nature research

Corresponding author(s): Noboru Mizushima

Last updated by author(s): Mar 1, 2021

# Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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<br>*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<br>*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 | Image collection: FV10-ASW (version 04.01, Olympus), Array Tomography Supporter (version 1.0.0.0, System In Frontier) |
| Data analysis | Image processing: FluoView software (FV10-ASW, version 04.02), Stacker NEO TEMography.com software (version 3.3.4.0, System In Frontier), Photoshop CC 2019 (version 20.0.6, Adobe), FIJI software (Image J, version 1.53c), GIMP (version 2.10.12)<br>Statistical analyses: GraphPad Prism (version 8.0)<br>Phylogenetic tree generation: Mega X (version 10.0.5) |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Microarray data that support the findings of this study have been deposited to the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE168029.
The animals and experimentally data that support the findings of this study are available from the corresponding authors upon reasonable request.

# 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 | The sample size was estimated from preliminary experiments or from our previous reports (Morishita et al, J Biol Chem. 2013). No statistical method was used to predetermine sample size. |
| Data exclusions | No data were excluded from any of the experimental studies presented in the manuscript. |
| Replication | All experiments in this study except for experiments in Extended Data Figs 6e, 9b, 10c, d were biologically repeated at least once independently and all attempts at replication were successful. |
| Randomization | Mice were grouped according to genotype, not randomized. Zebrafish selected for all studies were randomly chosen from siblings. |
| Blinding | Not performed as all groups were treated the same way and there was no analysis of subtle phenotypes. |

# 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 |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

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

## Antibodies

Antibodies used

For primary antibodies:
anti-complex III subunit core I (459140, Thermo Fisher Scientific)
anti-galectin 3 (ab2785, Abcam)
anti-GM130 (610822, clone 35/GM130, BD Transduction Laboratories)
anti-KDEL (ADI-SPA-827-D, Enzo)
anti-HRASLS3 (ab88447, Abcam)
anti-calnexin (2679, clone C5C9, Cell Signaling Technology, for culture cells)
anti-COX IV (4850, clone 3E11, Cell Signaling Technology)
anti-cytochrome c (ab133504, Abcam)
anti-KDEL (ab176333, Abcam)
anti-mitoHSP70/GRP75 (3593S, Cell Signaling Technology)
anti-Calnexin (ab22595, Abcam)
anti-LAMP1 (9091, clone D2D11, Cell Signaling Technology, for culture cells)
anti-calnexin (ab22595, Abcam, for tissues)
anti-PMP70 (PA1-650, Thermo Fisher Scientific)
anti-TOM20 (sc-11415, Santa Cruz Biotechnology)
anti-LAMP1 (ab25245, Abcam)

For secondary antibodies:
AlexaFluor 488-conjugated anti-mouse IgG (A32723, Thermo Fisher Scientific)
AlexaFluor 488-conjugated anti-rabbit IgG (A11008, Thermo Fisher Scientific)
AlexaFluor 568-conjugated anti-mouse IgG (A11031, Thermo Fisher Scientific)
AlexaFluor 568-conjugated anti-rabbit IgG (A11011, Thermo Fisher Scientific)
AlexaFluor 568-conjugated anti-rat IgG (A11077, Thermo Fisher Scientific)

