A new method for sensitive and accurate proteome quantification in neat plasma

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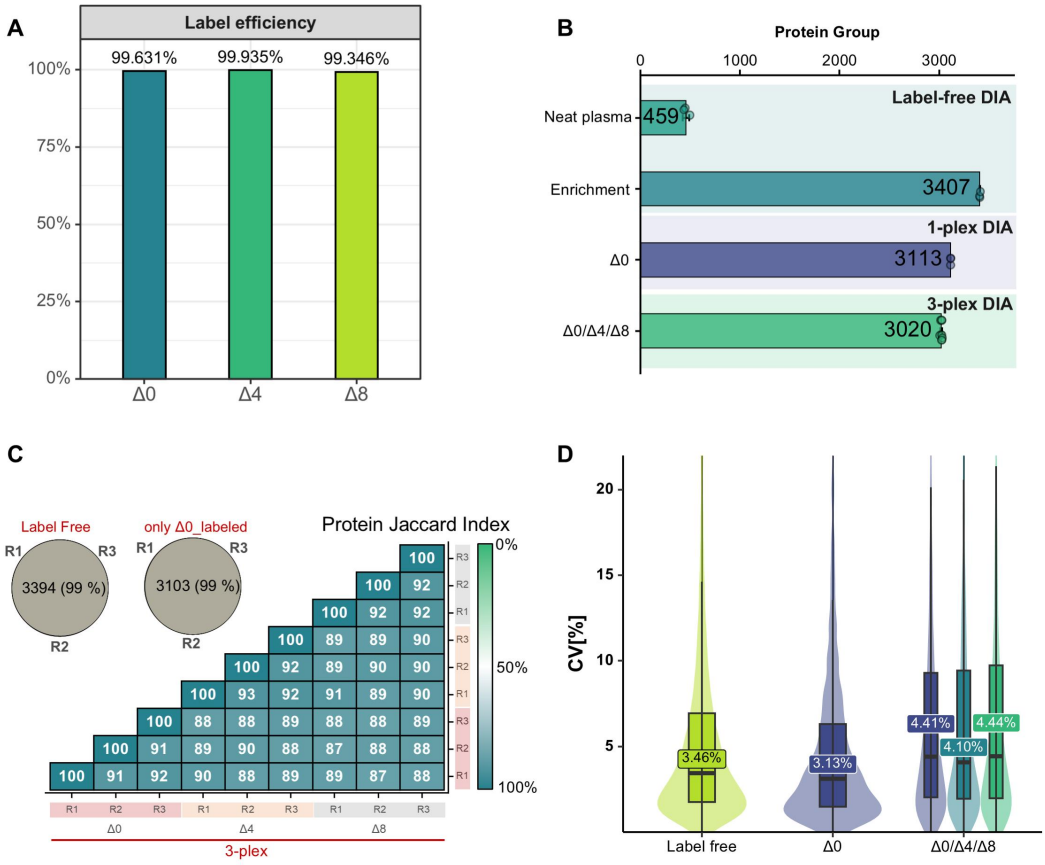

Figure S1. Impact of dimethyl labeling on plasma peptides. (A) Dimethyl labeling efficiency of plasma peptides. (B) Protein identification of the enriched plasma under different labeling combinations. (C) Pairwise Jaccard index heatmap and Venn plot showing qualitative reproducibility of proteins identified in the enriched plasma across labeling combinations. (D) Quantification precision of the enriched plasma under different labeling combinations.

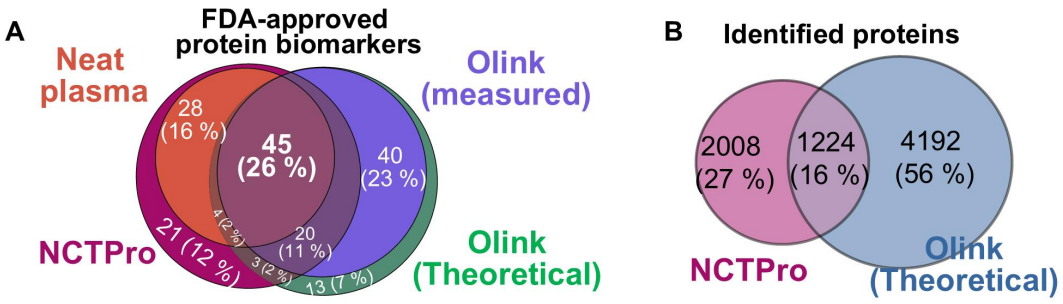

Figure S2. Overlap of FDA-approved protein biomarkers and plasma proteins identified by NCTPro and the Olink Explore HT platform. (A) Venn diagram showing the overlap of the FDA-approved protein biomarkers identified from neat plasma by NCTPro (123 biomarkers) and direct LF-DIA (79 biomarkers) with those covered by the Olink Explore HT platform under its theoretical (125 biomarkers within a 5416-protein panel) and measured

