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Corresponding author(s): Lori A Passmore

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	1. Deadenylation assays: Typhoon FLA 7000 Control Software Version 1.2.1.93 (GE) 2. Circular Dichroism assays: Spectra Manager 1.54.03 (JASCO Corporation)

Data analysis	1. Quantitation of deadenylation assays: FIJI, ImageJ version 2.0.0-rc-54/1.51h 2. Data plotting and analysis: GraphPad Prism Version 7.0b for Mac OS X (GraphPad Software, Inc.) 3. Crystallography suite: Ccp4i2 Version 1.0.2 4. Indexing and Integration: Xia2 DIALS Version 1.12 5. Scaling and Merging: AIMLESS Version 0.7.3 6. Crystallography suite: Phenix Version 1.14-3260 (www.phenix-online.org) 7. Molecular Replacement: Phaser Version 2.8 8. Refinement: phenix.refine 9. Map generation: Feature-enhanced map, simulated annealing composite omit map: Maps tools in Phenix version 1.14-3260 10. Building RNA: RCrane Version 1.1 11. Sequence alignment: T-coffee suite Version 11.00 12. Visualization of sequence alignment: Jalview Version 2.8 13. Structural superposition and model building: Coot Version 0.8.9.2 14. Structure visualization: PyMol Version 1.8.4.2 (Schrodinger LLC)

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The structures generated during the current study have been deposited in the wwPDB under accession codes 6R9I (apo), 6R9J (A7-bound), 6R9M (AAGGAA-bound), 6R9O (AAGGA-bound), 6R9P (AAUUAA-bound), and 6R9Q (AACCAA-bound). Source data for figures 1b-e, 2b-d, 4c, 5a, 7b, supplementary figures 1e-f, 2a, 6e-g, and 7a-b are available in tabular form with the paper online. Source data for figures 1a, 2a, and 6a-h are available in Supplementary Data Set 1 with the paper online. All annotated gels are available on Mendeley (DOI: 10.17632/zkfsh9nftk.1). All other data that support the findings of this study are available from the corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to L.A.P. (passmore@mrc-lmb.cam.ac.uk).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 previous experience to evaluate reproducibility of assays.
Data exclusions	No data were excluded.
Replication	All deadenylation assays were performed in triplicate. Error bars (standard deviation) are given.
Randomization	n/a
Blinding	n/a

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Requests for materials generated in this study will be fulfilled by the corresponding author, upon reasonable request.