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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 our Editorial Policies and the Editorial Policy Checklist.

## Statistics

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 | |
|---|---|---|
| ☐ | ☒ | 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<br>*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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*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 |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Deltavision acquisition software: Deltavision softWoRx version 6.5.2<br>OMX SoftWoRx software: GE Healthcare, V6.1, for OMX V3 Blaze SIM microscopy |
| Data analysis | Fiji/ImageJ: continually evolving software - see Schindelin et al doi:10.1038/nmeth.2012<br>Fiji/Image J with SIMCheck plug-in (for SIM microscopy quality control)<br>Huygens Professional version 17.04 |

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

Primary imaging data underlying widefield, SIM and STED images in Figs. 2, 3 and 6 have been deposited with Figshare. There are no restrictions on data availability.

# Field-specific reporting

Please select the one below 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 | No statistical methods were used to predetermine sample size, as this study did not include animal models or human participants. Sample size was determined based on standards in the field and experimental experience to obtain statistical significance and reproducibility. |
| Data exclusions | All data acquired for for this study were included in the analysis with rare exceptions where images did not pass quality controls: for 3D-SIM microscopy, images that showed more than 50% of bleaching during acquisition and did not pass the SIMcheck quality control step for artefact-free image reconstruction were discarded. |
| Replication | All experimental findings were reliably reproduced in multiple independent experiments. |
| Randomization | No randomization was done, because this study does not involve animals or human participants. Samples were organized into groups based on treatments (ie immunofluorescence, RASER, 4min, 4min+dry). Appropriate controls were included in all experiments. |
| Blinding | There was no blinded group allocation. All data that passed quality controls (see data exclusion) were analyzed by unbiased automated image analysis or under strict internal standards for objective manual image analysis. |

# 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.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | Rabbit anti-single-stranded DNA 1/50 (IBL International cat. no. JP17931) https://scicrunch.org/resolver/RRID:AB_2341405<br>Sheep anti-DIG FITC 1/50 (Roche cat. no. 11207741910) https://scicrunch.org/resolver/RRID:AB_514498<br>Rabbit anti-sheep FITC 1/100 (Vector cat. no. FI-6000) https://scicrunch.org/resolver/RRID:AB_2336218<br>mouse anti HP1alpha Millipore Cat. No. MAB3584 https://scicrunch.org/resolver/RRID:AB_94938<br>mouse anti fibrillarin Abcam ab4566 https://scicrunch.org/resolver/RRID:AB_304523<br>mouse anti 53BP1 Millipore MAB3802 https://scicrunch.org/resolver/ RRID:AB_2206767 |
| Validation | All antibodies are validated for their purpose by the suppliers. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | C127; mouse mammary epithelial cell line ATCC Cat No CRL-1804 https://scicrunch.org/resolver/RRID:CVCL_6580<br>HeLa; human epithelial like cervical carcinoma cell line ATCC Cat No CCL-2, https://scicrunch.org/resolver/RRID:CVCL_0030<br>mouse ES ROSA-Tir1 cells - gift from Prof R Klose, Oxford and as described in Rhodes et al 2020 doi:10.1016/j.celrep.2019.12.057<br>U2OS; human epithelial like osteosarcoma cell line ATCC Cat No HTB-96 https://scicrunch.org/resolver/RRID:CVCL_0042<br>RPE1; human epithelial retinal cell line ATCC Cat No CRL-4000 |

| | |
|---|---|
| Authentication | None of the cell lines used are known to be frequently misidentified or cross contaminated. All cells lines purchased from ATCC were authenticated by the provider. |
| Mycoplasma contamination | All cell lines tested negative for mycoplasma |
| Commonly misidentified lines (See ICLAC register) | Cell lines used in this study were not listed in the commonly misidentified category. |