nature portfolio

Corresponding author(s): Xing Liu

Last updated by author(s): Jan 17, 2025

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	Bio-Kine32 V4 was used for stopped-flow data collection. Image Studio Ver 5.2 was used for western blot data collection. Smart EPU 3 Software was used for cryo-EM micrographs collection.
Data analysis	GraphPad Prism 9 was used for statistical tests and regression. Image Studio Lite Ver 5.2 was used to quantify Western Blot bands. cryoSPARC version 3.2.0 was used for cryo-EM data processing. PyMOL 2.4.0. and ChimeraX version 1.5 was used for structural analyses. Proteome Discoverer 2.5 was used for proteomics analyses.

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 article and its Supplementary Information files.

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 were chosen based on standards in the field and records in prior studies: PMID: 23453757; PMID: 29499133; PMID: 29103612.
Data exclusions	Low-quality data in the global protein turnover experiment were excluded using quality screen procedures described in the method. No other data were excluded.
Replication	Number of replications for each experiment was noted in the figure legends.
Randomization	Not relevant. Our experiments involved examining the effect of a known factor on a defined biochemical or cellular process.
Blinding	Blinding was not applied. There were no prior assumptions for experimental outcomes. Samples were collected and processed uniformly regardless of treatment groups. Most of the data analyses were performed by computational softwares and were not affected by knowledge of the sample condition. Investigators were not blinded because investigators carried out the experiments.

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-IRP2 (Santa Cruz Biotechnology # 33682, 1:500),
anti-FBXL5 (Neoclone # N0036, 1:500; BioLegend # 672604, 1:500),
anti-GAPDH (Cell Signaling # 2118, 1:1000; Santa Cruz Biotechnology # sc-47724, 1:5000),
anti-CAND1 (Santa Cruz Biotechnology # 10672,1:2000; Bethyl Laboratories # A302-901A, 1:1000),
anti-CAND2 (Bethyl Laboratories # A304-046A, 1:1000),
anti-CUL1 (Invitrogen # 32-2400, 1:500; Bethyl Laboratories # A303-373A, 1:500),
anti-SKP1 (Cell Signaling # 12248, 1:1000),
anti-SKP2 (Cell Signaling # 2652, 1:1000),
anti-HA (Cell Signaling # 3724, 1:1000),
anti-FLAG (Sigma-Aldrich # F1804, 1:5000),
anti-StrepII (Abcam # ab76949, 1:500),
anti-IκBα (Abcam # ab32518, 1:1000),
anti-BCL10 (Santa Cruz Biotechnology sc-5273, 1:200)
anti-CUL2 (Thermo Fisher Scientific # 51-1800, 1:1000),
anti-CUL3 (Cell Signaling # 2759, 1:500),
anti-CUL4A (Cell Signaling # 2699, 1:1000)
Alexa Fluor 790 conjugated anti-mouse IgG (Abcam # ab186699, 1:10000)
Alexa Fluor 790 conjugated anti-rabbit IgG (Thermo Fisher Scientific # A11374, 1:10000)
Alexa Fluor 680 conjugated anti-rabbit IgG (Abcam # ab175772, 1:10000)
Alexa Fluor 680 conjugated anti-mouse IgG (Thermo Fisher Scientific # A10038, 1:10000).

