Mass spectrometry experimental workflows and data tables.

We have generated the following figures and tables to aid in the review of the quantitative mass spectrometry data presented in Davis *et al.*

Workflow figures:

Workflow 1: Data acquisition scheme. Schematic of 'discovery' DDA acquisition cycle (left) and 'SWATH' DIA acquisition cycle (right).

Workflow 2: DDA data processing and database searching scheme. Software tools were largely run through the Petunia interface to the Trans Proteomic Pipeline, with some individual software components run using linux shell scripts. Minor additional parsing and processing (not shown) was performed with homemade shell and Python scripts. Additional processing information (ASKA proteins, FASTA database utilized, comet and xTandem! parameters, iRT libraries) can be found in the attached file 'database_search_supplemental_files.zip'.

Workflow 3: qMS quantitation scheme for proteins in whole cell lysates. Software tools and analysis workflow used in the generation of figures 1C,D and S1D,E.

Workflow 4: qMS quantitation scheme for pulse-labeled samples in either whole cell lysates or purified particles. Software tools and analysis workflow used in the generation of figures 2D,E and S2D,E using MS^1 quantitation and isotope envelope fitting using Massacre. Figure 2E pool size curve fitting was performed using a series of Python scripts.

Workflow 5: qMS quantitation scheme using SWATH datasets. Software tools and analysis workflow used in the generation of Figure 3, S3A.

Workflow 5b: Supplemental scheme to calculate stoichiometry relative to a 70S particle. Abundance calculated herein was then used to convert the abundance from Workflow 5, which was relative to a cell lysate, into r-protein stoichiometry. This approach was also used in Figure S3B.

Workflow 6: qMS quantitation scheme for non-ribosomal proteins using SWATH datasets. Software tools and analysis workflow used in the generation of Figure S3C.

Data tables:

The following data tables were used to generate each figure presented. Transition lists [01a, 05a, 06a, 07a] were output by Skyline. They were filtered according to the workflows above, and desired isotope abundance ratios were calculated using Python scripts and reported in tables [01b, 01c, 05b, 06b, 07b]. For figures S1D,E, additional normalization relative to the 1.25 nM sample was calculated and is reported in tables 01d, 01e. Massacre fitting of MS^1 data is reported in tables [02a, 03a, 04a]. After filtering, appropriate isotope abundance ratios were then calculated and are reported in tables [02b, 03b, 04b]. All tables are included in the attached .zip file 'tables.zip'.