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	LAX S software version 1.0.0.12269; Zen Blue software version 2.1
Data analysis	ImageJ/FjJi software version 1.52a; Photoshop CC version 20.0.0 (Adobe); GraphPad Prism software version 8.0.2

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

Mass spectrometry data that support the findings of this study have been deposited in iProX with the accession code PXD070400, https://www.iprox.cn/page/SSV024.html;url=1762480420360UD6V. The data generated in this study are provided in the Supplementary Information and Source Data file. 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 pre-determine sample sizes, but our samples sizes are comparable to similar studies (PMID: 17653196; PMID: 23263443).
Data exclusions	No data were excluded from the analyses.
Replication	Yes, the experiments were replicated for 3 times independently and all attempts at replication were successful.
Randomization	Randomization was used to assign all experimental groups without bias based on any covariates.
Blinding	All experiments were conducted using a double-blind approach for data collection and statistical analysis.

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	Anti-CFL1 (66057-1-Ig, 1:1000) and anti-CFL1 (10960-1-AP, 1:1000) were purchased from Proteintech. Anti-SUMO1 (4940, 1:1000), anti-SAE1 (13585, 1:1000), anti-SAE2 (8688, 1:1000), anti-SENP3 (5591, 1:1000), anti-phospho-CFL1 (Ser3) (77G2, 1:1000), anti-pan-actin (4968, 1:1000), anti-HSP60 (12165, 1:1000), anti-Cyt c (4280, 1:1000), anti-Casp3 (9662, 1:1000), anti-C-Casp3 (9664, 1:1000),

anti-C-Casp9 (7237, 1:1000), anti-Bax (2772, 1:1000) and anti-GAPDH (2118, 1:5000) were purchased from Cell Signaling Technology. Anti-HA (H6908, 1:3000) and anti-SUMO2/3 (SAB3500488, 1:1000) was purchased from Sigma-Aldrich. Anti-Ubc9 (ab33044, 1:1000), anti-SENP1 (ab108981, 1:1000) and anti-COX IV (ab33985, 1:1000) were purchased from Abcam. Anti-Tom20 (sc-36211, 1:200), anti-Tom70 (sc-390545, 1:200), anti-Tim23 (sc-514463, 1:200) and anti-HSC70 (sc-7298, 1:200) were purchased from Santa Cruz. Anti-HSP70 (T55496, 1:1000), anti-HSP90 (M20032, 1:1000) and anti-CYC1 (PH1515, 1:1000) were purchased from Abmart (Shanghai, China).

Validation

Anti-HA, anti-SUMO1, anti-Ubc9 and anti-SENP1 have been extensively validated in previous studies (PMID: 25236484; PMID: 29670121; PMID: 32232156; PMID: 30813468; PMID: 15194470; PMID: 34759262).
Anti-SAE1 and anti-SAE2 have been extensively validated in previous studies (PMID: 32232156; PMID: 27348078; PMID: 33073888).
Anti-GAPDH has been extensively validated in previous studies (PMID: 37479684; PMID: 37468549; PMID: 37463911; PMID: 37291385).
Validation data for anti-CFL1 antibodies are available on vendor websites (https://www.ptglab.com/products/CFL1-Antibody-66057-1-Ig.htm and https://www.ptglab.com/products/CFL1-Antibody-10960-1-AP.htm)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

CHO-K1 cells (GNHa 7), HEK 293T cells (GNHu17), HeLa cells (TCHu187) were purchased from Cell Bank/Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). MEF cells were provided by J. Cheng (Shanghai Jiao Tong University School of Medicine, Shanghai, China).

Authentication

The CHO-K1 cells were authenticated by morphology. The MEF cells were authenticated by morphology. The HEK-293T cells were authenticated by STR profiling. The HeLa cells were authenticated by STR profiling. The DF-1 cells were authenticated by STR profiling.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines 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

The mouse strains used in this study were generated and maintained on the C57BL/6 background. The research included Senp1+/+ and Senp1−/− mice which were prepared as previously described in Nature Communications 5:4980,2014. CFL1 K34R (KI) mice were generated by CRISPR/Cas9-mediated genome editing (Gempharmatech Co., Ltd., Nanjing, China). Animal care and experimental protocol were approved by the Animal Ethics Committee of Shanghai Jiao Tong University School of Medicine, Shanghai, China. Embryos were removed from anaesthetized Senp1 and KI heterozygous mice at embryonic day 13.5 (E13.5) for WB assay.

Wild animals

The study did not involve wild animals.

Reporting on sex

No sex analysis was performed.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All experimental protocols with animals were carried out in accordance to the guidelines for the Care and Use of Laboratory Animals of Shanghai Jiao Tong University School of Medicine and approved by the Institutional Animal Care and Use Committee (IACUC) under Approval No. A-2022-040.

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

Plants

Seed stocks

None.

Novel plant genotypes

None.

Authentication

None.