Robust and High-Throughput Analytical Flow Proteomics Analysis of Cynomolgus Monkey and Human Matrices with Zeno SWATH Data Independent Acquisition

Weiwen Sun[1,*], Yuan Lin[1,*], Yue Huang[1], Josolyn Chan[1], Sonia Terrillon[1], Anton I. Rosenbaum[1] and Kévin Contrepois[1]

[1] Integrated Bioanalysis, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, South San Francisco, CA 94080, USA.

[*] Contributed equally

Running title: Robust and fast proteomics workflows with Zeno SWATH DIA

To whom correspondence should be addressed:

Kévin Contrepois: kevin.contrepois@astrazeneca.com

T: + 1(650)-313-1886

Anton I. Rosenbaum: anton.rosenbaum@astrazeneca.com

T: +1 (650)379-3099

SUPPLEMENTARY FIGURES

Figure S1. Sensitivity and protein coverage with Zeno SWATH DIA methods.

Figure S2. Similarity in protein composition across cynomolgus monkey tissues.

Figure S3. Evaluations of quantitative robustness and signal linearity in biofluid and *in vitro* samples.

Figure S4. Variability of significant proteins (FDR < 0.1) in Zeno SWATH DIA mode that were detected but not significant in SWATH DIA mode.

SUPPLEMENTARY TABLES

Table S1. Window number and width for SWATH and Zeno SWATH DIA experiments.

Table S2. Number of protein groups and precursors across LC-MS conditions for parameters optimization.

Table S3. Gain in pathway coverage and number of protein groups per pathway across peptide loads and matrices with Zeno trap activated.

Table S4. Demographics information for healthy and diseased individuals.

Table S5. Differential proteins in plasma from patients diagnosed with NASH and T2D.

Table S6. Differential pathways in patients diagnosed with NASH and T2D.

Figure S1

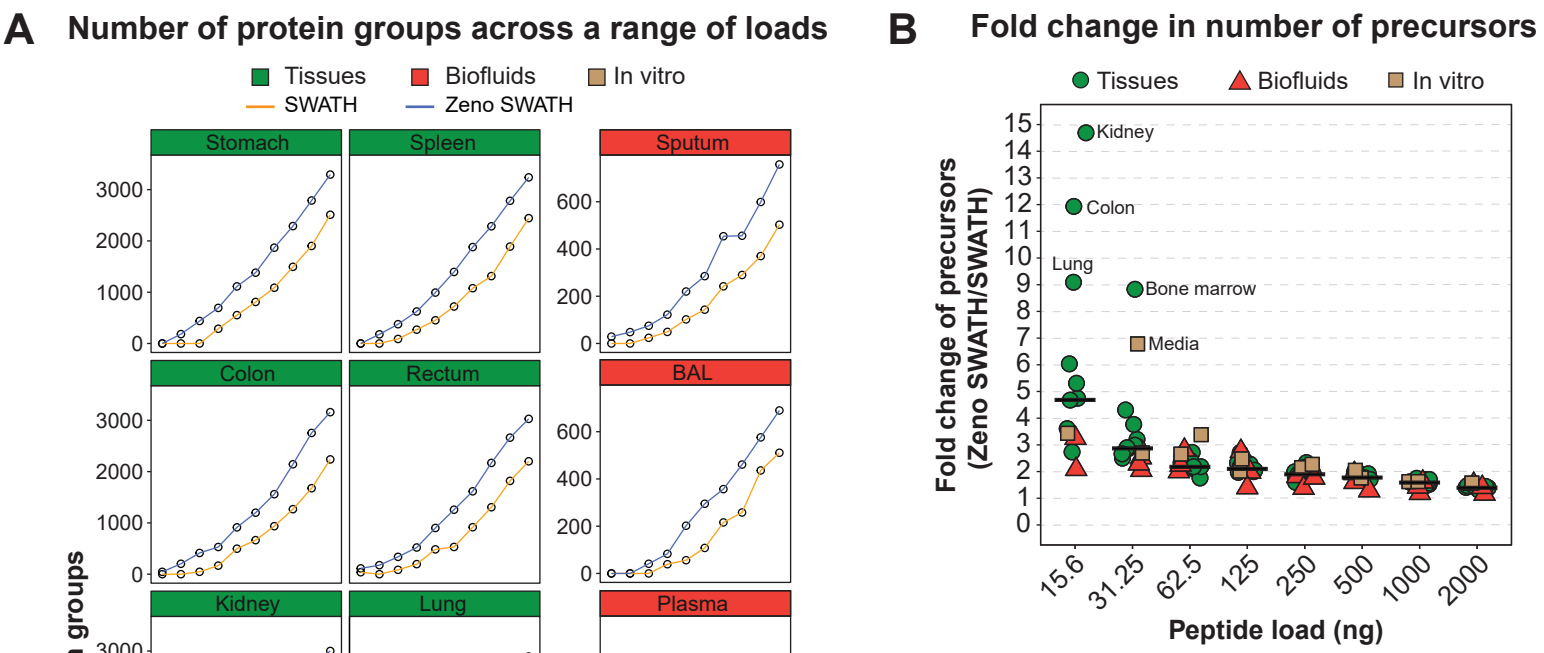

Figure S1. Sensitivity and protein coverage with Zeno SWATH DIA methods. (A) Number of protein groups identified across technical triplicates in various biological matrices at different peptide loads in SWATH and Zeno SWATH DIA modes. Peptide loads range from 3.9 ng to 2000 ng for all sample types except mucus that ranges from 3.9 ng to 1000 ng. (B) Fold change in precursors identified in Zeno SWATH DIA mode at different peptide loads. The horizontal bars represent the median.