Validation

All antibodies have been validated by the manufacturers and prior studies.
Santa Cruz Biotechnology # 33682;
https://www.citeab.com/antibodies/802004-sc-33682-irp-2-antibody-7h6?des=d90250dbd44942c6
BioLegend # 672604;
https://www.citeab.com/antibodies/6281176-672604-purified-anti-fbxl5-10f4h9d12-monoclonal?des=825a82aaad2ac164
Neoclone # N0036;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4047410/
Millipore # MAB374;
https://www.citeab.com/antibodies/226212-mab374-anti-glyceraldehyde-3-phosphate-dehydrogenase?des=e71958bb34c91665
Santa Cruz Biotechnology sc-47724;
https://www.citeab.com/antibodies/795239-sc-47724-gapdh-antibody-0411?des=628c9d7e0ff57c89
Bethyl Laboratories # A302-901A;
https://www.citeab.com/antibodies/657521-a302-901a-rabbit-anti-cand1antibody-affinity-purifi?des=e69ac986f288e80d Santa Cruz Biotechnology sc-10672;
https://www.citeab.com/antibodies/831216-sc-10672-tip120a-antibody-a-13
Bethyl Laboratories # A304-046A;
https://www.citeab.com/antibodies/1038634-a304-046a-rabbit-anti-tip120bantibody-affinity-pur?des=d78c5bab659b982a
Invitrogen # 32-2400;
https://www.citeab.com/antibodies/2399652-32-2400-cullin-1-monoclonal-antibody-2h4c9?des=92a633eca16bf0b3
Bethyl Laboratories # A303-373A;
https://www.citeab.com/antibodies/15807821-a303-373-rabbit-anti-cul1-antibody-affinity-purifi?des=04b3b20d53f702d3
Cell Signaling # 12248;
https://www.citeab.com/antibodies/975709-12248-skp1-d3j4n-rabbit-mab?des=c7e40e68fd4f14d3
Cell Signaling # 2652;
https://www.citeab.com/antibodies/123795-2652-skp2-d3g5-xpsup-sup-rabbit-mab?des=378dc610dda863c8
Abcam # ab76949;
https://www.citeab.com/antibodies/775679-ab76949-anti-strep-tag-ii-antibody? des=722e3a62ab6421e3
Cell Signaling # 3724;
https://www.citeab.com/antibodies/123379-3724-ha-tag-c29f4-rabbit-mab? des=8dd625905a818bcf
Sigma-Aldrich # F1804;
https://www.citeab.com/antibodies/2304935-f1804-monoclonal-anti-flag-r-m2-antibodyproduced-i?des=726d14f36e308379
Abcam # ab32518;
https://www.citeab.com/antibodies/766198-ab32518-recombinant-anti-ikb-alpha-antibody-e130?des=a34e410f3824f661
Santa Cruz Biotechnology sc-5273;
https://www.citeab.com/antibodies/810774-sc-5273-bcl10-antibody-331-3
Thermo Fisher Scientific # 51-1800;
https://www.citeab.com/antibodies/2400477-51-1800-cullin-2-polyclonal-antibody

Cell Signaling # 2759;
https://www.citeab.com/antibodies/124019-2759-cul3-antibody
Cell Signaling # 2699;
https://www.citeab.com/antibodies/123889-2699-cul4a-antibody
Abcam # ab186699;
https://www.citeab.com/antibodies/2359788-ab186699-donkey-anti-mouse-igg-h-l-alexa-fluor-790
Thermo Fisher Scientific # A11374;
https://www.citeab.com/antibodies/2401426-a11374-donkey-anti-rabbit-igg-h-l-highly-cross-ads
Abcam # ab175772;
https://www.citeab.com/antibodies/2359728-ab175772-donkey-anti-rabbit-igg-h-l-alexa-fluor-68
Thermo Fisher Scientific # A10038;
https://www.citeab.com/antibodies/2401388-a10038-donkey-anti-mouse-igg-h-l-highly-cross-adso

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Flp-In T-REx 293 Cell Line (RRID: CVCL_U427) was obatined from Thermo Fisher Scientific (Catalog number: R78007). JMSU1 (RRID: CVCL_2081) and JHOS4 (RRID: CVCL_4649) cell lines were obtained from RIKEN. TOV112D (RRID: CVCL_3612) cell line was obtained from ATCC.
Authentication	All human cell lines derived from the Flp-In T-REx 293 Cell Line were authenticated by STR Profiling analysis (service provided by labcorp). The other human cell lines were not authenticated.
Mycoplasma contamination	Cell lines were periodically tested for Mycoplasma contamination. No Mycoplasma contamination was detected.
Commonly misidentified lines (See ICLAC register)	None.

Plants

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