

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <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>
☒	☐ 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 <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	Wide-field images were acquired using a Zeiss LSM900 confocal microscope and subsequently deconvolved through python (V 3.11.0). For super-resolution imaging, structured illumination microscopy (SIM) was performed using either the Multi-SIM X system (NanoInsights-Tech) or the Polar SIM system (Airy Technology). Live-cell experiments were conducted in 8-well imaging chambers (CellVis) maintained at 37°C with a 5% CO2 atmosphere. Proteomic profiling was performed on an EASY-nLC 1200 system coupled to an Orbitrap Eclipse mass spectrometer (Thermo Scientific). Flow cytometric sorting was conducted using a BD FACSAria III system with a 100 µm nozzle. Cryo-CLEM was performed with an ELI-TriScope system and imaged at -180°C using a prototype triple-beam cryo-transmission electron microscope (Institute of Biophysics, Chinese Academy of Sciences). Quantitative PCR (qPCR) was performed on a LightCycler 96 (Roche), and Western Blot membranes were developed using ECL (GE Healthcare).
Data analysis	Fixed and live-cell images were processed in Fiji (V1.54s) and ZEN 3.0 (Blue edition). Three-dimensional reconstructions of fluorescence images were performed in Imaris Bitplane (V10.0, Oxford Instruments) and Amira (V2024.1, Thermo). Default settings were used for both. Quantifications of the number of SIMPAs were conducted on maximum intensity projections of images acquired using ImageJ (Fiji, V1.54s). Recruitment of different markers to SIMPAs was quantified manually using maximum intensity projections of SIM super-resolution images in Fiji (V1.54s). For cryo-CLEM, grids were subjected to cryo-fluorescence mapping (ELI-TriScope) to localize SIMPA-positive cells. Seahorse Metabolic Assays were normalized to cell count and analyzed using Seahorse Wave Software (V2.6.1). Statistical significance between two groups was assessed using a two-tailed Student's t-test (for data passing normality test) or the Mann-Whitney U test (for non-parametric distributions), as appropriate. The specific test used is indicated in the corresponding figure legend. All charts in this study were generated using GraphPad Prism (V9.0) and R (V4.2.2). Western blot data was quantified using Fiji (V1.54s).

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 provided in Article and its Supplementary Information. Full version of all gels and blots are provided in SI Figure 1. Raw data of all images presented in this manuscript, representative tomograms and proteomic datasets acquired in this study have been deposited in Figshare under accession numbers: 10.6084/m9.figshare.32050848, 10.6084/m9.figshare.32050956 and 10.6084/m9.figshare.32050986, respectively.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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](https://www.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 not predetermined by formal power analysis but were chosen based on standard practices in the field. For cell based experiments, at least three biologically independent replicates were performed, with each replicate including cell counts as indicated in the figure legends (e.g., n=30 cells per condition across three experiments). For cellular proportion analyses (e.g., percentage of SIMPA positive cells), each data point was derived from counting 100 cells per experimental condition per replicate. For animal studies, group sizes (n=5–6 mice per group) were selected based on pilot experiments to detect expected differences with adequate statistical power, consistent with previous publications in liver injury models. For high throughput data (proteomics), three independent biological samples were analyzed. The article has cited relevant references.
Data exclusions	No data were excluded from the analysis.
Replication	All experiments, including Western blot, immunofluorescence (IF), light microscopy, and electron microscopy, were conducted with at least three independent replicates, as detailed in the figure legends. All replication attempts were successful.
Randomization	For cell based experiments, cells from the same passage were seeded into multi well plates or culture dishes. Prior to any treatment, wells or dishes were randomly assigned to experimental groups. For siRNA or plasmid transfections, cells were plated in parallel, and each well was randomly allocated to receive either control or knockdown/overexpression constructs. For MitoTracker staining and recovery experiments, all wells were stained simultaneously, then randomly assigned to different recovery time points. For imaging based quantification, fields of view were selected randomly across each well or coverslip to avoid bias. The same software with identical settings was used for quantifications across conditions. The experimental setup included clearly defined conditions. For animal experiments, all mice were randomly assigned to the various experimental groups. Allocation concealment was maintained during treatment application.
Blinding	Data measurements for FACS or Confocal were blinded to the different persons who processed the assays at the time. Proteomic Profiling was conducted in a blinded manner. For other experiments (qPCR, Seahorse, animal histology, serum assays), blinding was implemented where

