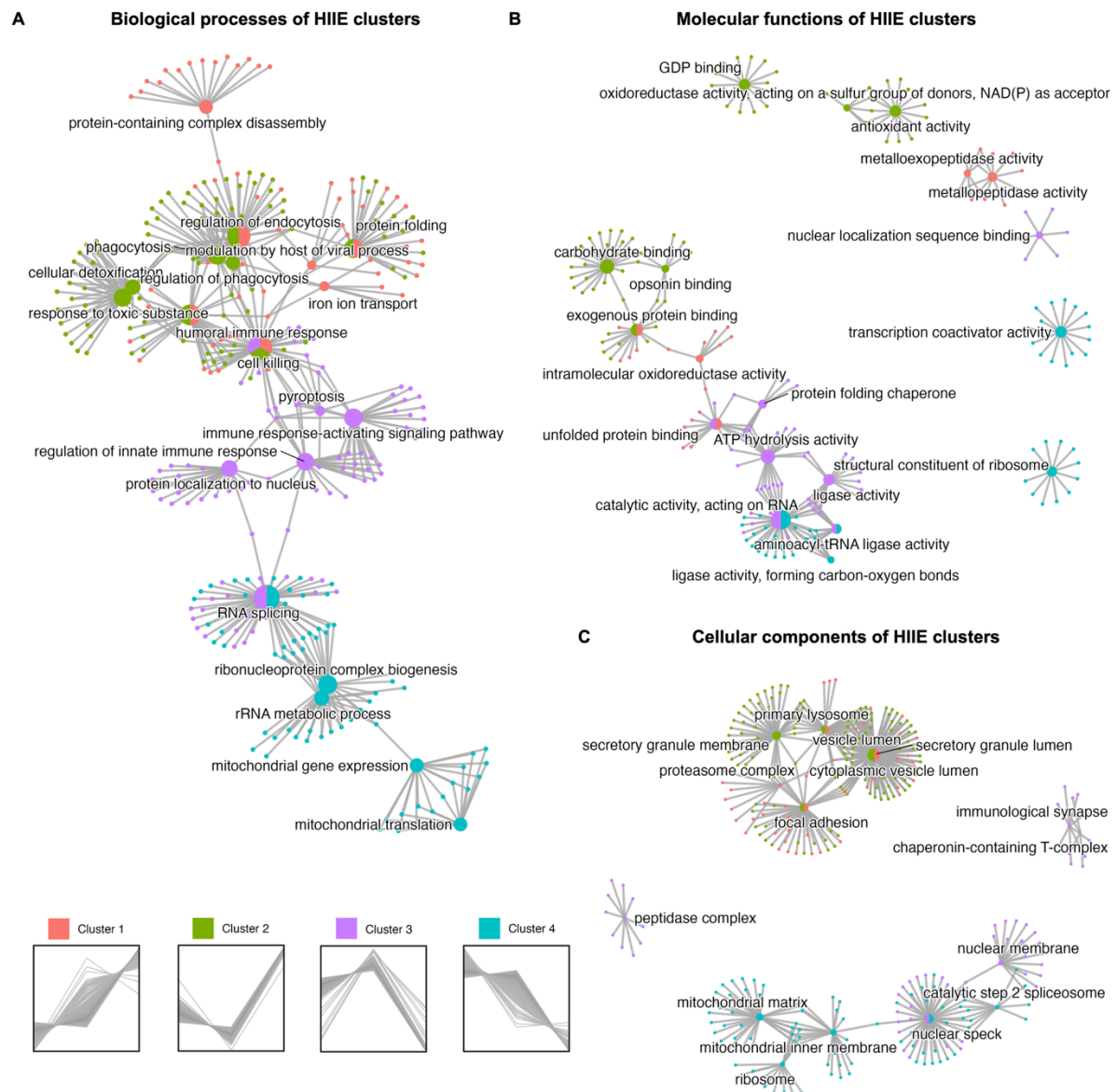


Figure S3 Biological theme comparison suggests shared and unique GO terms across protein clusters in HIIE

(A) Comparison of biological processes in clusters 1 – 4.

(B) Comparison of molecular functions in clusters 1 – 4.

(C) Comparison of cellular components in clusters 1 – 4.

