nature portfolio

Corresponding author(s): Susan Cotman

Last updated by author(s): Sep 26, 2024

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	Proteomics data were collected on an Easy nLC 1000 or 1200 nanoHPLC (Thermo Scientific) coupled online via a Nanospray Flex Ion Source (Thermo Scientific) equipped with a PRSO-V1 column oven (Sonation) and a Q-Exactive HF mass spectrometer (Thermo Scientific). Cell culture epifluorescence image data were collected on a Zeiss Axioscope. Microglial motility data were collected on an Incucyte Live-Cell Analysis System (Sartorius). Mouse tissue image data were collected on a Zeiss Celldiscoverer 7 microscope. Electron microscopy images were collected on a LEO906E electron microscope (Zeiss) or a JEOL JEM 1011 transmission electron microscope, where images were collected using an AMT digital camera and imaging system with proprietary image capture software (Advanced Microscopy Techniques). Immunoblots were imaged using an iBright FL1500 Imaging System (ThermoFisher) or on x-ray film, which was digitally scanned using a Bio5000 Plus scanner (Bioimager).
Data analysis	Proteomics data were analyzed with Spectronaut 12.0.20491.14.21367 using a microglia spectral library generated in Sebastian Monasor et al (reference 75). The statistical data analysis was performed in Perseus version 1.6.15.0 77. The gene ontology enrichment for cellular component (GOTERM_CC_FAT) and biological process (GOTERM_BP_FAT) were carried out with the webtool DAVID (reference 79). Image analysis was performed using Fiji:ImageJ and CellProfiler, as detailed in the Methods. Immunoblot quantification was performed using the GelAnalyzer tool in ImageJ.

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 authors declare that the data supporting the findings of this study are available within the paper and the associated supplementary information files. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the name "Proteomics of CLN3-deficient murine microglia identifies a disease associated phenotype with lysosomal alterations" and with the dataset identifier PXD048550.

Human research participants

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

Reporting on sex and gender	No human subjects research was performed in this study.
Population characteristics	No human subjects research was performed in this study.
Recruitment	No human subjects research was performed in this study.
Ethics oversight	No human subjects research was performed in this study.

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	Sample sizes were determined based on prior experience for the indicated studies. In general, a minimum of 3 biological replicates and 3 technical replicates were used. Details regarding sample size for each experiment are provided within the manuscript.
Data exclusions	No data were excluded from the study.
Replication	For all cell studies, a minimum of 3 independent experiments were performed to ensure reproducibility of the data.
Randomization	All samples for a given genotype were assigned to groups randomly.
Blinding	Where applicable, image acquisition was performed blinded to genotype/treatment by assigning random numbers to the samples where the personnel performing the imaging did not have access to the sample code. Analysis was then performed using automated image analysis tools to minimize any potential bias. If analysis had to be performed manually, the personnel performing the analysis were kept blinded to the code until the analysis was complete.

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

Antibodies

Antibodies used

The following antibodies were used: anti- Iba-1 [Western blot (WB): 1:1000, Wako 016-20001], anti- Iba-1 [immunofluorescence (IF): 1:500, Wako 019-19741], anti-Tuj-1 (WB: 1:1000, BioLegend MMS-435P), anti- GFAP (WB: 1:1000, Cell Signalling 3670S), anti-mCln3p (WB: 1:1000), anti- Apoe (WB/IF: 1:250, Invitrogen 16H22L18), anti- PLP [WB: 1:1000, immunohistochemistry (IHC): 1:300, Abcam ab28486], anti- MBP (IHC: 1:1000, Sigma Aldrich SMI-99), anti- actin (WB: 1:1000, Santa Cruz Biotech sc-81178), anti- subunit c (IF: 1:300, Abcam ab181243 (ATP Synthase C [EPR13907]), anti- Lamp1 (WB: 1:1000, IF: 1:100, Santa Cruz Biotech sc-19992), anti- Lamp2a (WB: 1:1000, IF: 1:500, Abcam ab18528), anti- GM130 (IF: 1:100, BD Transduction 610822), anti- GRP175 (IF: 1:100, Stressgen SPS-825), anti- LRP1 (WB: 1:50000, Abcam ab92544), anti- Galectin 3 (WB: 1:500, Santa Cruz Biotech sc-32790), anti- PDI (IF: 1:100, StressGen SPA-891), anti- Ganglioside GM1 (IF: 1:50, Abcam ab23943). Anti- Ganglioside GM2 (IF: 1:100) and anti-Ganglioside GM3 (IF: 1:50) were generous gifts from Dr. Kostantin Dobrenis, Albert Einstein College of Medicine. Secondary antibody goat anti-rat AlexaFluor 568 (1:800, Thermo Fisher Scientific Inc), secondary antibody donkey anti- rabbit AlexaFluor 488 (1:500, Thermo Fisher Scientific Inc) or 555 (1:800, Thermo Fisher Scientific Inc), secondary antibody donkey anti- mouse Alexa Fluor 488 (1:500, Thermo Fisher Scientific Inc) or 555 (1:800, Thermo Fisher Scientific Inc).

