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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	FiJi(ImageJ); Matlab version 2016b; Matlab version of Spot-On (v1.0; GitLab tag 92cdf210) (https://gitlab.com/tjian-darzacq-lab/spot-on-matlab)
Data analysis	FiJi(ImageJ); Matlab version 2016b; Matlab version of Spot-On (v1.0; GitLab tag 92cdf210) (https://gitlab.com/tjian-darzacq-lab/spot-on-matlab)

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/reviewers upon request. 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

Uncropped scans for all western blots are provided in Supplementary Figure 1. The raw slowSPT and spaSPT data are freely available in Spot-On readable CSV and

Matlab formats in the form of single-molecule trajectories at Zenodo: https://zenodo.org/record/1215836. The Spot-On Matlab code is available together with a step-by-step guide at Gitlab: https://gitlab.com/tjian-darzacq-lab/spot-on-matlab. All other data are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of trajectories used for SPT analysis was described in Fig. 2, and found to give statistically reliable findings. No sample size determination needed for rest.
Data exclusions	No data exclusions occurred in this study.
Replication	All experimental findings have been reliably reproduced at least three times and sometimes even more (e.g. SPT data). Any use of statistical methods have been described in relevant figure legends.
Randomization	This study does not involve randomization of samples/organisms/participants.
Blinding	This study does not require investigators to be blinded to group allocation during data collection and/or analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	AFF1: Bethylaboratories, Cat. #A302-344A, validated by WB, 1:1000 dilution AFF4: Abcam, Cat. # ab57077, validated by WB, 1:1000 dilution ELL2: Bethyl Laboratories, 238 Cat. # A302-505A, validated by WB, 1:1000 dilution MePCE: Bethyl Laboratories, Cat. # A304-184A, validated by WB, 1:1000 dilution RNA Pol II phospho-Ser2(3E10): Millipore, Cat. # 04-1571, validated by WB, 1:1000 dilution RNA Pol II phospho-Ser5(3E8): Millipore, Cat. # 04-1572, validated by WB, 1:1000 dilution Total RNA Pol II: Santa Cruz, Cat. # sc-56767, validated by WB, 1:1000 dilution CDK7: Sigma, Cat. # C7089-.2ML, validated by WB, 1:3000 dilution GST: CST, Cat. #2622, validated by WB, 1:1000 dilution Tubulin: EMD CHEMICALS, Cat. #CP06, validated by WB, 1:2000 dilution CycT1: Santa Cruz, Cat. # sc-10750, validated by WB (1:1000 dilution) and IF (1:200 dilution) Anti-Flag: Sigma, Cat. # F3165, validated by WB and IF, 1:500 dilution CDK9, LARP7, HEXIM1 and Brd4 abs: Homemade, validated by WB, 1:1000 dilution
Validation	All commercial antibodies were also validated by the manufactures as indicated on their web sites.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	ATCC
Authentication	UC Berkeley Cell Culture Facility
Mycoplasma contamination	No contamination as verified by UC Berkeley Cell Culture Facility
Commonly misidentified lines (See ICLAC register)	N/A