

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 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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 <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 Data were collected using Labview 2018.0.1 software

Data analysis Data were analyzed using Igor Pro 7.08 software

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 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

Data are available on figshare (DOI: 10.6084/m9.figshare.14614176)

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	7,269 unfolding and refolding curves were measured from 38 individual RNA molecules. The number of curves measured from a single molecule before it breaks varies widely, hence sample sizes could not be pre-determined. Approximate minimum sample sizes were chosen to ensure: (i) sufficient sampling to detect intermediates present at the 1% level (i.e., a minimum of 100-200 curves); (ii) sufficient sampling to obtain at least 10 measurements of contour length changes for every intermediate detected, to allow for reliable average values; and (iii) sufficient sampling to have at least 100 curves for defining the shape of the force distribution for each state, to allow for reliable fitting.
Data exclusions	No data from measurements of single RNA molecules were excluded. All measurements that did not involve correctly assembled single RNA molecules--as reflected via incorrect persistence length or contour length--were excluded.
Replication	Multiple molecules were measured for each experimental condition to show that the force-extension curves were reproducible across different molecules. Each curve represents an independent measurement of the unfolding/refolding. Measurements on different molecules represent replicates of the experiment. For each experimental condition, 180-3,650 independent measurements were done on 2-11 molecules (replicates), as listed in Supplementary Table 1. All the force-extension curves were reproducible across the different molecules (replicates).
Randomization	Randomization is not relevant to this study since there were no experimental groups. The individual molecules being studied were inherently randomized in solution.
Blinding	There were no experimental groups in this study. Investigators were not blinded during data analysis, but blinding was not necessary because all curves were analyzed in the same way using automated routines to remove potential bias.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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