(105 biomarkers within 2631 detected proteins)[1] configurations. (B) Venn diagram showing the overlap of the total plasma proteins identified by NCTPro (3232 proteins) and those theoretically claimed by the Olink Explore HT platform (5416 proteins).

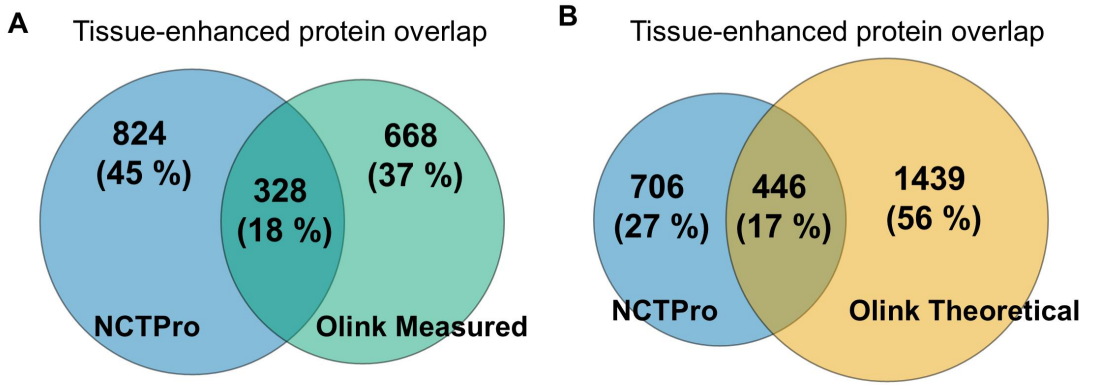

Figure S3. Overlap of tissue-enhanced proteins identified by NCTPro and covered by the Olink Explore HT platform. Tissue-enhanced proteins were annotated using the Human Protein Atlas after merging and deduplicating the male and female annotations. (A) NCTPro and measured Olink[1] shared 328 tissue-enhanced proteins, whereas 824 and 668 proteins were unique to NCTPro and measured Olink, respectively. (B) NCTPro and the theoretical Olink panel shared 446 tissue-enhanced proteins, whereas 706 and 1439 proteins were unique to NCTPro and the theoretical Olink panel, respectively.

