# natureresearch

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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

#### Statistical parameters

text	text, or Methods section).					
n/a	Coi	nfirmed				
	$\boxtimes$	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
	$\boxtimes$	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
$\boxtimes$		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 statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (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 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				
$\boxtimes$		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 ab	out availability of computer code
Data collection	Micro-Manager (1.4.22 Nightly-build) was used for device control and multi-dimensional data acquisition. LabVIEW 2011 was used to perform global exposures to the PCO Edge camera.
Data analysis	The raw 3D data is processed (descrewed, deconvolved and rotated) using Lattice Light Deconvolution Software (https:// www.flintbox.com/public/project/31374/). ChimeraX (0.5-daily https://www.cgl.ucsf.edu/chimerax/download.html) was used for view and demonstrate the volumetric date in 4D. Single molecule localization analysis was performed with home-written C++ software. Fuji was used to manually recognize the leading edge of actin. All other analysis code are custom written in Python 3.6.

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

The data that support the findings of this study are available from the authors 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

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

### Life sciences

### Study design

All studies must disclose on these points even when the disclosure is negative.						
Sample size	No sample size calculations were performed.					
Data exclusions	No data excluded from analyses.					
Replication	All experiments were repeated by at least three times independently.					
Randomization	Not applicable.					
Blinding	Not applicable.					

### Materials & experimental systems

#### Policy information about availability of materials

n/a	Involved in the study					
$\boxtimes$	Unique materials					
$\boxtimes$	Antibodies					
	Eukaryotic cell lines					
$\boxtimes$	Research animals					
$\boxtimes$	Human research participar	nts				
Eukaryotic cell lines						
Policy information about <u>cell lines</u>						
Cell line source(s)		All cell lines are purchased from ATCC.				
Authentication		All cell lines are authenticated cell lines from ATCC.				

 Mycoplasma contamination
 Cell lines were tested free of mycoplasma contamination.

 Commonly misidentified lines (See ICLAC register)
 Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

### Method-specific reporting

n/a	Involved in the study
$\boxtimes$	ChIP-seq
$\boxtimes$	Flow cytometry
$\boxtimes$	Magnetic resonance imaging