Figure S2

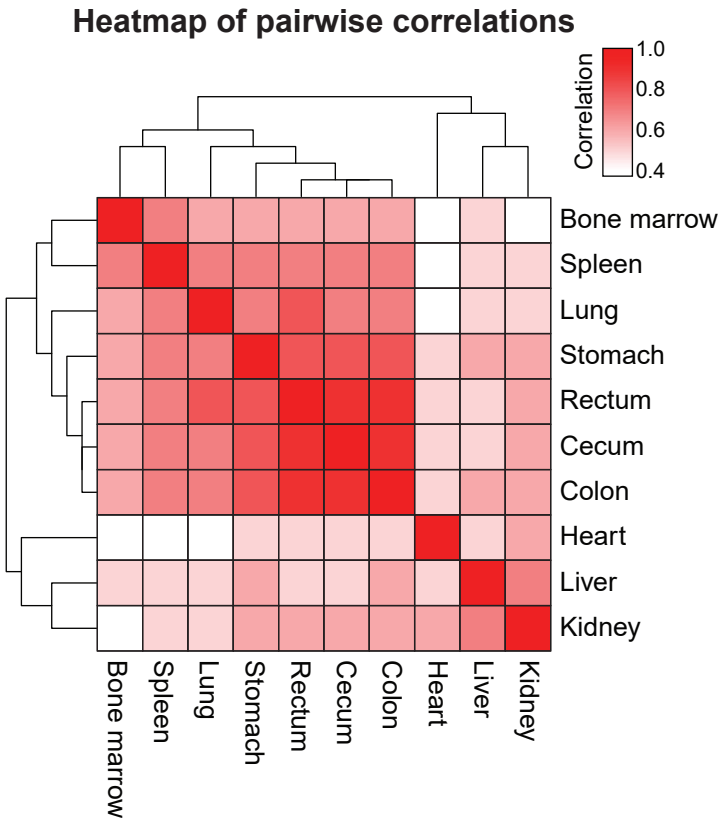

Figure S2. Similarity in protein composition across cynomolgus monkey tissues. Heatmap showing pairwise Pearson correlations between all 10 tissues. The median expression level across triplicates was used for each protein.

