

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	LC–MS data were collected using Orbitrap Astral mass spectrometer with Ultimate 3000 Liquid chromatography with Orbitrap Astral Tune Application v2.1.765.17 and Thermo Scientific Xcalibur v4.7.102.25
Data analysis	Raw MS data were analyzed using DIA-NN v2.2.0 Academia with UniProt human, yeast, and E. coli databases downloaded in December 2024 and a mouse UniProt database downloaded in March 2026. Downstream data filtering, normalization, statistical analysis, clustering, and visualization were performed using in-house Python 3.10 scripts with NumPy v1.26.4, pandas v2.2.3, Matplotlib v3.10.9, seaborn v0.13.2, SciPy v1.10.1, statsmodels v0.14.6, scikit-learn v1.6.1, tqdm v4.67.1, AnnData v0.11.4, Scanpy v1.11.5, and igraph v1.0.0. Custom analysis scripts are available on GitHub at https://github.com/RTKlab-BYU/NC_HighThroughput_LF_SCP . Input files, software requirements, and instructions are also included for reproducing the reported analyses and figures. The code is distributed under the MIT License, with no additional restrictions on access. The specific version of the code associated with this publication is archived in Zenodo and is accessible via DOI 10.5281/zenodo.22031389.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE75 partner repository with the dataset identifier PXD081782.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender n/a. Human (HeLa-CCL-2 from ATCC) and mouse (RAW264.7TIB-71 from ATCC) cell lines were used.

Reporting on race, ethnicity, or other socially relevant groupings n/a. No human subjects in this study

Population characteristics n/a. Immortalized cell lines were used

Recruitment n/a. No human subjects

Ethics oversight n/a. No animal or human studies were conducted

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No formal power analysis was performed because this study focused on technology development and analytical performance rather than hypothesis testing based on a prespecified effect size. Sample sizes for benchmarking experiments were chosen to assess technical reproducibility and consistency, with replicate numbers reported in the corresponding Methods sections and figure legends. For the large-scale single-cell experiments, approximately 1,000 HeLa cells and approximately 1,000 RAW 264.7 cells were analyzed, including approximately 200 PBS control cells and 800 LPS-treated cells. These sample sizes were selected to evaluate analytical throughput, reproducibility, and biological differences at the single-cell level. Overall platform robustness was further assessed across more than 4,000 LC-MS injections.
Data exclusions	Protein entries with > 33% missing values and outlier CV (> 1.5× IQR) were excluded before statistical testing. Exclusion criteria were pre-established.
Replication	All experiments were replicated as described in the text, ≥ 200 technical replicates for HeLa digest standard, and biological replicates of single-cell analyses (at least 200 cells per condition, the LPS treated RAW 267.7 cells have ~800 cells per condition.) Results were consistent across replicates.
Randomization	Samples were assigned to experimental groups according to the designated treatment condition (e.g., PBS control or LPS treatment), rather than by random allocation. Cells were derived from the same passage before treatment to minimize potential covariate differences. Samples from different groups were randomized across plate positions and LC-MS run order to minimize technical bias.
Blinding	Blinding was not applied because the primary outcomes, including protein identification and quantitative protein intensities, were determined computationally from the mass spectrometry data and did not involve subjective scoring or assessment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HeLa-CCL-2 and RAW264.7 TIB-71 were obtained from ATCC
Authentication	The identity of the HeLa and RAW264.7 cell line were not independently authenticated; however, cell morphology and responsiveness to LPS stimulation were consistent with reference characteristics described by ATCC.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination. The HeLa and RAW264.7 cells maintained in the laboratory showed stable morphology and growth, with no apparent signs of contamination throughout the study period.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed as commonly misidentified in the ICLAC register

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A