Supporting Information

Automated high throughput method for fast, robust and reproducible enrichment of newly synthesized proteins

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Content of Supporting Table S1 (XLSX)

Figure 2A. Extracted dataset used to create Figure 2A: loading speed comparison 2µL/min vs 5 µL/min, no FBS. Includes Uniprot ID, protein name and gene name.

Figure 2B. Extracted dataset used to create Figure 2B: matrix complexity comparison of cell lysate vs pseudo-secretome (FBS spiked-in cell lysate), at 5 µL/min. Includes Uniprot ID, protein name and gene name.

Figure 2C. Extracted dataset used to create Figure 2C: protein IDs per elution step. Includes Uniprot IDs per replicate.

Figure 2D. Extracted dataset used to create Figure 2D: distribution of eluted proteins by step of first elution. Includes Uniprot IDs.

Figure 3B. Extracted dataset used to create Figure 3B: protein identifications present in enriched secretome of IFN gamma treated A375 cells in the presence of p-SILAC + AHA, AHA only (label free) or no label (methionine control). Includes Uniprot IDs.

Figure 3C + CV. Extracted dataset and calculations used to create Figure 3C: quantitative comparison of protein identifications present in both label free and p-SILAC treatments. Intensities are log2 transformed. Includes coefficient of variation (CV) calculations, Uniprot ID, protein name and gene name.

Figure 3D. Extracted dataset used to create Figure 3D: statistical comparison of quantitative presence of proteins shared between label free AHA secretomes and their methionine (non-AHA) controls, after IFN gamma treatment. Includes information on the shared presence of the protein IDs in a p-SILAC labeled counterpart, for cross-validation. It also includes statistical calculations from T-test, statistical significance curve plot data, and log2 transform intensity data.

Datasets for Fig 4A, 4C. Extracted dataset used to create Figures 4A and 4C: unique and shared presence of secreted proteins by treatment and labeling condition. Includes Uniprot ID, protein name and gene name.

GO F4A + IFN gamma & control. Results of the GO enrichment made in GOrilla and used in Figure 4A, IFN alpha dataset. Includes results for IFN gamma and control.

Figure 4B. Extracted dataset used to create Figures 4B: statistical comparison of quantitative presence of proteins shared between label free AHA secretomes of IFN alpha vs IFN gamma treatments. Data per replicate. Includes significance results, Uniprot ID, protein name and gene name. It also includes statistical calculations from T-test, statistical significance curve plot data, and log2 transform intensity data.

Treatment specific changes. Extracted dataset used to create Figures 4C: secreted proteins up-regulated or down-regulated after IFN treatment. Includes gene names. Data was used for STRING network analysis.