nature portfolio

Corresponding author(s):	Shixin Liu and Michael E. O'Donnell
Last updated by author(s):	Jul 27, 2022

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

\sim			
\t	at	ıst	ICS

n/a	Confirmed						
	The exact	t sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement					
	A stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly					
	The statist	statistical test(s) used AND whether they are one- or two-sided common tests should be described solely by name; describe more complex techniques in the Methods section.					
\boxtimes	A descript	cription of all covariates tested					
\boxtimes	A descript	ption 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> values as exact values whenever suitable.						
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings						
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes						
		of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated					
	1	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					
So	ftware and	d code					
Poli	cy information a	about <u>availability of computer code</u>					
D	ata collection	Single-molecule data were collected on LUMICKS C-Trap TWOM and Bluelake software v 2.1.5 which are commercially available.					
D	ata analysis	Imaging data generated on LUMICKS were analyzed by home-written code publicly available on the LUMICKS Harbor platform, as well as Origin software (commercially available). Genomic data were analyzed by bowtie2, MACS2 and MATLAB (commercially available). MATLAB was also used to analyze potential ACS motifs within λ DNA. Statistical analysis was performed using MS excel and PRISM 9 (commercially available).					

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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.

- 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

Data supporting the findings of this study are available within the article, its supplementary figures, as well as in the source data files.

Field-spe	ecific re	porting	
Life sciences	В	s the best fit for your research. If you are not sure, read the appropriate sections before making your selection. ehavioural & social sciences	
Life scier	nces stu	udy design	
All studies must dis	sclose on these	points even when the disclosure is negative.	
Sample size	Sample sizes were chosen based on the generally accepted standard in the field and the technical difficulty associated with each experiment.		
Data exclusions	No data on qualified tethers was excluded from analysis.		
Replication	All experiments were confirmed with multiple biological replicates as described in figure legends.		
Randomization	This is not relevant because individual molecules were examined in this study and no grouping was used.		
Blinding	This is not relevant because our results are not subjective examinations by the experimenter.		
We require informati	on from authors a ted is relevant to	Decific materials, systems and methods about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
Materials & ex		·	
n/a Involved in th	,	n/a Involved in the study ☐ ChIP-seq	
Antibodies Karyotic cell lines		Flow cytometry	
_ _			
Animals and other organisms			
Human research participants			
Clinical data			
Dual use re	esearch of concer	n	
Eukaryotic c	ell lines		
Policy information	about <u>cell lines</u>		
Cell line source(s	l line source(s) Yeast strain OY001 was derived from the W303 strain.		

Yeast strain OY001 was derived from the W303 strain.
N/A
N/A
N/A