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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	LabVIEW 2017 (NI, USA) was used to control the single-molecule magnetic tweezers, manage experimental procedures, and collecting data.
Data analysis	Single-molecule data analysis was performed using a combination of commercial and custom code. MatLab R2022a (MathWorks, USA) was utilized for all primary data processing and analysis tasks, including force and extension data fitting, statistical analyses, and visualization. The custom code, developed specifically for this study, was written in MatLab and is provided as a supplementary data file. This custom code includes scripts for importing and preprocessing experimental data and generating the figures presented in the manuscript. The Gromacs 2022.4 software package was utilized for molecular dynamics simulations and the analysis of simulation trajectories.

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 authors declare that all data supporting the findings in this study are available within the article, within Supplementary Data files, or from the corresponding author on reasonable request.

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	In our ribozyme study using single-molecule magnetic tweezers, we did not predetermine the sample size through formal statistical methods. Instead, we collected data from a sufficient number of individual RNA molecules to ensure the reliability and stability of force histograms and changes in extension histograms. Typically, we collected data from 100 to 500 traces per condition, which we found to be adequate based on pilot experiments and practical considerations. This approach allowed us to verify the consistency of our measurements and detect meaningful differences in the force and extension profiles, while also maintaining consistency with previous studies in the field. The large number of data points ensured that our histograms were statistically robust and reliable for the analysis of key features.
Data exclusions	In our ribozyme study using single-molecule magnetic tweezers, we did not exclude any data from the analysis except for force-extension traces that were identified as originating from non-single molecules. This exclusion was necessary to ensure the accuracy and reliability of our single-molecule measurements. Non-single molecule traces, which can result from multiple molecules being tethered to the bead or partial detachment of the molecule, introduce artifacts and variability that would confound the interpretation of the data. The criteria for excluding these traces were pre-determined and based on clear visual inspection and characteristic features of the force-extension curves that deviated from the expected single-molecule behavior. By excluding non-single molecule traces, we maintained the integrity of our data and the validity of our conclusions.
Replication	To verify the reproducibility of our experimental findings in the single-molecule magnetic tweezers assays, we performed a large number of assays across multiple conditions. Specifically, we conducted 462 assays from 263 ribozyme molecules in the first condition, 339 assays from 209 ribozyme molecules in the second condition, 235 assays from 125 ribozyme molecules in the third condition, and 133 assays from 85 ribozyme molecules in the fourth condition. These extensive replications were designed to ensure that our results are consistent and reliable. Each set of assays was repeated multiple times under the same conditions, and the force-extension traces were analyzed to confirm that the key features, such as unfolding forces and changes in extension, were reproducible across the different trials. All attempts at replication were successful, and the histograms generated from these assays showed consistent patterns, supporting the reproducibility of our findings.
Randomization	In our single-molecule assays, the allocation of samples into experimental groups was not random. Instead, we prepared ribozymes and their substrate RNA in a controlled manner to ensure consistency across the different conditions. For each experimental condition, we prepared a fresh batch of ribozymes and substrate RNA, and these were used sequentially in the single-molecule assays. If the samples ran out during the

experiments, we prepared additional batches to continue the assays. Covariates such as the concentration of RNA, buffer conditions, and experimental setup were carefully controlled and kept constant across all groups to minimize variability and ensure that the differences observed were due to the experimental conditions of interest. This non-random allocation and control of covariates were necessary to maintain the integrity and reproducibility of our single-molecule measurements.

Blinding

In our single-molecule magnetic tweezers assays, blinding was not performed during data collection and analysis. Blinding was not possible due to the nature of the experimental setup and the need for real-time adjustments and visual inspections of the force-extension traces. Specifically, the investigators needed to observe the traces and ensure that the assays were being conducted correctly, which required them to be aware of the experimental conditions at all times. Additionally, preparing and handling the RNA samples necessitated a clear understanding of the conditions being tested, making blinding impractical.
We acknowledge that the lack of blinding could introduce bias, but we took several measures to minimize this potential bias. These measures include:
1. Standardized Protocols: We used standardized and well-documented protocols for sample preparation and data collection to ensure consistency across all experiments.
2. Data Analysis: Data analysis was performed using objective criteria and automated software tools to reduce subjective interpretation.
3. Replication: We conducted a large number of assays to ensure that our findings are robust and reproducible.
4. Independent Verification: Key findings were independently verified by multiple investigators to ensure reliability.

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-digoxigenin antibody (Cat#: 11093274910, Roche).

Validation

The anti-digoxigenin antibody (Cat#: 11093274910) from Roche is a goat IgG that has been purified by centrifugation exchange chromatography and further isolated through immunoadsorption. The Fab fragments, obtained by papain digestion, are purified by gel filtration, conjugated with specific tags, and stabilized in a buffer.
The working concentrations for the antibody vary depending on the application:
Dot Blot: 150 mU/ml
ELISA: 150 to 300 mU/ml
Immunohistochemistry: 250 to 500 mU/ml
In Situ Hybridization: 1.5 to 7.5 U/ml
Southern Blot: 150 mU/ml
Western Blot: 250 to 500 mU/ml
Working Solutions
Northern Blot, Southern Blot: 100 mM Maleic acid, 150 mM NaCl, pH 7.5
Western Blot: 50 mM Tris-HCl, 150 mM NaCl, pH 7.5
Other Applications: 100 mM Tris-HCl, 150 mM NaCl, pH 7.5
Blocking Reagents: To reduce non-specific binding, 1% (w/v) blocking reagent, 1 to 5% (v/v) heat-inactivated fetal bovine serum, or normal goat serum can be used.
Storage Conditions: Diluted conjugate is stable at 2 to 8°C for 12 hours. Fresh preparation is always required.
Analytical Specifications:
Cross-reactivity with digoxigenin: <1%
No cross-reactivity with other human estrogenic or androgenic steroids such as estradiol or testosterone.
Cross-reactivity with other steroids: Unknown
Self-binding of the conjugate: None
Binding capacity: Typically, one molecule of the conjugate binds to one molecule of digoxigenin, although there are two possible binding sites for digoxigenin.
Non-specific binding to RNA: Rare
By following the manufacturer's recommendations and performing internal validation, we ensured that the anti-digoxigenin antibody (Cat#: 11093274910) was suitable and reliable for our experimental needs.

Plants

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