# nature research

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Last updated by author(s):	Oct 13, 2020

## **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 our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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
	$\square$ 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  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 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 <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
$\boxtimes$	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 <i>d</i> , Pearson's <i>r</i> ), 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

A custom-built optical tweezers setup was used and data collection was controlled using a custom-written LabVIEW program as described previously in Nicholas et al. 2014 (Methods Mol. Biol.).

Data analysis

Data were analyzed using a custom-written MATLAB (version R2019a) program as previously described in Nicholas et al. 2015 (PNAS).

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 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

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Please select the or	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of t	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>
Life scier	nces study design
All studies must dis	sclose on these points even when the disclosure is negative.
Sample size	Sample sizes were determined to be able to draw statistically meaningful conclusion as previously described in Nicholas et al. 2015 (PNAS).
Data exclusions	No data were excluded.
Replication	For reproducibility, every genome was sequenced and all constructs were assayed from at least two independent preparations. All replication attempts were successful.
Randomization	Measured histograms of unbinding forces (number of events as a function of force) cannot be randomized.
Blinding	Data acquisition was automated in the absence of possible user bias. Two people analyzed the acquired data independently and obtained the same results.
We require information	g for specific materials, systems and methods on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ted 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 & exp	perimental systems  Methods  n/a   Involved in the study

Materials & experimental systems	Methods
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Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
Animals and other organisms	
Human research participants	
Clinical data	
Dual use research of concern	

### **Antibodies**

Antibodies used

Anti-GFP antibodies were purified from rabbit serum as previously described by Nicholas et al. 2015 (PNAS).

Validation

Antibodies were purified using an affinity column bearing purified GFP with a GST (glutathione S-transferase) tag (GSTGFP) and the GFP-tagged constructs used in this study only bound to trapping beads when coated with the purified anti-GFP antibody.