nature portfolio

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Last updated by author(s): Nov 28, 2025

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 (*n*) 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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 *d*, Pearson's *r*), 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	Intact glycopeptide were separated by an Easy-nLC 1200 system, followed by mass spectrometry analysis on an Orbitrap Fusion Lumos Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany). Tune application (v3.0.2041) and Xcalibur software (Thermo Fisher Scientific, v4.1.50) were used for instrument control and mass spectrometry data collection, respectively.
Data analysis	ALL LC-MS/MS data were searched against the reviewed Mus musculus reference proteome databases (UniProt,Proteome ID:UP000000589, downloaded on 18 Oct. 2023). Intact glycopeptides were identified using the StrucGP software. Briefly, the built-in glycan branch structure database (containing 17 branch structures) from StrucGP and the Mus musculus UniProt protein database were used for database search. Peptides with potential glycosites were screened using the N-X-S/T motif (where X is any amino acid except proline). Additional criteria included the presence of at least two oxonium ions among the top 10 fragment ions in MS/MS spectra for intact glycopeptide analysis. Mass tolerances were set to 10 ppm for precursor ions and 20 ppm for fragment ions. Identification results were filtered with a 1% FDR for both peptide sequences and glycan strustures, estimated by the decoy peptide and decoy spectra methods, respectively. Identified glycopeptide spectra were ranked by scores, with the top-scoring peptide/ glycan combination selected as the final identification. In addition, a probability-based evaluation was also applied to each module of the identified glycan structures.

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

All raw mass spectrometry data have been deposited in the iProX partner repositor69 under the dataset identifier IPX0011732000. The data are publicly accessible. Publicly available RNA-seq data from ArrayExpress (E-MTAB-10276) were used to assess the expression levels of glycosyltransferases across tissues.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

Life sciences study design

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

Sample size	This study analyzed 24 distinct mouse tissue types, including 16 major organs, 7 brain regions, and the serum, collectively representing all eight major anatomical systems. For each tissue, proteins were pooled from five male and five female C57BL/6N mice (8–9 weeks old), resulting in one representative sample per tissue type. The sample size was determined based on common practice in proteomics and the goal of achieving comprehensive tissue coverage rather than statistical inference across biological replicates.
Data exclusions	No sample was excluded.
Replication	Each tissue-specific pooled sample was subjected to at least three independent LC-MS/MS analyses following glycopeptide enrichment. For 22 tissues (excluding gallbladder and ovary), the HILIC-enriched glycopeptides were fractionated into six parts and analyzed by at least two LC-MS/MS runs per fraction, ensuring technical replication and data robustness. The glycoproteomic patterns observed were consistent across technical replicates, supporting the reproducibility of the findings.
Randomization	Mice were not randomized during sample collection, as the study design involved pooling equal amounts of protein from pre-defined tissues of male and female mice to generate comprehensive tissue-specific profiles. The analytical procedures, including LC-MS/MS runs, were randomized to minimize potential batch effects during instrument analysis.
Blinding	Blinding was not applicable, as the study aimed to generate an atlas of tissue-specific glycoproteomes from defined mouse tissues. Sample identity was known throughout the experimental procedures to ensure accurate data processing and annotation.

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	All samples were obtained from C57BL/6N mice (male and female), aged 8–9 weeks. Mice were maintained under specific pathogen-free (SPF) conditions with a 12-hour light/dark cycle and ad libitum access to food and water. All animal procedures were approved by the institutional animal care and use committee (IACUC) and performed in accordance with relevant ethical regulations for animal research.
Wild animals	This study did not involve wild animals.
Reporting on sex	Both male and female mice were included in this study. For each tissue, equal amounts of protein were pooled from five male and five female C57BL/6N mice to generate a representative sample, ensuring that both sexes were equally represented in the tissue-specific N-glycoproteome atlas.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	ALL procedures complied with ethical regulations and were approved by the Ethics Committee of Northwest University, China (Approval No.NWU-AWC-20241206M).

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA