nature portfolio

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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 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 *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 *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	No software was used for data collection
Data analysis	Prism Graphpad 9 used to perform statistical tests. Nikon Elements BR-4.40.00 64 bit used to measure lengths of DNA molecular combed fibers. ImageJ 1.52q was used to analyze autoradiograms and Western blots.

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.

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data supporting the findings of this study are available within the article and its Supplementary Information files. Source data showing unprocessed and uncropped gel and blot images are provided with this paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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 sample size calculation was performed. Biochemical experiments were performed at least twice with a representative result shown and, where relevant, quantification of multiple repeats. This is consistent with standard biochemical practice. For DNA fiber analysis in human cells at least 100 fibers were counted, which is consistent with standard practice and is a sufficiently large sample size to ensure that the mean will not appreciably change with additional measurements.
Data exclusions	No data were excluded from the analysis
Replication	All experiments were replicated at least twice. All results reproduced.
Randomization	Randomization is not relevant to this study. Xenopus egg extracts and cells were split evenly between different experimental conditions to ensure that essentially identical starting material was used in call cases.
Blinding	For DNA combing, all samples were measured blinded. For biochemical experiments samples could not be blinded because it was necessary to present individual samples in the appropriate order for visual analysis. Blinding also would not matter because these are bulk assays where the entire sample is measured using densitometry which is highly objective.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Antibodies targeting Xenopus CDC45, MCM6 and FAN1 were obtained from Johannes Walter. Antibody targeting Xenopus SAMHD1 and antibody (ii) targeting SMARCAL1 was obtained from Vincenzo Costanzo.

Antibody (i) targeting SMARCAL1 was generated by this study.
The secondary antibody used for Western blotting of Xenopus proteins was HRP Goat anti-rabbit (111-035-003) from Jackson Immunoresearch) in all cases.
Antibody targeting human RAD51 (AB63801) was obtained from Abcam and the secondary antibody was IRDye 800CW Goat anti-rabbit (LI-COR 926-32211).
Primary antibodies used for DNA fiber analysis were rat anti-CldU antibody (Abcam ab6326) and mouse anti-IdU antibody (BD B44).
Secondary antibodies used for DNA fiber analysis were Alexa Fluor 594 Goat anti-Rat (Invitrogen A11007) and Alexa Fluor Plus 488 Goat anti-mouse (Invitrogen A32723).

Validation

Antibody (i) targeting Xenopus SMARCAL1 was raised against a peptide of CKRRKIDDYFAL (New England Peptide 3850), affinity purified against its target peptide, and confirmed to immunoprecipitate only a single band that was also recognized by the previously-described Xenopus SMARCAL1 antibody (Kolinjivadi et al, 2017).
The following antibodies targeting Xenopus proteins were previously validated by Western blotting of Xenopus egg extracts in the indicated publication: RPA (Walter & Newport, 2000), CDC45 (Walter & Newport, 2000) , MCM6 (Dewar et al, 2017), SAMHD1 (Coquel et al, 2018) and FAN1 (Klein Douwel et al, 2014).
Antibody targeting human RAD51 was validated by Western blotting of HeLa cell lysates.
Anti-CldU antibody was validated by immunofluorescence of HEK293 cells (Condezo et al, 2017)
Anti-IdU antibody was validated by immunocytochemistry of chinese hamster ovary cells (Pinkel et al, 1985).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	U2OS (ATCC), HCT-116 (ATCC)
Authentication	All cell lines were verified using short tandem repeat profiling.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Xenopus Laevis, approximately 18 months and older
Wild animals	Study did not involve wild animals
Reporting on sex	N/A. Xenopus egg extracts are used as an experimental system for this study. These extracts are derived from frog eggs, prior to sex determination.
Field-collected samples	The study did not involve animals collected from the field
Ethics oversight	Animal protocols were approved by Vanderbilt Division of Animal Care (DAC) and Institutional Animal Care and Use committee (IACUC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.