| | |
|---|---|
| Validation | Validation statements are available from manufacturers:<br>anti-complex III subunit core I (459140, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/UQCRC1-Antibody-clone-16D10AD9AH5-Monoclonal/459140)<br>anti-galectin 3 (ab2785, Abcam, https://www.abcam.co.jp/Galectin-3-antibody-A3A12-ab2785.html)<br>anti-GM130 (610822, clone 35/GM130, BD Transduction Laboratories, https://www.bdbiosciences.com/jp/reagents/research/antibodies-buffers/cell-biology-reagents/cell-biology-antibodies/purified-mouse-anti-gm130-35gm130/p/610822)<br>anti-KDEL (ADI-SPA-827-D, Enzo, http://www.enzolifesciences.com/ADI-SPA-827/kdel-monoclonal-antibody-10c3/)<br>anti-HRASLS3 (ab88447, Abcam, https://www.abcam.co.jp/hrasls3-antibody-ab88447.html)<br>anti-calnexin (2679, clone C5C9, Cell Signaling Technology, https://www.cellsignal.jp/products/primary-antibodies/calnexin-c5c9-rabbit-mab/2679)<br>anti-COX IV (4850, clone 3E11, Cell Signaling Technology, https://www.cellsignal.com/products/primary-antibodies/cox-iv-3e11-rabbit-mab/4850)<br>anti-cytochrome c (ab133504, Abcam, https://www.abcam.co.jp/cytochrome-c-antibody-epr1327-ab133504.html)<br>anti-KDEL (ab176333, Abcam, https://www.abcam.co.jp/kdel-antibody-epr12668-ab176333.html)<br>anti-mitoHSP70/GRP75 (3593S, Cell Signaling Technology, https://www.cellsignal.com/products/primary-antibodies/grp75-d13h4-xp-rabbit-mab/3593)<br>anti-Calnexin (ab22595, Abcam, https://www.abcam.co.jp/calnexin-antibody-ab22595.html)<br>anti-LAMP1 (9091, clone D2D11, Cell Signaling Technology, for culture cells, https://www.cellsignal.jp/products/primary-antibodies/lamp1-d2d11-xp-rabbit-mab/9091)<br>anti-calnexin (ab22595, Abcam, https://www.abcam.co.jp/calnexin-antibody-er-marker-ab22595.html)<br>anti-PMP70 (PA1-650, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/PMP70-Antibody-Polyclonal/PA1-650)<br>anti-TOM20 (sc-11415, Santa Cruz Biotechnology, https://www.scbt.com/ja/p/tom20-antibody-fl-145)<br>anti-LAMP1 (ab25245, Abcam, https://www.abcam.co.jp/lamp1-antibody-1d4b-ab25245.html)<br><br>For secondary antibodies:<br>AlexaFluor 488-conjugated anti-mouse IgG (A32723, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32723)<br>AlexaFluor 488-conjugated anti-rabbit IgG (A11008, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008)<br>AlexaFluor 568-conjugated anti-mouse IgG (A11031, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11031)<br>AlexaFluor 568-conjugated anti-rabbit IgG (A11011, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11011)<br>AlexaFluor 568-conjugated anti-rat IgG (A11077, Thermo Fisher Scientific, https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11077) |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | We use HeLa, U2OS, and HEK293T cells that are authenticated by RIKEN |
| Authentication | HeLa, U2OS, and HEK293T cells were validated by STR profiling (RIKEN). |
| Mycoplasma contamination | The mammalian cell lines were confirmed to be negative for mycoplasma contamination by observation by fluorescence microscopy. |
| Commonly misidentified lines<br>(See ICLAC register) | No commonly misidentified cell lines were used. |

## Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| | |
|---|---|
| Laboratory animals | Mice (Mus musculus) were on C57BL/6J genetic background. RIKEN Wako wild-type strain (Danio rerio) were used. There was no bias between male and female mice used in our study. Wild-type or mutant neonatal mice were prepared by mating male and female mice at 2-10 months of age. For analysis of the lens of adult mice, we used mice at 2, 6, and 12 months of age. All mice were housed in a specific pathogen-free room maintained at a constant ambient temperature of 22-26 degree Celsius, 40-65% of humidity under a 12 h light/dark cycle with free access to food and drink. |
| Wild animals | This study did not involve wild animals. |
| Field-collected samples | This study did not involve field collected samples. |
| Ethics oversight | All experimental procedures and treatments were conducted in compliance with the Institutional Animal Care and Use Committee of the University of Tokyo (Medical-P17-084) and the Animal Care and Use Committee of the National Institute of Quantum and Radiological Science and Technology (1610111 and 1610121). |

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