

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	Micromanager (v2.0), Zen (blue v2.3), BlueLake (v1.6.11), Gromacs 2021 software, DAQmi (v4.2.1)
Data analysis	Python (v3.8), Spyder (v4.2.5) with the libraries numpy (v1.20.1), pandas (v1.2.4), scipy (v1.6.2), seaborn (v0.11.1), and matplotlib (v3.3.4) used for data analysis. ImageQuant (v5.0, Cytiva) and KaleidaGraph (v4.02, Synergy Software) were used for analysis in the activity assays. ACPYPE and an online R.E.D. server were used to generate parameters for molecular simulations. Fiji (v2.9.0) and Adobe Illustrator (v27.0) were used to prepare figures.

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 relevant to the findings of this manuscript are included in the manuscript and the supplementary appendix. Additional data are available from the authors upon reasonable request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

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](https://www.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

RNA is stored in concentrated stock solutions and diluted to a final working concentration. The sample size for microscopy experiments is approximately 3 microliters for all samples. This volume is adequate for imaging and chosen to be as conservative as possible with the amount of RNA consumed for each trial for reasons of cost and availability. For each turnover measurement of pre-tRNA cleavage by Pfu RPR, six aliquots were withdrawn during a time-course assay. The time points chosen ensured that the rate of the initial linear phase of pre-tRNA substrate cleavage could be measured. To determine average turnover numbers and standard deviation of pre-tRNA cleavage for each of the RPR annealing conditions assayed, at least three technical replicates were performed.

Data exclusions

No data was excluded.

Replication

All experiments reported in this manuscript have been repeated at least three times with all data points shown in the relevant figures. Samples for FRAP were prepared three separate times and at least three condensates were randomly chosen for FRAP measurements in each sample. Images and movies shown are representative of the sample and consistent with a larger set of data that was acquired.

Randomization

Randomization is not relevant to this study. Each system is homogeneous and studied individually therefore randomization is irrelevant.

Blinding

Blinding is not relevant to this study. There is no information that can influence the outcome of the experiment. For FRAP experiments, condensates are chosen randomly amongst a set of compositionally identical condensates. Parameters that are used in data acquisition are applied uniformly across all samples, which are observed in a random manner. Computer simulations are performed using predefined algorithms and parameters are also chosen algorithmically, therefore blinding is irrelevant.

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	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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