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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 *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	Topspin 3.2 and 4.0.6, AMBER19
Data analysis	Cara 1.9.1.5, Tospsin 3.2 and 4.0.6, NMRpipe 8.9, Matlab2019a (including the matNMR 3.9.144 package)., Pymol-2.3.4, Origin 9.0G, Redcat, Xplor-NIH 2.49, eNORA, Sparky 3.114

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

NMR resonance assignments of Dz5C·RNA2'F are available via the BMRB with accession code: 34654. Atomic coordinates of the Dz5C·RNA2'F ensemble (cluster I) are deposited in the PDB under accession code: 7PDU. Coordinates of additional cluster and scripts for data analysis are available from the corresponding author upon reasonable request.

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 NMR data acquisition, individual samples for each condition were used relaying on the intrinsic ensemble averaging of the method. Sample stability was assessed for all conditions via characteristic spectral properties. Sample reproducibility was explicitly checked via different sample preparations. For mutation studies all possible (natural) nucleotide substitutions were used at all individually tested positions. The EPR experiments focused on the condition representing the central element of the study. Additional conditions have been reported before (ref. 20). For MD simulations 10 independent runs for each condition were computed to ensure sufficient sampling space and assess reproducibility.
Data exclusions	Only NMR peaks with unresolved signal overlap where excluded from analysis.
Replication	Key samples for NMR were tested by preparation with different stocks at different days showing high reproducibility of NMR spectra. Time-resolved 1D NMR results were repeated in two independent experiments. Cross validation of NMR data was carried out by analysis of the total observed spectral changes, which agree well with the respective kinetics observed via FRET. The same behavior was also detected in the 2D NMR measurement series confirming reproducibility of the experimental setup. Reproducibility of used kinetic assays was previously confirmed (ref. 20). EPR measurements of selected conditions were repeated at different days using independent sample preparations confirmed high reproducibility of EPR read out parameters. Activity of mutations were determined in triplicates. In general, all attempts at replication were successful including NMR, EPR, FRET, and PAGE data.
Randomization	No experimental design that required randomization was used in our study.
Blinding	Blinding was not used since it was not feasible (experimental design, data acquisition, and analysis required expertise of same highly trained researcher, i.e., for NMR, EPR, and MD experiments) and/or not relevant for the experimental design (in vitro activity assays). Instead, all data interpretation resulted from objective data analysis consistently involving all acquired data points.

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