nature portfolio

Corresponding author(s):	Shixin Liu
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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.

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For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
		The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	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.
\boxtimes		A description of all covariates tested
	\boxtimes	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)
	\boxtimes	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\times		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
	'	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Single-molecule data was acquired using LUMICKS Bluelake software version 1.6.16. Mass photometry movies were acquired using AcquireMP

Data analysis

Kymographs were processed and analyzed using a custom script (https://harbor.lumicks.com/single-script/ c5b103a4-0804-4b06-95d3-20a08d65768f) which incorporates tools from the lumicks.pylake Python library and other Python modules (Numpy, Matplotlib, Pandas) to generate tracked lines using the kymotracker greedy algorithm. Python 3 was used to perform the mean squared displacement (MSD) analysis. Raw native mass spectrometry spectra were visualized using Thermo Xcalibur Qual Browser version 4.2.47. Native mass spectrometry data processing and spectra deconvolution were performed using UniDec version 4.2.0. Mass photometry raw data were converted to frequency distributions using Prism 9 (GraphPad). Statistical analysis of data was performed using Prism 10 (GraphPad).

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

Source data (raw values, uncropped gels) are provided with this paper. All kymographs used for analysis have been deposited as datasets in Zenodo (https://doi.org/10.5281/zenodo.11557684; https://doi.org/10.5281/zenodo.11559182).

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

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	v 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 single-molecule experiments, 15-30 kymographs were collected for each condition as standard practice in the field. The number of measurements was chosen to ensure sufficient statistics for each sample.
Data exclusions	For single-molecule experiments, tethers that display abnormal force-extension behaviors were discarded.
Replication	For single-molecule experiments, 15-30 kymographs were collected for each condition with comparable behaviors that were further quantified and described. Attempts at replication were successful.
Randomization	Randomization was not applicable as there was no grouping in this study.
Blinding	Blinding was not applicable to this study since the results are not subjective examinations by the experimenter.

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.

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Materials & experimen	ntal systems	Methods
n/a Involved in the study		n/a Involved in the study
Antibodies		ChIP-seq
Eukaryotic cell lines		Flow cytometry
Palaeontology and a	rchaeology	MRI-based neuroimaging
Animals and other or	rganisms	
Clinical data		
Dual use research of	concern	
Plants		
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Seed stocks	N/A	
Novel plant genotypes	N/A	
Authentication	N/A	