Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size
   Describe how sample size was determined.
   Sample sizes were determined empirically for RNA structure profiling analysis.

2. Data exclusions
   Describe any data exclusions.
   No data were excluded from the analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Each experiment was replicated in triplicate.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   N/A

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   N/A

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

☐ ✔ The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

☐ ✔ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ ✔ A statement indicating how many times each experiment was replicated

☐ ✔ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

☐ ✔ A description of any assumptions or corrections, such as an adjustment for multiple comparisons

☐ ✔ The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted

☐ ✔ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

☐ ✔ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.
7. Software

Describe the software used to analyze the data in this study. Semi-Automated Footprinting Analysis (SAFA) was used for mapping RT stops in gels. Pymol was used for model interpretation.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

No restrictions on materials

9. Antibodies

N/A

10. Eukaryotic cell lines

HeLa cells were used. Cells were purchased from ATCC and all protocols were followed from NIH standards

Cells were authenticated using the human STR profiling service offered from ATCC

Cells were tested for mycoplasma contamination using a commercially available myco testing kit.

None

11. Description of research animals

N/A

12. Description of human research participants

N/A