possible by sample coding and randomization. Blinding was not possible for treatment administrators due to visible differences, but outcome assessors remained blinded. For live imaging where blinding was not feasible (fluorescence directly reveals condition), automated quantification was used to reduce bias. For western blotting, blinding was not performed because the same investigator ran the gels and analyzed the bands; however, all images were processed using consistent threshold settings and were verified by a second investigator.

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 Western Blotting: β -actin (proteintech, 81115-1-RR, 23039755), GAPDH(CST, 2118, 16), HSP60 (CST,12165, 4), CYP2E1(ABclonal, A23030, ARC58001)diluted 1:2000; GRP75 (SantaCruz, sc-133137, K2524), GRP78(CST, 3177, 11), VDAC1 (abcam, ab14734, GR3296736-22), ITPR3 (abcam, ab264283, GR3363015), MFN1 (Abnova, H00055669-m04, N2131-3C9), MFN2 (Abnova, H00009927-m03, BS21082061) diluted 1:1500; DRP1 (CST, 8570, 6), LONP1(CST, 28020, 1), FIS1 (OriGene, TA332938, 3561157003), OPA1 (proteintech, 27733-1-AP, 00072448), COX2 (CST, 12282, 7), COX3 (ThermoFisher, PA5-103392, 30FFEA31), CYPD (ThermoFisher, MA5-35640, 80307620), VCP (CST, 2648, 1) diluted 1:1000; P62 (MBL, m162-3, 018), LAMP1 (CST, 9091, 8), LC3 (MBL, pm036, 034), IRE1 α (CST, 3294, 12), eIF2 α (CST, 5324, 15), ATF4 (GeneTex, GTX641410, 42788), CLPP(CST, 14181, 1), ATF5(ABclonal, A3563, ARC2043), PERK (proteintech, 20582-1-AP, 00126845), ATF6 (CST, 65880S, 6), UFD1 (CST, 13789S, 11), NPLOC4 (ThermoFisher, PA5-54749, R09692) diluted 1:750; p-IRE1 α (Sigma-Aldrich, ZRB1072, Q3604808), p-eIF2 α (CST, 3398, 8), p-PERK (proteintech, 82534-1-RR, 23007454) diluted 1:500

For Immunofluorescence: TOM20 (1:500; Proteintech, 66777-1-Ig, 10041813), HSP60 (1:500; CST, 12165, 4), GRP75 (1:200; Santa Cruz, sc-133137, K2524) (1:200;CST, 3593, 2), VDAC1 (1:200; abcam, ab14734, GR3296736-22), IP3R(1:200; abcam, ab5804, GR3363015-1), dsDNA (1:200; Abcam, ab27156, GR3337290-1), HSPA8 (1:200; Sigma-Aldrich, MABE1120, 620899), HSPA1L (1:200; ThermoFisher, PA5-106899, ZK4555360), ATP6 (1:200; proteintech, 55313-1-AP, 00059946), p-IRE1 α (1:100; Sigma-Aldrich, ZRB1072, Q3604808), p-eIF2 α (1:100; CST, 3398, 8)

Validation

All the antibodies purchased have been validated in multiple previous studies accessible at the manufacturer's Website. Each antibody has the corresponding species (human and mouse) and function (western blot and IF) capabilities required for the experiment. Relevant certification information is available on the official websites of the antibodies used in this study. Detailed antibody website links are provided as follows.

