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	Data were acquired using software supplied by instrument manufacturer LSM710, LSM780 (ZEISS) and FV3000 (EVIDENT) for immunofluorescence. ImageQuant LAS4000 (Perkin Elmer) and Fusion SOLO S system (Vilber) for immunoblotting. BD LSR Fortessa X-20 cell sorter (BD) for Flowcytometry.
Data analysis	ZEISS ZEN system (3.9), Microsoft 365, ImageJ v.1.54 (FIJI), Adobe Photoshop 2024 and illustrator 2024, GraphPad Prism10, FACSDiva.

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 supporting the findings of this study are available within the paper and its Supplementary Information. Source data are provided with this paper.

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	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 size. The sample size in different experimental groups were determined based on previous studies or preliminary studies.
Data exclusions	No raw image data was excluded.
Replication	Each experiment was repeated for two or more repeats to confirm reproducibility. All repeat experiments were successful.
Randomization	To estimate significance of data, at least random 50 cells were selected for each condition group on imaging.
Blinding	n/a, Random Images are acquired.

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

<For immunoblotting>
Anti-PMP70 Mouse monoclonal 70-18 SAB4200181 SIGMA AB_10639362 1:500
Anti-PEX14 Rabbit polyclonal (-) ABC142 Millipore (-) 1:1000

Anti-PEX16 Rabbit polyclonal (-) 14816-1-AP Proteintech AB_2162250 1:250
Anti-PEX19 Rabbit polyclonal (-) 14713-1-AP Proteintech AB_2162265 1:500
Anti-Tubulin Rat monoclonal YL1/2 ab6160 abcam AB_305328 1:3000
Anti-β-Actin Mouse monoclonal 6D1 M177-3 MBL AB_10697039 1:2000
Anti-USP30 Rabbit polyclonal (-) NBP1-81914 Novus Biologicals AB_11011210 1:500
Anti-FAF2 Rabbit Polyclonal (-) 16251-1-AP ProteinTech AB_2262469 1:1000
Anti-Ubiquitin Mouse monoclonal P4D1 sc-8017 SantaCruz AB_628423 1:1000
Anti-p97//VCP Mouse monoclonal 5 ab11433 abcam AB_298039 1:1000
Anti-HA Mouse monoclonal TANA-2 M180-3 MBL AB_10951811 1:1000
Anti-HA Rat monoclonal 3F10 12158167001 Roche AB_390915 1:1000
Anti-Flag Rabbit Polyclonal (-) PM020 MBL AB_591224 1:1000
Anti-Flag Mouse monoclonal FLA-1 M185-3L MBL AB_11123930 1:2000
Anti-p62 Rabbit Polyclonal (-) PM066 MBL AB_10896692 1:1000
Anti-OPTN Rabbit Polyclonal (-) 10837-1-AP Proteintech AB_2156665 1:2000
Anti-NBR1 Mouse monoclonal 4BR sc-130380 SantaCruz AB_2149402 1:200
Anti-NPLOC4 Rabbit Polyclonal (-) 11638-1-AP Proteintech AB_10597107 1:1000
Anti-UFD1 Rabbit Polyclonal (-) 10615-1-AP Proteintech AB_2213944 1:1000
Anti-NSFL1C/p47 Rabbit Polyclonal (-) 15620-1-AP Proteintech AB_2878158 1:1000
Anti-PEX26 Rabbit polyclonal (-) NBP1-32743 Novus Biologicals AB_2268086 1:500
Anti-HaloTag Mouse monoclonal (-) G9211 Promega AB_2688011 1:2000
Anti-TAX1BP1 Rabbito monoclonal D1D5 5105S Cell Signaling Technology AB_11178939 1:500
Anti-NDP52 Rabbito monoclonal D1E4A 60732 Cell Signaling Technology AB_2732810 1:1000
HRP-conjugated goat anti-mouse Goat polyclonal 115-035-003 Jackson ImmnoResearch Inc. AB_10015289 1:5000
HRP-conjugated goat anti-rabbit Goat polyclonal 111-035-144 Jackson ImmnoResearch Inc. AB_2307391 1:5000
HRP-conjugated donkey anti-rat Donkey polyclonal 712-035-153 Jackson ImmnoResearch Inc. AB_2340639 1:5000
<For immunocytochemistry>
Anti-Catalase Mouse monoclonal 1A1 LF-MA0003 AbFrontier AB_1611839 1:1000
Anti-PMP70 Mouse monoclonal 70-18 SAB4200181 SIGMA AB_10639362 1:200
Anti-PEX14 Rabbit polyclonal (-) ABC142 Millipore (-) 1:500
Anti-Hsp60 Goat polyclonal (-) sc1052(N-20) santa cruz AB_631683 1:250
Anti-LC3B Rabbit polyclonal (-) PM036 MBL AB_2274121 1:1000
Anti-p62 Rabbit Polyclonal (-) PM066 MBL AB_10896692 1:1000
Anti-OPTN Rabbit Polyclonal (-) 10837-1-AP Proteintech AB_2156665 1:400
Anti-NBR1 Mouse monoclonal 5C3 ab55474 abcam AB_2149404 1:100
Anti-NDP52 Rabbit Polyclonal (-) GTX115378 GeneTex AB_10620266 1:400
Anti-TAX1BP1 rabbit monoclonal D1D5 5105S Cell Signaling Technology AB_11178939 1:250
Anti-Flag Rabbit Polyclonal (-) PM020 MBL AB_591224 1:1000
Anti-HA Rat monoclonal 3F10 12158167001 Roche AB_390915 1:500
Anti-LAMP1 Mouse monoclonal H4A3 sc-20011 santa cruz AB_626853 1:200
Anti-FAF2 Rabbit Polyclonal (-) 16251-1-AP ProteinTech AB_2262469 1:500
Anti-Ubiquitin Mouse monoclonal P4D1 sc-8017 SantaCruz AB_628423 1:100
Goat anti-Rabbit IgG Alexa Fluor 488 conjugated A-11034 Invitrogen AB_2576217 1:2000
Goat anti-Rabbit IgG Alexa Fluor 568 conjugated A-11036 Invitrogen AB_10563566 1:2000
Goat anti-Rabbit IgG Alexa Fluor 647 conjugated A-21245 Invitrogen AB_2535813 1:2000
Goat anti-Mouse IgG Alexa Fluor 488 conjugated A-11029 Invitrogen AB_2534088 1:2000
Goat anti-Mouse IgG Alexa Fluor 568 conjugated A-11031 Invitrogen AB_144696 1:2000
Goat anti-Mouse IgG Alexa Fluor 647 conjugated A-21236 Invitrogen AB_2535805 1:2000
Donkey anti-Goat igG Alexa Fluor 647 conjugated A-21447 Thermo Fisher Scientific AB_2535864 1:2000

