nature portfolio

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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	All softwares for data collection are publicly or commercially available: Agilent QQQ, Lecia LSM980 ZEN microscopy software Zen3.6 (blue edition), Olympus microscope BX46, Leica Biosystems Bond vIII, Thermo Scientific Orbitrap mass spectrometer v3.3.2782.34
Data analysis	All softwares for data analysis are publicly or commercially available: Microsoft Excel v16.72, Agilent quantitative software v10.0, ImageJ v2.1.0, Cellprofiler v4.7.2, QuPath v0.4.3 and GraphPad Prism v10.0.

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 data needed to evaluate the conclusions stated in the paper are present in the paper and/or the Supplementary Materials. This paper does not report original code. Cell lines can be requested, and requests will be fulfilled by, the lead author, Monther Abu-Remaileh (monther@stanford.edu). All sequencing datasets from

the haploid screens have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1177366. Data from untargeted lipid profiling are deposited at Zenodo under accession number 14170856.

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	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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	Based on previously published work (https://pubmed.ncbi.nlm.nih.gov/36131016/), we used a minimum of size of n = 3 for quantitative experiments. For in vivo experiments, the final numbers were determined based on availability of each genotype, experimental design and animal housing conditions and we observed that sample sizes of 5 to 12 animals were adequate to obtain statistical power. For cell based experiments, each independent culture of cells was defined as biological replicate.
Data exclusions	In rare cases where we had poor detection across majority of the lipids, the whole sample was excluded.
Replication	For most of our figures, at least three independent experiments were performed and have not encountered discrepancy in results and conclusions
Randomization	For targeted mass spectrometry acquisition, samples were often randomized to prevent any systematic bias and column carry over. For pairwise comparisons, samples were processed in internally controlled pairs (i.e Wild type vs KO), while matching age and sex. For other experiments where samples were not allocated randomly, group allocation and randomization were unnecessary because all samples were measured independently in the same way in an internally controlled manner
Blinding	Sample blinding was not performed for most experiments as they did not involve formal qualitative scoring. Furthermore, all measurement modalities were quantitative and all samples in all experiments were performed in the same way regardless of group or treatment. Immunofluorescence (IF) assessments were qualitative and based on identifiable features. In at least one replicate for each IF experiment, samples were independently evaluated by one other investigator whose interpretation supported the conclusions in the manuscript.

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

Antibodies

Antibodies used

His: CST 2365, Mouse HRP: CST 7076, Rabbit HRP: CST 7074, LAMP2; SC-18822, Vinculin: SC-73614, CTSB: CST-31718, CLN5: Ab170899, GFAP: Ab7260, Calbindin: Ab229915, MBP: Ab218011, Iba1: Ab178846. All CST are from Cell Signaling Technology, SC from Santa Cruz Biotechnology) and Ab from Abcam.

Validation

All antibodies used in this study are acquired from commercial sources and were validated for specificity and species reactivity by the manufacturer. The information is readily available on the website of the manufacturer through the above-mentioned cat#. and listed below

https://www.cellsignal.com/products/primary-antibodies/his-tag-antibody/2365/
https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076
https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
https://www.scbt.com/p/lamp-2-antibody-h4b4
https://www.scbt.com/p/vinculin-antibody-7f9
https://www.cellsignal.com/products/primary-antibodies/cathepsin-b-d1c7y-xp-rabbit-mab/31718
https://www.abcam.com/en-us/products/primary-antibodies/cln5-antibody-epr12197b-ab170899
https://www.abcam.com/en-us/products/primary-antibodies/gfap-antibody-ab7260
https://www.abcam.com/en-us/products/primary-antibodies/calbindin-antibody-epr22698-236-ab229915
https://www.abcam.com/en-us/products/primary-antibodies/myelin-basic-protein-antibody-epr21188-ab218011
https://www.abcam.com/en-us/products/primary-antibodies/iba1-antibody-epr16588-ab178846

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK 293T and HeLa cells were from ATCC. Expi 293F cells (Thermo Fisher Scientific A14527) were a gift from Peter Kim at Stanford University. HAP1 cells were acquired under license with the Whitehead Institute and the Netherlands Cancer Institute.

Authentication

STR profiling and genetic analysis

Mycoplasma contamination

Test routinely and negative results obtained

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study

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

Experiments were performed in the following mouse strains: Lysotag mice, Npc1+/+ Pla2g15-/-, Npc1-/- Pla2g15+/+, Npc1-/- Pla2g15-/-, Npc1-/- Pla2g15+/-, Gba D409V and wild type mice. All mice had a average age of 2months and were maintained on a standard light-dark cycle with access to food and water. Cages were cleaned regularly and supplies of water and food were checked daily

Wild animals

None

Reporting on sex

Sex of mice are indicated in the figure legends and/or supplementary tables for each experiment.

Field-collected samples	None
Ethics oversight	All procedures involving mice were carried out in accordance with the approved guidelines by Stanford University, University of Wisconsin and Animal Care and Welfare committee in Austria

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

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable