Supporting Information

Systematic assessment of the effect of internal library in targeted analysis of SWATH-MS
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Figure S1. Overview of generation of spectral library of whole proteome of murine L929 cell line.
Figure S2. Quantified proteins in L929 fixed windows SWATH-MS dataset.
Figure S3. Pearson correlation of protein or peptide intensities identified in two samples using L929 library or internal library.
Figure S4. Comparison of properties of identified peptide precursors using L929 library or internal library.
Figure S5. XICs of six product ions used for quantification for several common peptides from two libraries.
Figure S6. CVs of quantified proteins using the combined library and comparisons of identified proteins and peptides using combined library, internal library or L929 library.
Figure S7. Comparison of charges or hydrophobicity of peptides detected by L929 library or internal library.
Figure S8. The percent of detected peptides per protein with combined library compared with SSL or human library.
Figure S9. Quantitative values of mouse plasma proteins ranked according to their abundance.
Figure S10. Overlap of mouse plasma proteins detected by the internal library and mouse plasma proteins in PeptideAtlas.
Figure S11. The numbers of peptides identified by DDA or pseudo-spectra files in phosphoproteomic SWATH-MS data.
Figure S12. Pearson correlation of phosphopeptides intensities identified in two samples using DDA library.
Figure S13. Analysis of biological pathways in which TNF-induced proteins are involved in L929 cells and differential expression analysis of quantitative proteins in TNF-treated Hela cells.
Figure S14. Temporal profiles of upregulated proteins in TNFR1 IP dataset.
Figure S15. Validation of confidence of peptides and proteins in the combined library.
Table S1. Peptides and proteins in L929 dataset.
Table S2. Peptides and proteins in Hela dataset.
Table S3. Peptides and proteins in TNFR1 IP dataset.
Table S4. Peptides and proteins in plasma dataset.
Table S5. Phosphopeptides in phosphoproteomics dataset.
**Figure S1.** Overview of generation of spectral library of whole proteome of murine L929 cell line.

**Figure S2.** Quantified proteins in L929 fixed windows SWATH-MS dataset. **A.** Heatmap of quantified proteins using L929 library. **B.** Heatmap of quantified proteins using the internal library. **C.** CV of quantified proteins in three replicates using L929 library. **D.** CV of quantified proteins in three replicates using the internal library.

**Figure S3.** **A.** Pearson correlation of protein intensities identified in two samples using L929 library or internal library. **B.** Pearson correlation of peptide intensities identified in two samples using L929 library or internal library.

**Figure S4.** **A.** Comparison of identified peptide precursors using L929 library or internal library. **B.** Comparison of iRT values of the peptide precursors from two libraries. **C.** The percentage of different numbers of overlapped product ions per peptide from two libraries.

**Figure S5.** XICs of six product ions used for quantification for several common peptides from two libraries.

**Figure S6.** **A.** CV of quantified proteins in three replicates using the combined library. **B.** Comparison of identified proteins using L929, internal or combined libraries. **C.** Comparison of identified peptides using L929, internal or combined libraries.

**Figure S7.** Comparison of charges or hydrophobicity of peptides detected by L929 library or internal library. **A.** Comparison of charges of peptides detected by L929 library or internal library. **B.** Comparison of hydrophobicity of peptides detected by L929 library or internal library.

**Figure S8.** The percent of detected peptides per protein with combined library compared with SSL or human library. **A.** The percent of detected peptides per protein with combined library compared with SSL. **B.** The percent of detected peptides per protein with combined library compared with internal library.

**Figure S9.** Quantitative values of mouse plasma proteins ranked according to their abundance.

**Figure S10.** Overlap of mouse plasma proteins detected by the internal library and mouse plasma proteins in PeptideAltas.

**Figure S11.** The numbers of peptides identified by DDA or pseudo-spectra files in phosphoproteomic SWATH-MS data.

**Figure S12.** Pearson correlation of phosphopeptides intensities identified in two samples using DDA library.

**Figure S13.** **A.** Analysis of biological pathways in which upregulated proteins are involved in TNF-treated L929 cells. **B.** Differential expression analysis of quantitative proteins in Hela cells treated with TNF for 3h.

**Figure S14.** Temporal profiles of upregulated proteins in TNFR1 IP dataset.

**Figure S15.** **A.** Overlap of identified peptides by OpenSWATH using the combined library or the internal library in 100VW L929 dataset. **B.** Overlaps of proteins and peptides between the combined library and re-search library.
Supplementary Figure 1

Depth and coverage of murine L929 proteome

- **Immunoprecipitation**
  - Affinity purification
    - Peptides
    - MS analysis

- **Single run**
  - HILIC fractionation
    - Proteins
    - Peptides
    - MS analysis

- **SEC followed by HILIC fractionation**
  - Proteins
  - HILIC fractionation
    - 8 fractions
    - Peptides
    - Digestion
    - MS analysis

- **All experiments combined**
  - All data are combined
  - 10~40 HILIC fractions per SEC fraction
  - Peptides
  - Digestion
  - MS analysis

Supplementary Figure 1
Supplementary Figure 4

**A**

![Venn diagram showing the overlap of peptide precursors identified by two libraries.](image)

- L929 library: 112,406 identified peptide precursors
- Internal library: 1172 identified peptide precursors
- Overlap: 10747 identified peptide precursors

**B**

![Scatter plot showing the correlation of iRT values.](image)

- Pearson correlation: 0.99881
- R²: 0.99763

**C**

![Bar chart showing the distribution of numbers of overlapped product ions per peptide.](image)

- Percent (%) distribution:
  - 6: 0
  - 5: 10
  - 4: 30
  - 3: 20
  - 2: 10
A

% Coefficient of Variation

0 0.5 1 2 2.5 3 3.5 4

TNF(h)

B

Combined Proteins

8 716 2231 168 57

L929 Internal

C

combined Peptides

220 3996 8006 604 3861

L929 Internal

Supplementary Figure 6
Supplementary Figure 13

A

-Log10(p-value)

0 1 2 3 4 5 6

TNF signaling pathway
Malaria
African trypanosomiasis
NOD-like receptor signaling pathway
Osteoclast differentiation
Legionellosis

B

-Log10(p-value)

Log2(fold change)