Validation

All antibodies were validated for immunofluorescence or immunoblotting. Validation reports of the commercially available antibodies were provided on the manufacturer's website; https://www.sigmaaldrich.com/JP/ja/products/protein-biology/antibodies, https://www.merckmillipore.com/JP/ja/life-science-research/antibodies-assays/antibodies-overview/l6Wb.qB.p6cAAAFOKNAqQvST,nav, https://www.ptglab.co.jp/products/primary-antibodies/, https://www.abcam.co.jp/nav/primary-antibodies, https://ruo.mbl.co.jp/bio/ins/A/, https://www.novusbio.com/, https://www.scbt.com/ja/home, https://www.cellsignal.com/, https://www.abfrontier.com/, https://www.genetex.com/, https://www.roche.com/topic/antibodies.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HeLa cell line (ATCC), HEK293T cell line (ATCC, CRL-3216), HCT116 cell line (ATCC, CVCL_0291)

Authentication

HeLa cells used in this study (Cell No. KBN0573-01) were authenticated by the Japanese Collection of Research Bioresources Cell Bank (JCRB) cell bank at the National Institute of Biomedical Innovation (Osaka, Japan) as being the same as the HeLa cell registered in ATCC.
HEK293T cell line and HCT116 cell line were purchased from ATCC and the authentication was done by ATCC.

Mycoplasma contamination

All cell lines have been tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

There was no misidentified cell line in this study.

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cells were resuspended in sorting buffer (phosphate buffer with 2.5% FBS).
Instrument	BD LSRFortessa X-20 cell sorter (BD)
Software	FACSDiva
Cell population abundance	For each sample, 10,000 mKeima-SKL positive cells were collected.
Gating strategy	FSC/SSC, PE-Texas Red/PE-Cy7, FITC/BV605, BV605/PETexas Red

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.