Figure S3

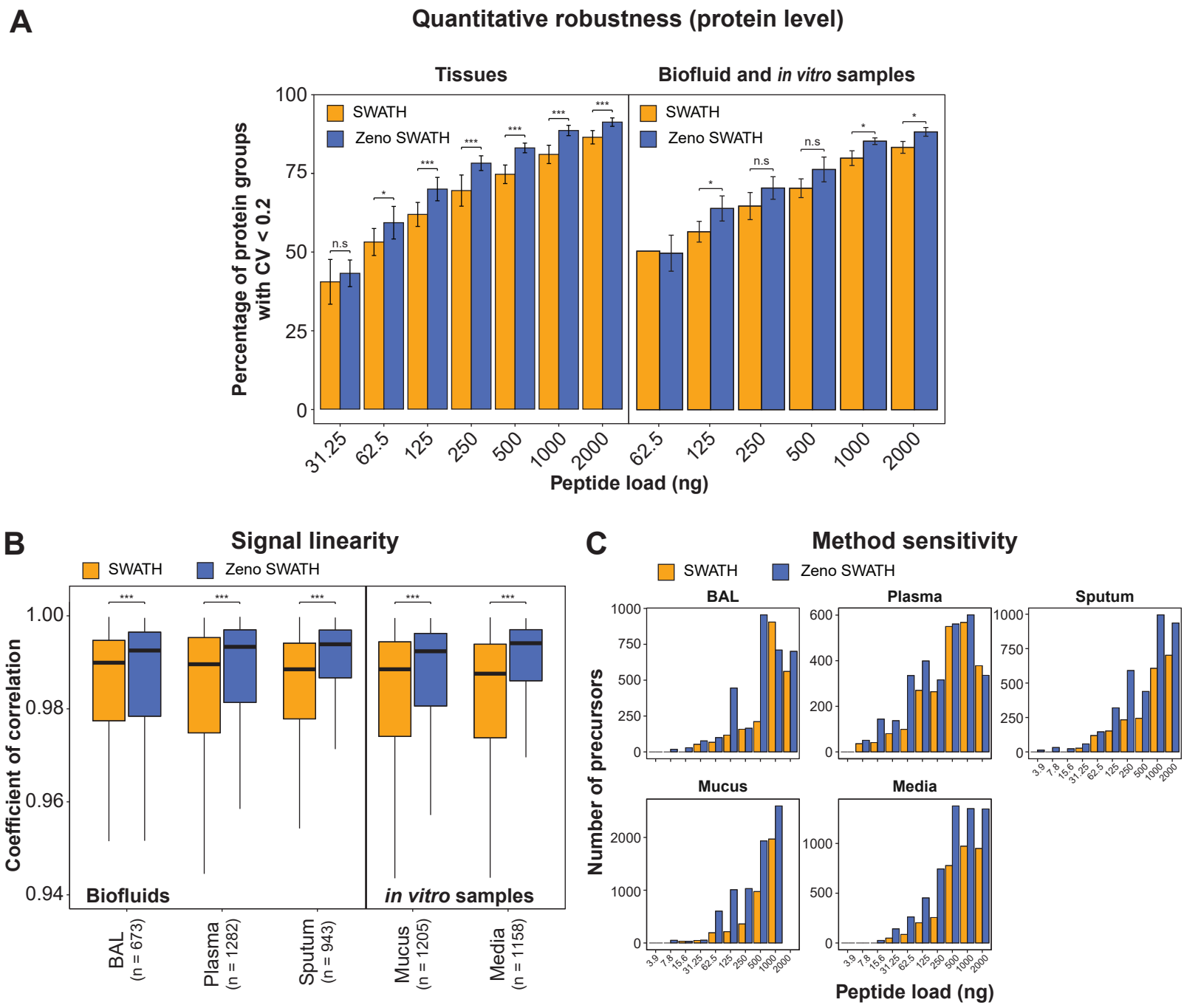

Figure S3. Evaluations of quantitative robustness and signal linearity in biofluid and in vitro samples. (A) Proportion of proteins with a coefficient of variation (CV) below 0.2 calculated across technical triplicates at different loads in SWATH and Zeno SWATH DIA modes. Only proteins detected in 100% of the triplicates were considered and only conditions with more than 50 proteins identified were plotted. Bars represent the mean across tissues or biofluid and in vitro samples and the error bars represent the standard deviation. A two-sided Welch's t-test was used for differential analysis. n.s: not significant, *: P-value < 0.05, **: P-value < 0.01, *** P-value < 0.001. (B) Boxplot showing the distribution of Pearson coefficients of correlation of precursor abundances against dilution factor for biofluids and in vitro samples. Only precursors detected in at least 3 levels in both SWATH and Zeno SWATH DIA modes were considered in the analysis. The number of precursors is indicated in parenthesis. The box illustrates the first and third quartile, the whiskers are 1.5 times the interquartile range and the horizontal bar depicts the median of the distribution. A two-sided Mann–Whitney U test was used for differential analysis. *** P-value < 0.001. (C) Ability of Zeno SWATH and SWATH methods to robustly quantify precursors across loads in individual biofluids and in vitro matrices.

Figure S4

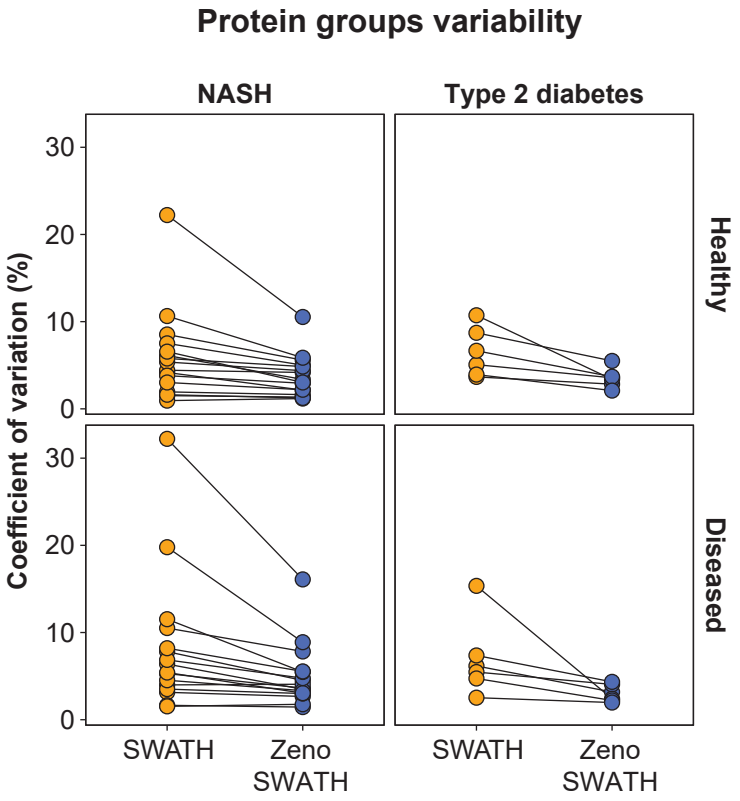

Figure S4. Variability of significant proteins (FDR < 0.1) in Zeno SWATH DIA mode that were detected but not significant in SWATH DIA mode.