

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
<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	The freely available software, micromanager version 1.4 (https://micro-manager.org), was used to operate the microscope system and collect the movie files.
Data analysis	All data was analyzed using python 3.10 based, lab-built software freely available on the lab's github page. vbscope: https://github.com/GonzalezBiophysicsLab/vbscope-paper tMAVEN: https://github.com/GonzalezBiophysicsLab/tmaven

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 analyzed in this study are available in the manuscript and Extended

Data. The number of molecules included and the parameters for the raw survival curve fit for each replicate, uncorrected for framerate and labeling efficiency, are available in Supplemental Table 1. The large, raw movie files are available upon request to R.L.G.

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](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	No statistical methods were used to determine sample size. Trace counts of approximately 100 molecules are sufficient to accurately determine rates. Therefore, every single-molecule experiment was performed in biological duplicate to verify reproducibility and each replicate contained a sufficient (~>100) single molecule trajectories upon which survival analysis was performed. Two experiments, ejection of Cy5-eIF4G:E from the internal mRNA with and without eIF4A and ATP, contained a low number of trajectories and were repeated an additional time. The ejection experiments with the second internal labeling position rpl41a A211 was repeated four times due to low trace counts.
Data exclusions	Trajectories were filtered for signal-to-noise ratio and the characteristics of a single-molecule (single-step photobleaching or photoblinking). All single-molecule trajectories with a signal-to-noise ratio >5 were included in the analysis.
Replication	All single-molecule experiments were performed in biological duplicate. Additionally, many experiments were performed where the fluorophore was moved to another protein in the complex (Cy5-eIF4G:E vs eIF4G:E-Cy5). The titration-regime anisotropy experiments were performed once each, but with two different concentrations of RNA in the titration-regime. All attempts at replication were successful.
Randomization	For the type of quantitative, biochemical experiments performed in this article assignment of groups is not necessary for accurate analysis of the data. Therefore no group randomization was performed.
Blinding	For the type of quantitative, biochemical experiments performed in this article, no group assignment is performed and consequently, blinding the experimenter to the group is not necessary.

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
☒	☐ Plants

Methods

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A