β -actin (proteintech, 81115-1-RR, 23039755), <https://www.ptgcn.com/products/beta-actin-Antibody-81115-1-RR.htm>
HSP60 (CST,12165, 4), <https://www.cellsignal.cn/products/primary-antibodies/hsp60-d6f1-xp-rabbit-mab/12165>
CYP2E1(ABclonal, A23030, ARC58001), <https://abclonal.com.cn/catalog/A23030>
GRP75 (SantaCruz, sc-133137, K2524), <https://www.scbt.com/zh/p/grp-75-antibody-d-9;> (CST, 3593, 2), <https://www.cellsignal.cn/products/primary-antibodies/grp75-d13h4-xp-rabbit-mab/3593>
VDAC1 (abcam, ab14734, GR3296736-22), <https://www.abcam.cn/products/primary-antibodies/vdac1porin--vdac3-antibody-20b12af2-ab14734.html>
GRP78(CST, 3177, 11), <https://www.cellsignal.cn/products/primary-antibodies/bip-c50b12-rabbit-monoclonal-antibody/3177>
GAPDH(CST, 2118, 16), <https://www.cellsignal.cn/products/primary-antibodies/gapdh-14c10-rabbit-monoclonal-antibody/2118>
LONP1(CST, 28020, 1), <https://www.cellsignal.cn/products/primary-antibodies/prss15-lonp1-d8w1j-rabbit-monoclonal-antibody/28020>
ATF5(ABclonal, A3563, ARC2043), <https://abclonal.com.cn/catalog/A3563>
ITPR3 (abcam, ab264283, GR3363015), <https://www.abcam.cn/products/primary-antibodies/itpr3-antibody-ab264283.html>
IP3R(abcam, ab5804, GR3363015-1), <https://www.abcam.cn/products/primary-antibodies/ip3-receptor-antibody-ab5804>
MFN1(Abnova, H00055669-m04, N2131-3C9), <https://www.abnova.com/en-global/product/detail/H00055669-M04>
CLPP(CST, 14181, 1), <https://www.cellsignal.cn/products/primary-antibodies/clpp-antibody/14181>
MFN2(Abnova, H00009927-m03, BS21082061), <https://www.abnova.com/en-global/product/detail/H00009927-M03>
DRP1 (CST, 8570, 6), <https://www.cellsignal.cn/products/primary-antibodies/drp1-d6c7-rabbit-mab/8570>
FIS1 (OriGene, TA332938, 3561157003), <https://www.origene.cn/catalog/antibodies/primary-antibodies/ta369131/ttc11-fis1-rabbit-polyclonal-antibody>
OPA1 (27733-1-AP, proteintech, 00072448), <https://www.ptgcn.com/Products/OPA1-Antibody-27733-1-AP.htm>
COX2 (CST, 12282, 7), <https://www.cellsignal.com/products/primary-antibodies/cox2-d5h5-rabbit-monoclonal-antibody/12282>
COX3 (ThermoFisher, PA5-103392, 30FFEA31), <https://www.thermofisher.cn/cn/zh/antibody/product/COX3-Antibody-Polyclonal/PA5-103392>
CYPD (ThermoFisher, MA5-35640, 80307620), <https://www.thermofisher.cn/cn/zh/antibody/product/Cyclophilin-40-Antibody-clone-407P5-Recombinant-Monoclonal/MA5-35640>