Validation

Iba-1 antibodies: validation for indicated applications summarized on Wako website (labchem-wako.fujifilm.com); Tuj-1 antibody: validation for indicated applications summarized on Biolegend website (Biolegend.com); GFAP antibody: validation for indicated applications summarized on Cell Signalling website (cellsignal.com); mCln3p antibody: validation for indicated applications shown in reference 10; Apoe antibody: validation for indicated applications summarized on Invitrogen/Thermo Fisher website (thermofisher.com); PLP antibody: validation for indicated applications summarized on Abcam website (abcam.com) and in reference 33; MBP antibody: validation for indicated applications summarized on Sigma website (Sigmaaldrich.com); actin antibody: validation for indicated applications summarized on Santa Cruz website (scbt.com); subunit c antibody: validation for indicated applications summarized on Abcam website (abcam.com) and in references 10, 11, 37, 50; Lamp1 antibody: validation for indicated applications summarized on Santa Cruz website (scbt.com); Lamp2a antibody: validation for indicated applications summarized on Abcam website (abcam.com); GM130 antibody: validation for indicated applications summarized on BD Biosciences website (bdbiosciences.com); GRP175 antibody: validation for indicated applications summarized on Bio-Techne website (bio-techne.com) and in references 6 and 11; LRP1 antibody: validation for indicated applications summarized on Abcam website (abcam.com); Galectin 3 antibody: validation for indicated applications summarized on Santa CMichruz website (scbt.com); PDI antibody: validation for indicated applications summarized on Enzo website (enzo.com); GM1 antibody: validation for indicated applications summarized on Abcam website (abcam.com); GM2 and GM3 antibodies: validation summarized in Micsenyi et al., J Neuropathol Exp Neurol. 2009 Feb; 68(2)pp125-135. doi:10.1097/NEN.0b013e3181942cf0; Secondary antibodies: validation for indicated applications summarized on Thermo Fisher website (thermofisher.com).

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

Cln3Δex7/8 mice have been previously described and are publicly available at The Jackson Laboratory (Strain #: 017895) (reference 34). Cln3Δex7/8 mice were maintained on a C57BL/6J congenic background (>20 generations). Mice were collected at the ages indicated in the text, which ranged from 3 months to 20 months.

Wild animals

Not applicable.

Reporting on sex

Both sexes were used for the studies of roughly equal numbers. Although in humans disease progression has shown some variation based on sex, CLN3 disease equally affects both sexes and therefore both sexes were included in our samples and in our analyses. Mice used for proteomics studies included 5 females and 16 males, with sexes balanced between control and mutant groups, as indicated in samples data tab in Supplementary Table 1. An additional ~102 mice (~50% female; ~50% male) were used to generate isolated microglial samples to support the studies described in the manuscript.

Field-collected samples

Not applicable.

Ethics oversight

Studies involving use of mice underwent thorough review and approval by the Subcommittee on Research Animal Care at MGH, ensuring adherence to government regulations and guidelines for animal welfare (Protocol # 2008N000013).

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