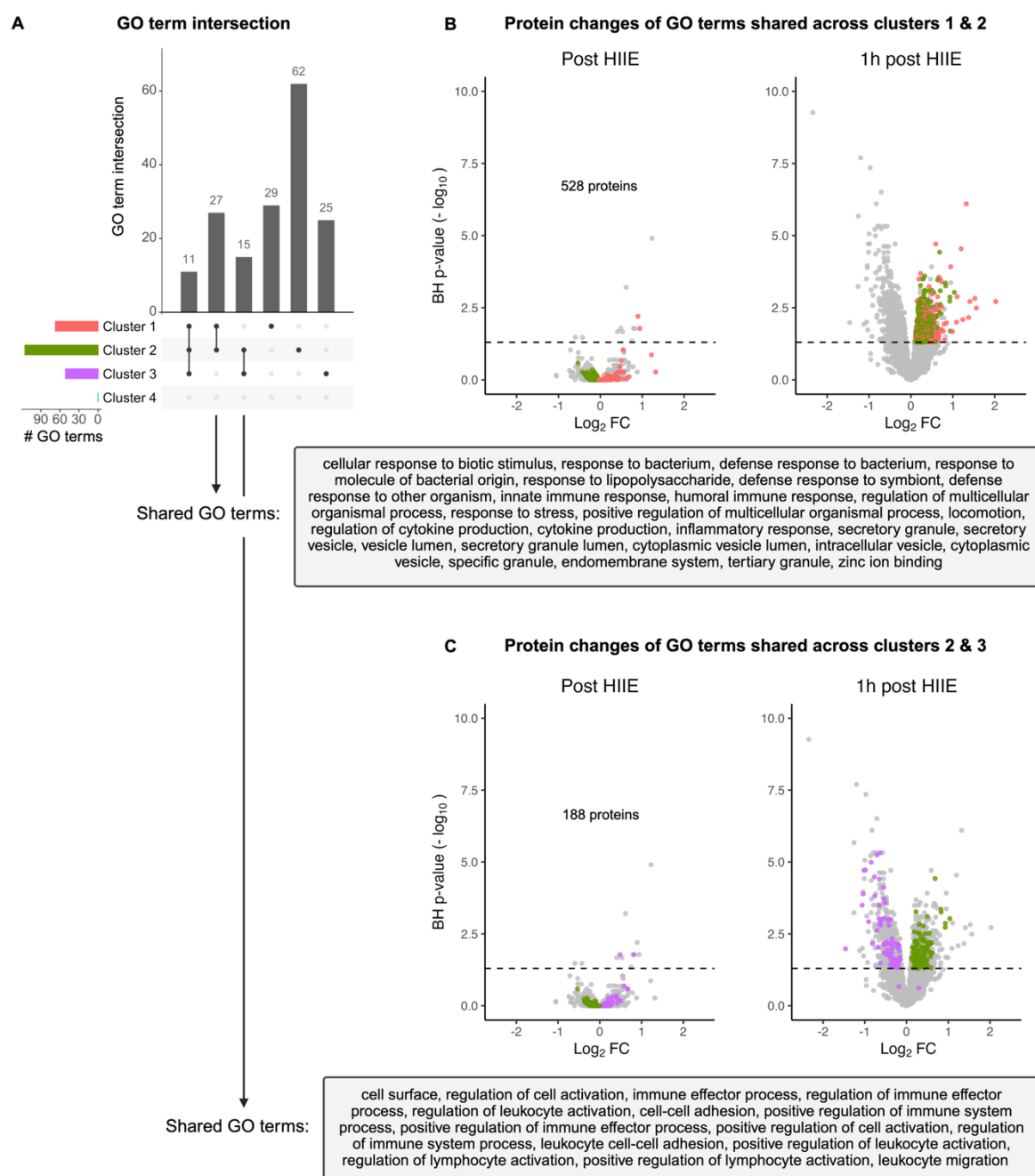


Figure S4 GO terms overlap between clusters 1 & 2 and 2 & 3, respectively

(A) Overview of shared and unique GO terms across all clusters.

(B) GO terms shared between clusters 1 and 2.

(C) GO terms shared between clusters 2 and 3.

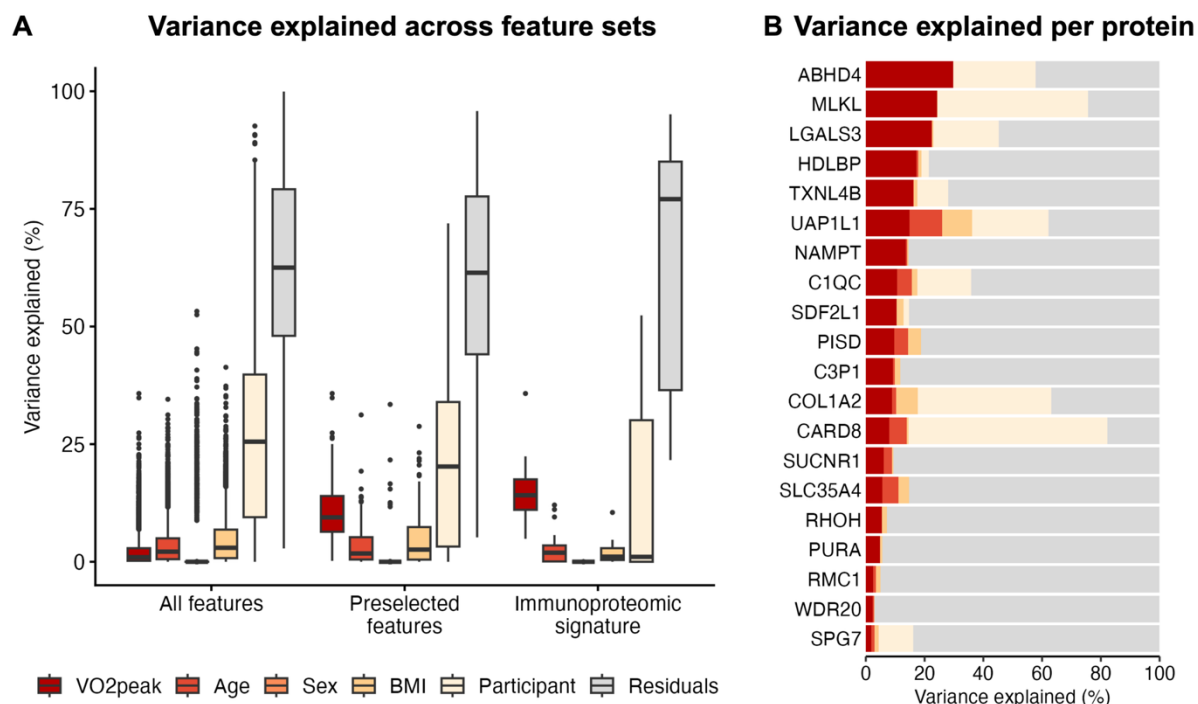


Figure S5 Full results of variance partitioning and correlation between $\dot{V}O_{2peak}$ and NAMPT protein levels obtained by untargeted proteomics
 (A) Variance explained by participant characteristics and residual variance across different feature sets (all features, preselected features, immunoproteomic signature).
 (B) Variance explained by participant characteristics and residual variance for each protein of the immunoproteomic signature.