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	Scans of radioactive gels were acquired using Typhoon TM FLA 9500 software, version 1.0. Single molecule DNA Replication tracks were imaged on a Nikon Eclipse 90i microscope fitted with a PL Apo 40X/0.95 numerical aperture (NA) objective.
Data analysis	The radioactive gels and single molecule DNA replication tracks used in this study were analyzed using ImageJ 1.53g and the results were plotted using Graph Pad Prism 9.0.2. software.

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 relevant data generated or analyzed during this study are included in this article and its supplementary information file.

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	Sample size (or number of repeats) was chosen based on what is common in the field, and what was practical to do.
Data exclusions	In general, no data were excluded unless there was a valid reason to do so, e.g. experiments with failed positive controls indicating technical problems, or when loading control indicated unequal loading that invalidated the analysis or other technical issues (broken gels, collapsed wells in gels etc.).
Replication	The experiments were replicated several times as indicated in figure legends. Oftentimes, even within a single experiment, multiple enzyme concentrations were analyzed, or samples were compared at multiple time points. This also contributes to the robustness of the data.
Randomization	N/A
Blinding	N/A

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	Primary Antibodies Mouse anti-RAD51B (Santa Cruz sc-377192), Mouse anti-XRCC2 (Santa Cruz sc-365854), Mouse anti-XRCC3 (Santa Cruz sc-271714), Rabbit anti-RAD51C (Abcam ab95069), Rabbit anti-RAD51D (Abcam ab202063), Rat anti-BrdU (Abcam ab6326), Mouse anti-BrdU (Becton Dickinson BD347580), Mouse anti-SMARCAL1 (Santa Cruz sc-376377), Mouse anti-BRCA1 (Santa Cruz sc-6954), Rat anti-TUBULIN (Abcam ab-6160), Mouse anti-his (MBL D291-3), Mouse anti-FLAG (Sigma F3165), Mouse anti-MBP (MBL M091-3). Secondary Antibodies Goat anti-mouse Alexa Fluor 488 (Thermo Fisher A-11029), Goat anti-rabbit Alexa Fluor 594 (Thermo Fisher A-11008).
Validation	All antibodies used in this study are commercially available. In addition, the antibodies directed against the RAD51 paralogs were further confirmed by purified recombinant proteins. SMARCAL1, BRCA1, Tubulin were further validated by using an siRNA directed against the protein of interest and/ or the corresponding knock-out cell line. The CldU (BrdU), IdU (BrdU) and rest of the antibodies are well validated as mentioned in the manufacturer's website. https://www.abcam.com/brdu-antibody-bu175-icr1-proliferation-marker-ab6326.html https://www.bdbiosciences.com/en-ch/products/reagents/flow-cytometry-reagents/clinical-discovery-research/single-color-antibodies-ruo-gmp/purified-mouse-anti-brdu.347580 https://www.abcam.com/tubulin-antibody-yl12-loading-control-ab6160.html https://www.mblintl.com/products/d291-3/ https://www.sigmaaldrich.com/CH/en/product/sigma/f3165 https://www.mblbio.com/bio/g/dtl/A/?pcd=M091-3

https://www.scbt.com/p/rad51b-antibody-f-12
https://www.scbt.com/p/xrcc2-antibody-f-4
https://www.scbt.com/p/xrcc3-antibody-d-7
https://www.abcam.com/rad51c-antibody-ab95069.html
https://www.abcam.com/rad51d-antibody-epr16205-ab202063.html
https://www.scbt.com/p/smarcal1-antibody-a-2
https://www.scbt.com/p/brca1-antibody-d-9

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MCF10A SMARCAL1 KO cells were previously used (Taglialatela et al., 2017 and catalog number not available), HEK293T (Cat#CRL-11268) cells were obtained from ATCC.
Authentication	Cell lines were not independently authenticated.
Mycoplasma contamination	Cell lines were not independently tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study (ICLAC, v11).