

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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	SerialEM 4.1beta (https://bio3d.colorado.edu/SerialEM/) for cryo-EM data (cryo-ET data were acquired on a G3i Titan Krios equipped with a Falcon 4i and Selectris-X energy filter) webUI v1.1 Thermo Fisher Scientific) for identification of lamella sites and milling Zen Blue and Zen Black (Zeiss) for Airyscan and confocal imaging Volocity 4.3 for live imaging EVOS™ M5000 (Invitrogen) for COMET assays Zeiss Axio Scan.Z1 slide scanner system (Zeiss) Olympus SLIDEVIEW VS200 slide scanner (Olympus Life Science) LI-COR software package for Western blot imaging
Data analysis	Microsoft Excel Microsoft 16.89.1 (Microsoft) and GraphPad Prism versions 8.0.1 and 10.2.0 (GraphPad Software) for statistical analysis ImageJ version 1.54 (github.com/imagej/ImageJ) and Fiji 2.14.0 (github.com/fiji/fiji) for image analysis (unless otherwise stated, see below) MATLAB version R2023b (MathWorks) for TUNEL image analysis RELION-5.0 beta (https://github.com/3dem/relion/tree/ver5.0), CTFFIND4 version 4.14, AreTomo version 1.3.3, CryoCARE, ChimeraX for cryo-EM data visualization and processing (see Methods for references and detailed information) SNAPGene viewer 8.1.1. for DNA sequence analysis NEB Tm Calculator, New England Biolabs (NEB) Imaris 10.0 for data visualization Adobe Illustrator 2023 for figure design Photoshop (2024; version 25.5.1) for figure design BioRender BioRender (Toronto, Ontario, Canada) for model design

Image Lab BioRad (Hercules, California, USA)
 cellSens 3.2 Olympus (Waltham, Massachusetts, USA)
 Microsoft 365 (version 16.89.1)

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 data that support the findings of this study are available from the corresponding author upon reasonable request.

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	Sample sizes are described in the figure legends for each dataset. Imaging experiments: at least three (often more, see Figure legends for specific datasets) independent experiments were conducted with a minimum of 15 cells imaged per condition (often more, see Figure legends). Western blot experiments: a minimum of three independent experiments (often more, see Figure legends and graphs - each spot represents one experiment) were conducted with one sample/condition per experiment. Cryo-ET experiments: 38 tomograms were collected in total, among which 7 showed buds with the narrow neck.
Data exclusions	For fluorescence imaging data displayed as box plots with individual cells shown, outliers were identified using the ROUT test, which fits the data with nonlinear regression and applies a 1% false discovery rate (Q) to pooled experimental replicates. Identified outliers (sometimes none) were excluded from subsequent analysis. No other data were excluded.
Replication	All experiments were performed at least three times, often more - see figure legends for details on each dataset (on different rounds of fibroblasts, frozen and processed separately). Similar/comparable results were obtained in each experiment.
Randomization	For each experiment and treatment, an appropriate experimental group was used. No randomization into experimental groups was performed prior to freezing for EM experiments.
Blinding	Investigators were blinded during acquisition and analysis whenever feasible, or just during the analyses (imaging data). Note that results are so transparently different between conditions that in some cases blinding was not effective - investigators could realize fast which dataset belongs to dynamin-TKO samples. That is why we performed selective imaging data analyses independently by 2-3 investigators in 2 different institutions.

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

Primary

Mouse monoclonal anti-acetylated α -Tubulin Sigma-Aldrich T7451
 Mouse monoclonal anti-dynamin (pan) BD Transduction Laboratories 610245
 Mouse monoclonal anti-HSP60 BD Transduction Laboratories 6111563
 Mouse monoclonal anti-Lamin A/C Santa Cruz Biotechnology sc-376248
 Mouse monoclonal anti-Lamin B1 Santa Cruz Biotechnology sc-374015
 Mouse monoclonal anti-PCNA Santa Cruz Biotechnology sc-56
 Mouse monoclonal anti-SQSTM1 Abcam ab56416
 Mouse monoclonal anti-vinculin Millipore MAB3574
 Rabbit monoclonal anti-alpha 1 Sodium Potassium ATPase Abcam Ab76020
 Rabbit monoclonal anti-alpha-Tubulin Cell Signaling Technology 2125
 Rabbit monoclonal anti-cleaved caspase-3 (Asp175) Cell Signaling Technology 9664
 Rabbit monoclonal anti-p21 Abcam ab188224
 Rabbit monoclonal anti-RAD51 Cell Signaling Technology 8875
 Rabbit polyclonal anti-ATM Cell Signaling Technology 2873
 Rabbit polyclonal anti-calnexin Enzo ADI-SPA-800-D
 Rabbit polyclonal anti-GAPDH Sigma-Aldrich G9545
 Rabbit polyclonal anti-LAMP1 Sigma-Aldrich L1418
 Rabbit polyclonal anti-LC3B Cell Signaling Technologies 2775
 Rabbit polyclonal anti-p53 Proteintech 10442-1-AP
 Rabbit polyclonal anti-phospho-ATM (Ser1981) Cell Signalling Technologies 5883
 Rabbit polyclonal anti-phospho-histone H2A.X (Ser139) (γ-H2AX) Cell Signalling Technologies 2577

Secondary

Goat anti-rabbit IgG, HRP-linked Antibody Cell Signaling Technology 7074
 Goat anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568 Invitrogen, Thermo Fisher Scientific A11031
 Goat anti-mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 Invitrogen Fisher Scientific, Thermo A11029
 Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568 Invitrogen Fisher Scientific, Thermo A11036
 Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 Invitrogen Fisher Scientific, Thermo A11034
 Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 Invitrogen Fisher Scientific, Thermo A21245
 Horse anti-mouse IgG, HRP-linked Antibody Cell Signaling Technology 7076

Validation

Primary antibodies used in this study antibodies were validated/used in our previous studies, received from collaborators in whose labs they were validated, or purchased from manufacturer who provided ample data on antibody validation, including references. We did not validate the secondary antibodies.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Dynamin 1,2,3 triple conditional knockout mouse fibroblasts, a kind gift from Pietro de Camilli (Yale University, New Haven, USA), were published previously e.g.
 1. Park, R. J. et al. Dynamin triple knockout cells reveal off target effects of commonly used dynamin inhibitors. J. Cell Sci. 126, 5305–5312 (2013).
 2. Fonseca, T. B., Sánchez-Guerrero, Á., Milosevic, I. & Raimundo, N. Mitochondrial fission requires DRP1 but not dynamins. Nature 570, E34–E42 (2019).
 Dynamin-TKO cells were obtained by a 4-hydroxitamoxifen (4-OHT)-induced (Sigma-Aldrich) knock-out of all three dynamin genes in cells in culture protocol as described previously (see 1,2).

	Wild-type (WT) mouse (C57BL/6J; SCRC-1008) and human fibroblasts were obtained from ATCC.
Authentication	Each round of dynamin TKO cell production was validated by Western blots, as described in Methods and shown in Extended Data Figure 1.
Mycoplasma contamination	All cell lines were routinely tested for mycoplasma contamination, and found to be negative.
Commonly misidentified lines (See ICLAC register)	N/A

Plants

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