nature portfolio

Corresponding author(s): Benjamin A. Garcia

Last updated by author(s): May 5, 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	Glycans were analyzed using a Vanquish Neo UHPLC System (Thermo Scientific, CA) coupled with an Orbitrap Exploris 240 mass spectrometer (Thermo Scientific, CA), and Xcalibur v4.0 was used for data collection. LI-COR image studio 5.x CLx/DLx was used for protein and RNA gel scanning. NanoDrop 2000 was used for the determination of RNA concentration.
Data analysis	For DDA results, the glycans were identified and quantified using GlycoNote and Agilent MassHunter Qualitative Analysis software (v.B08), the settings for GlycoNote were described in the previous publication and the range of monosaccharide compositions Hex 2-15; HexNAc 2-10; dHex 0-5; Neu5Ac 0-4 was used for general N-glycan analysis. For DIA results, Glycan Product Generator was used to predict the singly charged glycan fragments, ProteoWizard MSConvert (v3.0) was used for demultiplexing the spectrum, and Thermo Scientific FreeStyle (v1.8) and Skyline (v21.0) software was used for viewing MS1 and MS2 Information. GlycanDIA Finder was used to identify and quantify the glycans. For GlycanDIA Finder, we adapted parameters from GlycoNote, including mass shift range, number of decoy sets, FDR, tolerance fragment, and tolerance precursor. We chose a mass shift range of 50% because our precursor mass is 600-1800 m/z. The number of decoy sets was set as 100, which is a balance between simulation time and variability of the decoy set. Smaller tolerances for fragment and precursor ions, 20 ppm and 10 ppm, respectively, were used in our software for resolving the data acquired from high-resolution mass spectrometry instruments. An FDR of 2% was chosen as a balance between run time and controlling false-positive identifications. Mass tolerance, intensity threshold, and maximum charge range were set to 10 ppm (MS1), 20 ppm (MS2), 1e4 level, and 3 charges, respectively. The signal-to-noise (S/N) ratio above 5 was chosen for quantification (peak area), and the apex of the peak was used as the peak height.

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 supplementary information includes all data generated or analyzed during this study. The raw mass spectrometry data generated in this study have been deposited in the Mass Spectrometry Interactive Virtual Environment (MassIVE) database under accession code MSV000093677 [https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=9f5160f9b4574db9a9abde9d922fcb5d].

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	No statistical methods were used to predetermine sample sizes. For all the DIA analysis, 3 samples were used.
Data exclusions	No data were excluded.
Replication	Three biological replicates of the mouse glycoRNA samples were analyzed in DIA mode. All attempts at replication were successful.
Randomization	Not applicable. No randomization is needed in this study.
Blinding	No blinding was performed during this study. Blinding is not relevant to the study since the same criteria were used to all samples and no samples with different conditions were used.

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

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)	HCT116, HEK293T, and HeLa cells were used. All cell lines are from ATCC.
Authentication	No specific authentication of cell lines used apart from separate handling of original obtained vials throughout entire project.
Mycoplasma contamination	A representative set of growing cell lines in the lab selected randomly is subjected to mycoplasma screening bi-monthly, and within the last 3 years no infected cells have been found
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed in the ICLAC database.

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	C57BL/6 mice (24-28 weeks old)
Wild animals	n/a
Reporting on sex	All male mice in this study.
Field-collected samples	n/a
Ethics oversight	All mouse procedures and protocols were approved by the Animal Care and Use Committee of Boston Children's Hospital and followed all relevant guidelines and regulations.

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