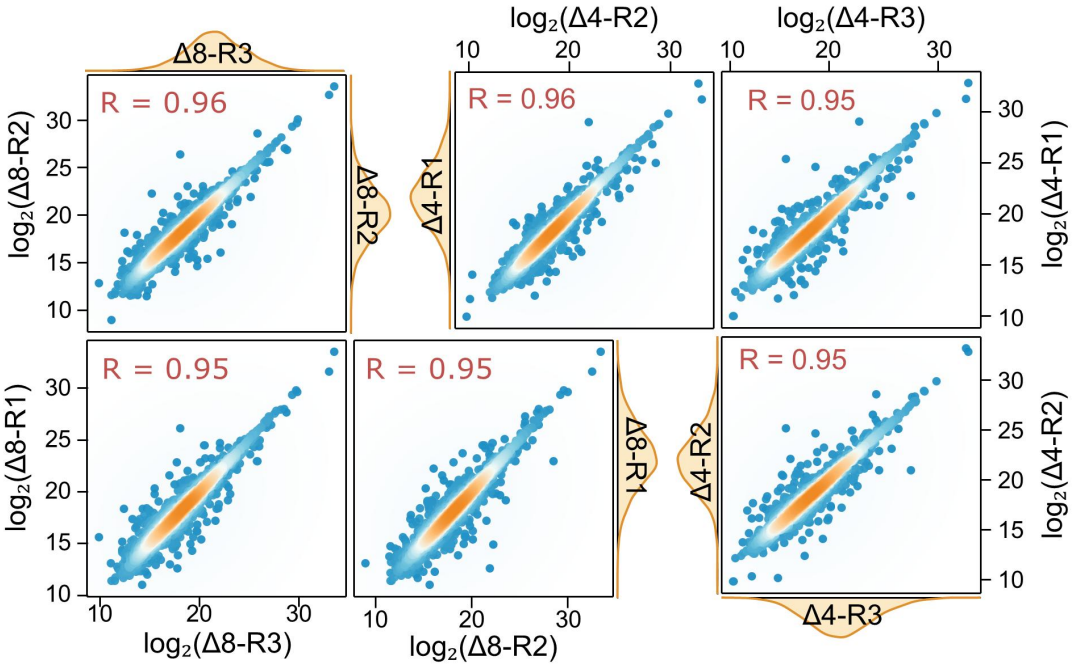

Figure S4. Cross-channel quantitative correlation analysis of NCTPro. The pearson correlation was calculated using three technical replicates of human plasma.

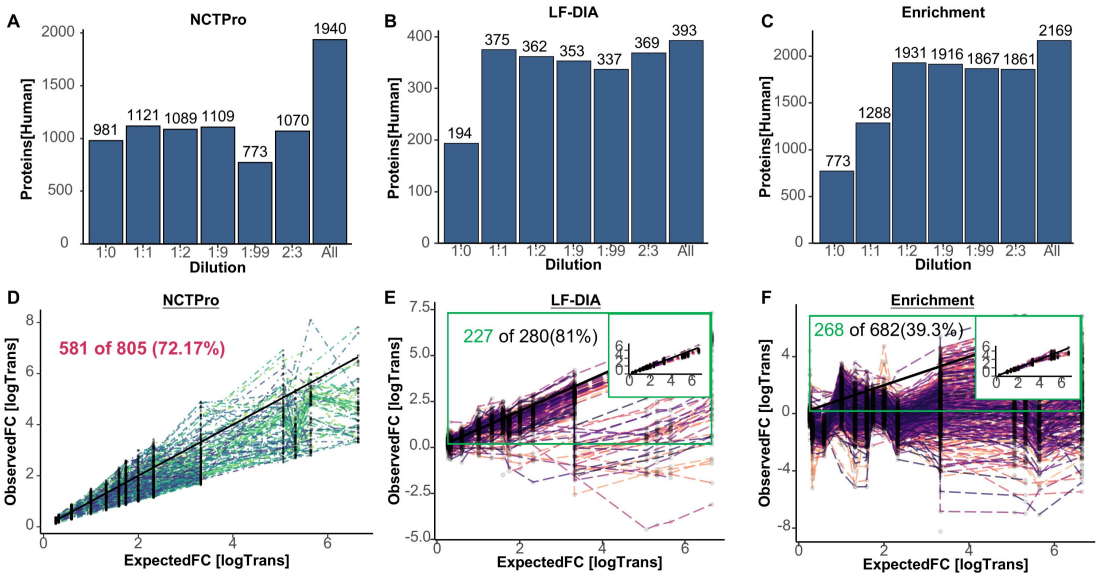

Figure S5. Quantification accuracy evaluation in spike-in experiments using NCTPro, enrichment and LF-DIA methods. (A) Schematic of the experimental design in which bovine plasma proteins were spiked into a human plasma background at defined ratios. (B) Number of proteins identified from the bovine-human plasma mixtures processed using the LF-DIA method. (C) Number of proteins identified from the same mixtures using the enrichment method. (D-F) Quantification accuracy and linearity of the serial spike-in samples for NCTPro, the nanomaterials-enrichment method, and LF-DIA. Only proteins quantified at five or more dilution points were included. Fold-change accuracy was assessed by comparing the observed and expected $\log_2$ fold changes (fold-change accuracy error, FCAE, %). The numbers indicate the proteins meeting the predefined accuracy criterion (FCAE $\leq$ 25%) relative to all evaluable proteins.

Reference

1 Beimers, W. F. *et al.* Technical Evaluation of Plasma Proteomics Technologies. *J Proteome Res* **24**, 3074–3087 (2025). https://doi.org/10.1021/acs.jproteome.5c00221