nature portfolio

Corresponding author(s): Chun Tang

Last updated by author(s): Sep 30, 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	Thermo Scientific Xcalibur (version 4.2.47)
Data analysis	pLink2 (version 2.3.11), pFind3 (version 3.0.11), pymol (version 2.5), Xplor-NIH (version 3.7), AlphaFold3, AlphaFold-Multimer, Chai-1, GRASP, xiSEARCH1.8.7, Kojak2.1, xiFDR2.3.5, pLabel, pGlycoQuant(version 202407)

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD054983.

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	Approximately 7 × 10^8 cells were subjected to in-vivo cross-linking, as this quantity was determined based on the detection limit of mass spectrometry.
Data exclusions	No data were excluded from the analyses.
Replication	We verified the reproducibility of all cross-links, compartment-specific cross-links (cytoplasmic vs. nuclear) for all proteins, proteins involving the proteasome, and within the proteasome. All attempts at replication were successful.
Randomization	There were no distinct experimental groups; therefore, randomization was not relevant.
Blinding	There were no distinct experimental groups; therefore, blinding was not relevant.

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	EIF3M (Proteintech, 11423-1-AP, 1:100) ; Rpn11 (Santa Cruz, sc-100464, 1:100); RPS5 (Proteintech, 16964-1-AP, 1:200); Alexa Fluor 488 anti-rabbit IgG and Alexa Fluor 594 anti-mouse IgG (Jackson, both diluted by 1:200)
Validation	All primary antibodies have been validated by the manufacturers for use in human cells and Western blot. Validation information is available on the manufacturer's websites (EIF3M: https://www.ptglab.com/Products/Pictures/pdf/11423-1-AP.pdf; Rpn11: https://datasheets.scbt.com/sc-100464.pdf; RPS5: https://www.ptglab.com/products/RPS5-Antibody-16964-1-AP.htm) and has been

confirmed by our own Western blot data shown in Fig 8a.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Construction of Rpn11-HTBH HEK293 stable cell lines: The starting vector pRk5/iGluR2-IRES-EGFP was used to construct the expression plasmid pRk5/hRpn11-HTBT-IRES-EGFP. PEI was used to transfect this plasmid into HEK293 cells, and then single-clone HEK293 cells stably transfected with the recombinant plasmid pRK5/hRpn11-HTBT-IRES-EGFP were screened using the limited dilution method in 96-well plates.
Authentication	Fluorescence analysis of GFP, qPCR and western blot analysis of his-tag to characterize the Rpn11-HTBH HEK293 stable cell lines.
Mycoplasma contamination	The cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were commonly misidentified lines.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A