nature portfolio

Corresponding author(s): Kathy Fange Liu, Yale E. Goldman

Last updated by author(s): Aug 20, 2024

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	FretBursts v0.7 (multiparameter confocal spectroscopy data collection)
Data analysis	Fiji v2.14.0/1.54f, GraphPad Prism 9, MaxQuant 1.6.17.10, Metascape 3.5, custom Python scripts for the analysis of multiparameter confocal spectroscopy data (Zenodo 13334020)

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

Source Data are provided with this paper. The APEX2-MS proteomics data generated in this study have been deposited in the MassIVE database under accession code MSV000090789 [ftp://massive.ucsd.edu/v05/MSV000090789]. All other data generated in this study are provided in the Source Data file.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	For in vitro studies, at least three independent measurements from independent reactions were taken to ensure robustness. An n of at least for in vitro studies is sufficient to ensure reproducibility. For imaging experiments, at least 20 frames of each slide or well were taken in an unbiased manner (left to right, top to bottom) to ensure even coverage of the slide. Transfections were performed three times in total to ensure robustness. This is inline with our previous study (Mol Cell. 2022 Jul 21; 82(14) 2588-2603.e9)
Data exclusions	EMSAs: an individual band was excluded from analysis of binding data if something clearly went wrong with sample loading (i.e. a clogged well or a bubble in the gel) that affected the position of the unbound RNA band (shifted relative to other bands). No more than one data point (if any) was removed among the three repeated binding experiments for a given construct.
Replication	All experiments have at least n of three. In vitro experiments were performed with by at least two different individuals to ensure that the reported trends were accurate. Cell experiments were repeated with three different transfections on different days to ensure reproducibility. All replication attempts were successful
Randomization	No randomization was necessary
Blinding	Blinding was not relevant for this study as the measurements are not subjective

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

HRP conjugated anti-FLAG (Invitrogen MA1-91878-HRP, dil. 1:1000), Rabbit polyclonal anti-GAPDH (Invitrogen PA1-16777, dil. 1:1000), Goat anti-rabbit IgG (H&L) (HRP) (Abcam ab6721, dil. 1:20000), mouse anti-flag (Invitrogen MA1-91878 FG4R, dil. 1:1000), anti-mouse 594 secondary antibody (Invitrogen A32742, dil. 1:1000), Rabbit anti-DDX3Y (Invitrogen PA5-90055, dil. 1:3000) .

Validation

Validation statements taken from the manufacturer's websites:
MA1-91878-HRP: This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated.
PA1-16777: This Antibody was verified by Knockdown to ensure that the antibody binds to the antigen stated.
ab6721: 36 validation Western blot images reported.
MA1-91878: This Antibody was verified by Cell treatment to ensure that the antibody binds to the antigen stated.
A32742: This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated.

PA5-90055: This antibody was previously validated by our lab to be cross-reactive with DDX3X and DDX3Y (Mol Cell. 2022 Jul 21; 82(14) 2588-2603.e9)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HeLa (ATCC CCL-2) and HEK293T (ATCC CRL-3216)
Authentication	Cell lines were authenticated by ATCC
Mycoplasma contamination	None tested
Commonly misidentified lines (See ICLAC register)	Not used

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.