VCP (CST, 2648S, 1), <https://www.cellsignal.com/products/primary-antibodies/vcp-antibody/2648>
P62 (MBL, m162-3, 018), <https://www.mblbio.com/bio/g/dtl/A/index.html?pcd=M162-3>
LAMP1 (CST, 9091, 8), <https://www.cellsignal.cn/products/primary-antibodies/lamp1-d2d11-xp-rabbit-mab/9091>
LC3 (MBL, pm036, 034), <https://www.mblbio.com/bio/g/dtl/A/?pcd=PM036>
IRE1α (CST, 3294, 12), <https://www.cellsignal.cn/products/primary-antibodies/ire1a-14c10-rabbit-mab/3294>
eIF2α (CST, 5324, 15), <https://www.cellsignal.cn/products/primary-antibodies/eif2a-d7d3-xp-rabbit-mab/5324>
ATF4 (GeneTex, GTX641410, 42788), <https://www.genetex.cn/Product/Detail/ATF4-antibody-HL3513/GTX641410>
PERK (proteintech, 20582-1-AP, 00126845), <https://www.ptgcn.com/products/PERK-Antibody-20582-1-AP.htm>
ATF6 (CST, 65880S, 6), <https://www.cellsignal.com/products/primary-antibodies/atf-6-d4z8v-rabbit-monoclonal-antibody/65880>
UFD1 (CST, 13789S, 11), <https://www.cellsignal.cn/products/primary-antibodies/ufd1-antibody/13789>
NPLOC4 (ThermoFisher, PA5-54749, R09692), <https://www.thermofisher.cn/antibody/product/NPLOC4-Antibody-Polyclonal/PA5-54749>
p-IRE1α (Sigma-Aldrich, ZRB1072, Q3604808), <https://www.sigmaaldrich.cn/CN/zh/product/sigma/zrb1072>
p-eIF2α (CST, 3398, 8), <https://www.cellsignal.cn/products/primary-antibodies/phospho-eif2a-ser51-d9g8-xp-rabbit-mab/3398>
p-PERK (proteintech, 82534-1-RR, 23007454), <https://www.ptgcn.com/products/Phospho-PERK-EIF2AK3-Thr982-Antibody-82534-1-RR.htm>
TOM20 (Proteintech, 66777-1-Ig, 10041813), <https://www.ptgcn.com/products/TOM20-Antibody-66777-1-Ig.htm>
dsDNA (Abcam, ab27156, GR3337290-1), <https://www.abcam.cn/products/primary-antibodies/ds-dna-antibody-35i9-dna-bsa-and-azide-free-ab27156.html>
HSPA8 (Sigma-Aldrich, MABE1120, 620899), <https://www.sigmaaldrich.cn/CN/zh/product/mm/mabe1120>
HSPA1L (ThermoFisher, PA5-106899, ZK4555360), <https://www.thermofisher.cn/antibody/product/HSP701L-Antibody-Polyclonal/PA5-106899>
ATP6 (proteintech, 55313-1-AP, 00059946), <https://www.ptgcn.com/products/ATP6-Antibody-55313-1-AP.htm>

Eukaryotic cell lines

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

Cell line source(s)	U-2OS cells (ECACC 92022711), Cos-7 (ECACC 87021302), and HeLa cells (ECACC 08011102) were obtained from the ECACC. Split-GFP(MAM) U-2OS cells were provided by the Chao Tong laboratory from College of Life Sciences of Zhejiang University. The split-MAM-GFP U-2OS cell line was generated in house and was not commercially authenticated. However, the parental U-2OS cells were obtained from ECACC (92022711).
Authentication	The cell lines utilized in this study exhibit diverse morphologies and growth characteristics, and all have been passaged fewer than 20 times.
Mycoplasma contamination	All cells were routinely tested for mycoplasma contamination using a MycoAlert Kit (Lonza). Cultures found to be contaminated were immediately discarded.
Commonly misidentified lines (See ICLAC register)	No ICLAC cells 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	C57BL/6J male mice (4–8 weeks old, SPF-grade; Charles River Laboratories) were housed under specific pathogen-free conditions at a controlled temperature (20–26 °C) and humidity (40–70%) with a 12 h light/dark cycle and provided γ-irradiated feed and autoclaved water ad libitum. All animal experiments were approved by the Science and Technology Ethics Committee of the University of Health and Rehabilitation (KFDX: NO.2025-1022).
Wild animals	No wild animals were involved in this study.
Reporting on sex	The sex of the mice did not influence the experimental results.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All animal experiments were approved by the Science and Technology Ethics Committee of the University of Health and Rehabilitation (KFDX: NO.2025-1022).

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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	Cell pellets were resuspended in an isotonic buffer, homogenized using a Dounce homogenizer (Wheaton, pestle B), and the crude mitochondrial fraction was obtained through differential gradient centrifugation. Detailed steps can be found in the Methods section.
Instrument	Flow cytometric sorting was performed using a BD FACSARIA III system equipped with a 100 µm nozzle.
Software	FlowJo was used to analyze the data.
Cell population abundance	A minimum threshold of 1×10^7 sorted particles per group was collected.
Gating strategy	The FSC/SSC parameters initially excluded debris by selecting particles ranging from 0.5-2 µm, followed by fluorescence thresholding to isolate dual-positive FITC/PE SIMPA populations from FITC single-positive controls.

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